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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 are 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.

Keywords

Borrelia burgdorferi; ospC; Lyme disease; biodiversity; dilution effect; multiple niche polymorphism

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. 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, 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). The 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, MNP, 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 mammalian host diversity and did not find a relationship between Lyme disease risk and fragment size (LoGiudice et al. 2008). A recent paper 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, 2014).

In addition, 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 km² 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 *Ixodes scapularis* in the mainland, is limited on the island to a 4 ha site (Enser, 2000); thus most tick habitat on the Block Island is restricted to shrublands and shrub edges with sufficient leaf litter accumulation for tick survival (Finch et al., 2014). The mammalian community is composed exclusively of the white-footed mouse (*P. leucopus*), house mouse (*Mus musculus*), meadow vole (*Microtus pennsylvanicus*), brown rat (*Rattus norvegicus*), muskrat (*Ondatra zibethicus*), and white-tailed deer (*Odocoileus virginianus*) on the island (Comings, 2006; DeGraaf and Yamasaki, 2001). In contrast, the mammalian community of northeastern Connecticut is comprised of over thirty-five species of small and medium-sized mammals, including all known *I. scapularis* host species in the northeastern United States (DeGraaf and Yamasaki, 2001).

Small mammal sampling and collection of *I. scapularis* nymphal and larval ticks were performed in four peridomestic sites in both island and mainland regions (‘primary’ sites). Host-seeking nymphs were also collected at five additional peridomestic and natural sites (‘secondary’ sites) to improve estimates of nymphal densities, infection prevalence and *B. burgdorferi* OMG genotype diversity. Finally, an additional 69 nymphs collected from 17 sites on the island in 2010 and 2011 were genotyped to better characterize the OMG genotype frequency distribution (Table S1).

2.2 Small mammal sampling and larval burden estimation

Mark-recapture of small mammals via live-trapping was conducted at the four primary peridomestic sites in each of the study regions at approximately monthly intervals. In 2010, two two-night trapping sessions occurred in both regions. Mainland trapping ran from 8 July to 20 August 2010, while island trapping occurred simultaneously from 14 July to 27 August 2010. In 2011, three two-night trapping sessions ran from 26 May to 18 August 2011 at mainland sites and from 8 June to 18 August 2011. All trapping and handling procedures were approved by the Yale University Institutional Animal Care and Use Committee. At each site/night, 65 Sherman box traps (3" × 3.5" × 9", H.B. Sherman Traps, Inc. Tallahassee, FL) were distributed at 10 m intervals in three transects along residential, lawn-forest/shrubland edge, and forest/shrubland vegetation. Traps were set at dusk and checked the following morning no later than 7:30 am. All captured mammals were ear-tagged, aged, sexed, weighed, and searched for ticks before release at the point of capture. All attached ticks were removed with forceps and preserved in 70% ethanol. The species identity of larvae removed from hosts was determined morphologically using taxonomic keys (Durden and Keirans, 1996).

2.3 Host-seeking *I. scapularis* nymphal collections

We collected host-seeking *I. scapularis* nymphs at the eight primary sites, four secondary island sites (two peridomestic and two natural) and one secondary mainland site (natural). Island sites were sampled from 25 May to 29 July in 2010 and 9 June to 5 July in 2011; mainland sites from 15 June to 27 July in 2010 and from 2 June to 24 August in 2011. Host-seeking nymphs were collected by dragging a 1 m² corduroy cloth along transects of approximately 100 m through forest/shrubland and edge vegetation (Daniels et al., 2000; Schulze et al., 1997; Talleklint-Eisen and Lane, 2000). Attached nymphs were collected, counted and preserved in 70% ethanol, and identified to species using taxonomic keys (Durden and Keirans, 1996).

2.4 *B. burgdorferi* molecular screening

We extracted genomic DNA from *I. scapularis* nymphs and *P. leucopus*-derived larvae using the Macherey-Nagel Nucleospin Tissue Kit and its provided protocol (Macherey-Nagel, Düren, Germany) after homogenization of single ticks in liquid nitrogen using sterile pestles. All available host-seeking *I. scapularis* nymphs were processed; whereas only larvae that were determined as partially or fully engorged were further analyzed. Tick DNA extracts were screened for *B. burgdorferi* DNA by real-time PCR using a primer and probe combination that targets the 16S rDNA of *B. burgdorferi* following (Hoen et al., 2009) on a Applied Biosystems 7500 Real-Time PCR machine (Applied Biosystems, Foster City CA).

2.5 *B. burgdorferi* OMG determination

In addition to host-seeking nymphs collected from primary and secondary sites, an additional 69 nymphs collected from 17 sites on the island in 2010 and 2011 were included in this analysis to maximize the number of genotypes per region. To determine genetic diversity of *B. burgdorferi* strains, we characterized the OMG genotypes from the DNA template of host-seeking nymphs and *P. leucopus*-derived larvae using terminal restriction

fragment length polymorphism (tRFLP) analysis (Tsao et al., 2013). We restricted our OMG analyses to samples with known *B. burgdorferi* infection status and that were previously identified as containing a single *B. burgdorferi* strain as determined by Sanger sequencing at another locus (data not shown).

2.6 Data analysis

2.6.1 Small mammal sampling and tick burden estimation—*P. leucopus*

abundance was represented as mean captures/trap night and compared between mainland and island using a t test. A Wilcox test was used to compare *I. scapularis* larval burdens on *P. leucopus* between island and mainland sites.

2.6.2 Density of host-seeking *I. scapularis* nymphs and *B. burgdorferi*

infection—We calculated the density of *I. scapularis* host-seeking nymphs (DON), density of infected nymphs (DIN), and nymphal infection prevalence (NIP) using transect data from both island and mainland sites in 2010 and 2011. The DON and DIN were calculated as the number of host-seeking *I. scapularis* nymphs and the number of host-seeking *I. scapularis* nymphs infected with *B. burgdorferi* per 1000 m², respectively. NIP was calculated as the proportion of *B. burgdorferi*-infected nymphs of all nymphs collected per transect.

We used a logistic regression model with host-seeking nymphal infection status as the response variable to compare NIP between the island and mainland regions and across years (R software, version 2.15.2). We used a Poisson regression model with the number of nymphs per transect and the number of *B. burgdorferi*-infected nymphs per transect as the response variable to respectively compare DON and DIN between regions and years. We included transect area in the Poisson models as an offset or exposure variable, to adjust for sampling effort. We assessed univariate and multivariate models including main and interaction effects and identified the best fit model as the one with the lowest Akaike information criterion (AIC) (Akaike, 1974; Burnham and Anderson, 2004; Hurvich and Tsai, 1989).

2.6.3 OMG genotype richness and diversity—We assessed whether OMG richness and diversity differed between island and mainland sites. We treated OMG genotypes as different ‘species’ and used EstimateS version 9.1.0 (Colwell, 2013) to calculate established indices of species richness and diversity. We included OMG genotype data derived from nymphal ticks collected from all primary and secondary sites. Because richness analysis calculates presence/absence of OMG genotypes, data were pooled for both years for each region to increase the likelihood that all genotypes present were captured. Each site within a region represented a sampling unit, and the abundances of genotypes within each site were used to calculate sample-based richness and diversity statistics. We estimated species richness as the expected number of genotypes on the island and mainland with sample-based rarefaction and extrapolation using the Bernoulli product model (Colwell et al., 2012). Because of the lower number of sites sampled in the mainland, we used an extrapolation estimator to allow comparisons of genotype richness between the island and mainland communities. We extended the extrapolation conservatively to two times the reference sample size of the island. This procedure produced an extrapolated genotype richness

estimate and unconditional standard errors to produce 95% confidence intervals (Colwell et al. 2012). We also compared genotype richness and diversity between island and mainland by deriving the Chao2 and Jackknife2 richness estimators and Shannon diversity index. The Chao2 estimator is a commonly used nonparametric estimate of the total number of species for sample-based data while accounting for the number of unique species and duplicates (species that are detected twice) (Colwell et al., 2012; Colwell and Coddington, 1994). We also include the Jackknife richness estimator because it has lower standard deviation at lower sampling intensity and therefore represents a more conservative estimator (Hortal et al., 2006). The Shannon index is a common measure of species diversity that considers both species richness and evenness (Magurran and McGill, 2011).

2.6.4 Comparison of OMG frequency distribution between island and mainland nymphs—We used a Fisher exact test for count data to compare OMG genotype frequency distributions between regions and years. We compared the proportions of individual OMG genotypes between mainland and island sites using a binomial probability function to generate log-likelihood estimates of 95% confidence intervals.

2.6.5 Comparison of OMG frequency distribution between host-seeking nymphs and *P. leucopus*-derived larvae on Block Island—To assess the degree of host specialization of certain OMG genotypes to *P. leucopus*, we compared OMG genotype frequencies in *P. leucopus*-derived larvae to frequencies in host-seeking nymphs from all island sites, using a Fisher exact test for each year of the study. If certain OMG genotypes are adapted to *P. leucopus*, *P. leucopus*-derived larvae should be rich in these genotypes relative to the background genotype distribution that is found in host-seeking nymphs. Alternatively, similar frequency distributions in nymphs and larvae of the same year would indicate that *B. burgdorferi* transmission by *P. leucopus* alone can perpetuate OMG genotypes at the observed frequencies in the nymphal population. Nymphal *I. scapularis* samples were the same as described in the previous section; larval samples were derived from *B. burgdorferi*-infected larval ticks collected from *P. leucopus* during trapping sessions as described above.

3. Results

3.1 Small mammal sampling and tick burden estimation

The sampled host community composition varied between the two regions. On island sites, *P. leucopus* accounted for 96% of total captures in both years (86 and 154 total captures in 2010 and 2011, respectively), the meadow vole (*M. pennsylvanicus*) was the only other host captured in 2010 (4%) and meadow voles (2%) and avian hosts (2%) were captured in 2010 (Table S2). In mainland sites, *P. leucopus* was the most commonly captured host (201 and 418 captures in 2010 and 2011, respectively), comprising 75% and 76% of all captures in 2010 and 2011, respectively. Other host species captured on the mainland were the short-tailed shrew (*Blarina brevicauda*), eastern chipmunk (*Tamias striatus*), American red squirrel (*Tamiasciurus hudsonicus*), meadow vole (*Microtus pennsylvanicus*), and southern flying squirrel (*Glaucomys volans*), which comprised 25% and 24% of captures in 2010 and 2011, respectively (Table S2).

P. leucopus mean captures/trap night were significantly higher in the mainland (0.05) than the island (0.02) in 2010 (Welch two sample t-test: $t = -2.92$, $df = 18.93$, $P = 0.01$) and 2011 (Mainland: 0.03, Island: 0.02; $t = -4.07$, $df = 46.61$, $P = 0.01$) (Table S3). *I. scapularis* larval burdens on *P. leucopus* were significantly greater on island than mainland sites in 2010 (Wilcox Test: $W = 12535$, $P < 0.001$) and 2011 (Wilcox Test: $W = 43227$, $P < 0.001$) (Table S3). In 2010, a total of 1372 and 903 larvae were collected from *P. leucopus* in island and mainland sites, respectively; in 2011, 618 and 290 larvae were collected in island and mainland sites, respectively. Relatively few larvae were found on other hosts. Across both years, we found a total of 125 and 93 larvae on other small mammal hosts at island and mainland sites, respectively, representing 6% and 7.2% of the total larvae collected in these two regions.

3.2 Density of host-seeking *I. scapularis* nymphs and *B. burgdorferi* infection prevalence

In 2010, a total of 161 host seeking nymphs were collected from 64 island transects and 487 nymphs were collected from 80 mainland transects. In 2011, 122 host-seeking nymphs were collected from 35 island transects and 343 nymphs were collected from 36 mainland transects. The Poisson model with the lowest AIC for DON/1000 m² included a significant effect for region, with greater DON at mainland compared to island sites; year and the interaction term were not significant (Table 1, Figure 1). Similarly, the Poisson model with the lowest AIC for DIN/1000 m² also included a significant effect for region; DIN was greater in mainland sites compared to island sites, and year was not significant (Table 1, Figure 1). The logistic regression model with the lowest AIC indicated a significant effect for year and the interaction between region and year on the probability of a nymph being infected with *B. burgdorferi* (NIP). The significant interaction indicates that regional differences in NIP varied by year, with higher NIP in mainland sites in 2010 and higher NIP on island sites in 2011 (Table 1, Figure 1).

3.3 OMG genotype richness and diversity

We estimated OMG genotype richness from 221 host-seeking nymphs collected at 8 island sites and 129 nymphs from 5 mainland sites (primary and secondary sites). We identified a total of 16 OMG genotypes, three of which (J, T, and U) were only detected in mainland sites. OMG genotype richness rarefaction and extrapolation curves and associated 95% confidence intervals indicate significantly greater species richness in mainland sites than in island sites (Figure 2). Consistently, both Chao 2 and Jackknife 1 richness estimators were greater for the mainland than the island (island Chao 2 = 13, 95% CI = 13.00-13.42; island Jackknife 1 = 13, SD=0; mainland Chao 2 = 16.6, 95% CI = 16.05 – 23.14, mainland Jackknife 1 = 15.5, SD = 2.4). The Shannon diversity index indicated that mainland genotype diversity was greater than island diversity (2.49 and 2.31, respectively).

3.4 Comparison of OMG frequency distribution from island and mainland nymphs

The frequency distributions of OMGs in nymphal ticks were significantly different between island and mainland sites in 2010 (Fisher exact test: 100,000 Monte Carlo steps $P = 0.002$), but not in 2011 ($P = 0.223$). Within region, OMG frequencies were significantly different among years on the island (Fisher exact test: 100,000 Monte Carlo steps $P = 0.034$) but not

on the mainland ($P=0.774$) (Figure 3). Among *P. leucopus* persistent/human invasive OMGs (A, B, I, K, N) (Derdakova et al., 2004; Dykhuizen et al., 2008; Hanincova et al., 2013; Hanincova et al., 2008) frequencies of OMGs A, B, and N were not different between regions for either year, whereas genotype K and genotype I were found more often than expected by chance in the mainland in 2010 and in 2011, respectively. Additionally, in 2010, OMG genotype C, which has been reported as both invasive and non-invasive (Dykhuizen et al., 2008; Hanincova et al., 2013), was found more often on the island than the mainland than would be expected by chance.

3.5 Comparison of OMG frequency distribution from island nymphs and *P. leucopus*-derived larvae

We obtained 37 *B. burgdorferi*-infected larvae from 18 mice in 2010 and 76 *B. burgdorferi*-infected larvae from 29 mice in 2011. We found that overall, 10 of the 13 OMGs were present in larvae, though I and N were only present in 2010, while F was only detected in 2011 (Figure 4). OMG genotype frequencies in host-seeking nymphs and fed larvae were significantly different in 2010 (Fisher exact test: 100,000 Monte Carlo steps $P = 0.25$); but not in 2011 (Fisher exact test: 100,000 Monte Carlo steps $P < 0.001$).

4. Discussion

Host communities with low species diversity that are dominated by *P. leucopus* mice are predicted to maintain high *B. burgdorferi* NIP and DIN according to the dilution effect hypothesis, and low OMG genetic diversity with overrepresentation of *P. leucopus*-persistent strains according to the MNP hypothesis. Our results provided no support for the dilution effect hypothesis: *B. burgdorferi* NIP at island sites (0.24 ± 0.27) was not only similar to the mainland sites sampled in this study (0.28 ± 0.30), but was also comparable to the national average (0.20), as determined by a nationwide survey across three years including 5,328 tested nymphs (Diuk-Wasser et al., 2012). DIN was lower at island sites during the two-year study, contrary to the dilution effect predictions. We found mixed support for the MNP hypothesis: OMG diversity was lower on the island than the mainland community, supporting the MNP hypothesis; however, the frequency distributions of OMGs were only different in one of two years and these differences were not due to overrepresentation of *P. leucopus*-persistent/human invasive OMGs. The results of this study thus indicate a limited impact of mammalian host species diversity on human Lyme disease risk.

Similar *B. burgdorferi* NIP in regions of contrasting mammalian host species diversity suggests that other ecological processes may play an equal or stronger role in driving *B. burgdorferi* transmission dynamics than variation in reservoir competence. Two processes that can reduce the proportion of feeding larvae that become infected which are rarely accounted for in models of the dilution effect are the decline in reservoir host competence over time and host demographic turnover (Schauber et al. 2002, Hamer et al. 2012, Ogden and Tsao 2009). Larval peak feeding occurs on average two months after hosts are exposed to infectious nymphs in the northeastern US (Gatewood et al. 2009). During this period, some *B. burgdorferi* strains are subject to immune clearance (Hanincova et al. 2006, Derdakova et al. 2004) and there can be significant demographic turnover in the host

population (Schauber et al. 2002). These two mechanisms may partly explain the difference between the approximately 0.20 NIP observed on Block Island sites and the 0.92 NIP predicted in a mouse and deer host community from LoGiudice et al. (2003) model, which does not consider mechanisms of pathogen loss beyond the dilution effect.

The low NIP observed in a human modified landscape such as Block Island may result from high abundance of human-adapted dilution hosts. While the dilution effect proposes that human modified landscapes will be dominated by the highly competent white-footed mice (Nupp and Swihart, 1998, Allan et al., 2003), these landscapes may also have higher abundance of white-tailed deer that can both increase tick densities and reduce NIP (Telford III et al., 1988, LoGiudice et al., 2003, Ogden and Tsao, 2009), as well as higher abundances of bird species that may serve as dilution hosts (Brinkerhoff et al., 2011; Giardina et al., 2000; Ginsberg et al., 2005; Richter et al., 2000). The role of deer as a larval (dilution) host in addition to an adult tick host has been poorly quantified in the United States, mainly due to limited hunting during the August peak larval season; conversely it has been theoretically and empirically shown in European tick-borne disease epidemiological contexts (Cagnacci et al., 2012; Carpi et al., 2008). In our study, aerial infrared deer surveys estimated Block Island deer densities of 52.4 deer/mi² (unpublished data), more than double the density at the mainland sites (24.6 deer/mi²) (Gregonis 2007). Clarifying the role of deer as potential dilution hosts and of changes in the densities of avian and other low competence mammalian hosts in human modified landscapes is needed to improve our understanding of the associations between anthropogenic landscape modification, biodiversity and Lyme disease risk.

The other outcome measure of human Lyme disease risk, DIN, was lower on the island than the mainland, contrary to the prediction of the dilution effect hypothesis. Lower DIN on the island was mainly related to lower DON because of less efficient dragging in the dense understory vegetation. We therefore focus our conclusions on the more valid comparison between island and mainland NIP rather than DIN. The increased larval burdens on mice observed on the island site are consistent with the dilution effect hypothesis, because increased tick burdens would be expected by the absence of alternative hosts to divert questing ticks from mice (Brunner and Ostfeld, 2008). However, higher tick burdens on mice on the island may also be explained by the lower mouse densities observed there and a negative correlation between tick burden and mouse density as previously described (Ostfeld et al., 1996; Rosa and Pugliese, 2007; Rosa et al., 2007).

Our study provides mixed support for a role of MNP in maintaining OMG diversity. The maintenance of most OMGs on Block Island at similar frequencies as in the mainland is consistent with increasing evidence that most OMGs can infect most vertebrate hosts (Anderson and Norris, 2006; Hanincova et al., 2006; Vuong et al., 2013). On the other hand, the lower OMG richness and diversity on island than mainland sites and the absence on the island of three OMGs (J, T and U) that were previously found to be absent or rare in *P. leucopus* mice (Brisson and Dykhuizen, 2004) are consistent with the MNP hypothesis. However, two of these three missing genotypes (T and U) were detected on *P. leucopus* in two other studies (Hanincova et al., 2006; Vuong et al., 2013). Furthermore, their low frequency even in mainland sites suggests that their absence may be due to sampling effects,

even if narrow 95% confidence intervals on Block Island suggests that sampling effort was sufficient to characterize OMG variation in this region. Further research is needed to determine whether the trend towards lower OMG richness and diversity on Block Island may in fact represent a biologically significant phenomenon.

Although the MNP hypothesis would predict more genetic diversity in a system containing more opportunities for transmission (i.e. host types), this phenomenon could be obscured by other processes. For example, even if differential selection imposed by multiple and evolutionarily divergent hosts is strong and a main driver of diversity, importation of non-mouse-adapted genotypes by birds or other immigrating hosts to the species-poor system would serve to increase genotype richness and diversity. Conversely, lower genotype diversity in the species-poor island system may reflect founder effects rather than host-driven selection. Our finding of different OMG frequencies on the island in different years indicates that, while selective forces may drive increased frequencies of certain genotypes, these frequencies are dynamic and may vary significantly across space and time, as shown in other studies (Gatewood et al., 2009; MacQueen et al., 2012; Qiu et al., 1997).

The relatively high OMG diversity maintained in our study by a common reservoir host provides support for an alternative hypothesis for the maintenance of *B. burgdorferi* OMG diversity: the negative frequency-dependent selection hypothesis. Negative frequency-dependent selection is a form of balancing selection that occurs when rare genotypes in a population have a selective advantage over common genotypes, as might result from a weaker adaptive immune response from a host that is infected with multiple pathogen genotypes (Gromko, 1977; Wang et al., 1999). The negative frequency-dependent selection hypothesis is consistent with the large number of existing OMGs, which is greater than the number of hosts species typically infected by *B. burgdorferi*, and has been supported by a recent theoretical analysis (Haven et al., 2011). Longitudinal studies in ecologically contrasting settings are required to identify the ecological and evolutionary factors influencing the population dynamics of *B. burgdorferi* OMGs (Kurtenbach et al., 2006).

Our study does not support the prediction that there would be higher frequencies of *P. leucopus*-persistent OMGs on the island than the mainland communities. Since some of these genotypes have also been found to be invasive in humans, this implies no increased risk of human disease. However, the associations between these OMGs and invasiveness in humans do not appear to be strong (Hanincova et al., 2013). Furthermore, 24.7% of our samples in both island and mainland were composed of the OMG type C, which has mixed invasiveness phenotypes and was rare in most previous reports, so it remains to be fully characterized and its apparent emergence further investigated (Dykhuizen et al., 2008; Hanincova et al., 2013). One limitation of our study was the restriction to single infections only, which may introduce bias in the OMG composition if certain OMGs tend to occur more frequently in single vs. multiple infections. However, our population-based comparison of OMG frequencies should still be valid because potential biases should similarly influence island and mainland populations. Finally, although some OMG types have been detected during field studies being apparently over-represented in *P. leucopus* (or other hosts), this by itself is not necessarily evidence of adaptation to these host types or differential transmission. Evidence suggests that some strains persist better than others in

mice (*P. leucopus* and *Mus musculus*) (Derdakova et al., 2004; Hanincova et al., 2008), but comparative transmission experiments have not been performed for the majority of OMGs and kinetics of infection are largely unknown.

5. Conclusion

We found no support for the dilution effect when comparing NIP in two study systems that varied in mammalian host richness and abundance. We propose that similar NIP in contrasting mammal host communities suggests that alternative determinants of NIP should be further explored, such as host demographic turnover and the role of deer or birds as dilution hosts. Variation in OMG diversity between these systems is more challenging to interpret. The trend towards lower richness and diversity of OMG genotypes in the island system is consistent with MNP such that the narrower breadth of host types in the low host diversity system leads to lower diversity of OMGs. However, both systems were dominated by *P. leucopus* and we detected a number of OMGs that have not been previously associated with *P. leucopus* and thus not identified as potentially adapted to this host type. Even if host-mediated selection drives OMG diversity, the selectively favored genotype may vary over time through NFDS, resulting in long-term maintenance of high genotype diversity. It is apparent that there are multiple causes of NIP, DIN and OMG diversity and that these are not driven solely by the relative abundance and diversity of small mammal hosts. Teasing out the factors that are key to the maintenance of *B. burgdorferi* genetic diversity and variable infection prevalence in host-seeking ticks will require long-term, in-depth studies in a variety of environmental contexts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Ixodes scapularis nymphal density and *Borrelia burgdorferi* infection at peridomestic sites on Block Island, Rhode Island (“Island”) and northeastern Connecticut (“Mainland”) in 2010 and 2011. Density of host-seeking *I. scapularis* nymphs (DON) per 1000m² (top), density of *B. burgdorferi*-infected host-seeking *I. scapularis* nymphs (DIN) per 1000m² (middle), *I. scapularis* nymphal infection prevalence, estimated as the mean proportion of infected nymphs per transect (NIP) (bottom).

Figure 2.

Rarefaction and extrapolation curves (solid lines) and 95% confidence intervals (dotted lines) of estimated richness of *Borrelia burgdorferi* ospC major group (“OMG”) genotypes using increasing number of infected *I. scapularis* nymphal samples. Nymphal samples were collected on Block Island (“Island”) and northeastern Connecticut (“Mainland”) sites in 2010 and 2011. The filled circles indicate the number of genotypes actually obtained from each region.

Figure 3.
Borrelia burgdorferi ospC major group (“OMG”) genotype frequency distribution and binomial log-likelihood confidence intervals from host-seeking *Ixodes scapularis* nymphs collected at Block Island (“Island”) and northeastern Connecticut (“Mainland”) sites in 2010 (top) and 2011 (bottom).

Figure 4.

Borrelia burgdorferi ospC major group (“OMG”) genotype frequency distribution and binomial log-likelihood confidence intervals from host-seeking *Ixodes scapularis* nymphs and *Peromyscus leucopus*-derived *I. scapularis* larvae from sites on Block Island, Rhode Island in 2010 (top) and 2011 (bottom).

Table 1

Comparison between Connecticut sites and Block Island sites of the density of *Ixodes scapularis* nymphs (DON), density of nymphs (DIN) and nymphal infection prevalence (NIP) using poisson and logistic models.

	Estimate	Std error	z value	P
Poisson - DON^a				
Intercept	-3.46	0.08	-43.94	< 0.001
Region	0.733	0.1	7.16	< 0.001
Year	-0.12	0.12	-1.01	0.31
Region * Year	0.28	0.15	1.92	0.06
offset = log(transect area) ^d				
Poisson - DIN^b				
Intercept	-4.81	0.13	-37.65	< 0.001
Region	0.85	0.14	6.22	< 0.001
Year	0.09	0.13	0.71	0.48
offset = log(transect area) ^d				
Logistic - NIP^c				
Intercept	-1.24	0.19	-6.58	< 0.001
Region	0.04	0.22	0.17	0.87
Year	0.67	0.27	2.52	0.01
Region * Year	-0.7	0.32	-2.23	0.03

^a Density of nymphs,

^b Density of infected nymphs,

^c Nymphal infection prevalence,

^d In the Poisson models transect area is an offset or exposure variable to adjust for sampling effort