Ecology, 89(10), 2008, pp. 2841–2849

IMPACT OF HOST COMMUNITY COMPOSITION ON LYME DISEASE RISK

KATHLEEN LOGIUDICE,[1,2,4] SHANNON T. K. DUERR,[2] MICHAEL J. NEWHOUSE,[2] KENNETH A. SCHMIDT,[2,3] MARY E. KILLILEA,[2] AND RICHARD S. OSTFELD[2]

[1]*Department of Biological Sciences, Union College, Schenectady, New York 12308 USA*
[2]*Cary Institute of Ecosystem Studies, Box AB, Millbrook, New York 12545 USA*
[3]*Texas Tech University, MS 3131, Lubbock, Texas 79409 USA*

Abstract. The drivers of variable disease risk in complex multi-host disease systems have proved very difficult to identify. Here we test a model that explains the entomological risk of Lyme disease (LD) in terms of host community composition. The model was parameterized in a continuous forest tract at the Cary Institute of Ecosystem Studies (formerly the Institute of Ecosystem Studies) in New York State, USA. We report the results of continuing longitudinal observations (10 years) at the Cary Institute, and of a shorter-term study conducted in forest fragments in LD endemic areas of Connecticut, New Jersey, and New York, USA. Model predictions were significantly correlated with the observed nymphal infection prevalence (NIP) in both studies, although the relationship was stronger in the longer-term Cary Institute study. Species richness was negatively, albeit weakly, correlated with NIP (logistic regression), and there was no relationship between the Shannon diversity index (H') and NIP. Although these results suggest that LD risk is in fact dependent on host diversity, the relationship relies explicitly on the identities and frequencies of host species such that conventional uses of the term biodiversity (i.e., richness, evenness, H') are less appropriate than are metrics that include species identity. This underscores the importance of constructing interaction webs for vertebrates and exploring the direct and indirect effects of anthropogenic stressors on host community composition.

Key words: biodiversity; Borrelia burgdorferi; *dilution effect;* Ixodes scapularis; *Lyme disease;* Peromyscus leucopus; *ticks; vector borne; white-footed mouse; zoonoses.*

INTRODUCTION

The rise of zoonotic diseases in recent decades underscores the importance of ecology in understanding threats to human health (Daszak et al. 2000, Kruse et al. 2004, Patz et al. 2004, Dobson et al. 2006). However, despite much study, determining the drivers of variable disease risk in natural systems has proven to be challenging. Zoonotic cycles are integrated into complex webs of interactions involving numerous species on multiple scales, influenced by abiotic conditions and the vagaries of human behavior, placing them among the most difficult systems in community ecology. The difficulty in predicting disease risk is much in evidence in the case of Lyme disease (LD), one of the most studied zoonotic diseases in recent years. In scores of papers, investigators have probed the impact of abiotic features such as climate (Jones and Kitron 2000, Ogden et al. 2005), microhabitat (Stafford 1994, Bertrand and Wilson 1997, Randolph and Storey 1999, Schulze et al. 2002), and soils (Guerra et al. 2002, Bunnell et al. 2003), as well as biotic factors such as the population dynamics of *Borrelia burgdorferi*, the etiologic agent (Schauber and Ostfeld 2002, Brisson and Dykhuizen 2004, Tsao et al. 2004, Kurtenbach et al. 2006), the tick vectors (Benoit et al. 2005, Hornbostel et al. 2005, Ostfeld et al. 2006*a*), and their many vertebrate hosts (Daniels et al. 1993, Goodwin et al. 2001, Schulze et al. 2001, 2005, LoGiudice et al. 2003, Ostfeld and LoGiudice 2003, Perkins et al. 2006). Yet no consensus has been reached on the causes of the extreme temporal and spatial variation in Lyme disease risk.

Lyme disease is transmitted to humans primarily by nymphs of the *Ixodes ricinus* complex of ticks (Barbour and Fish 1993). Research has therefore focused on the factors that determine the density of the nymph vector (DON; Ostfeld and Keesing 2000*a*) and nymphal infection prevalence, (NIP), or proportion of the vector population infected with the disease agent. Together, these determine the density of infected nymphs (DIN). DIN is generally considered the primary measure of relative risk to humans when human behavior is held constant, although there is some indication that NIP may be a better predictor than DIN of human LD incidence (Connally et al. 2006).

The composition of the vertebrate host community and, by extension, the factors that shape that community, are likely to be pivotal in determining entomological risk. Important features of vertebrate communities that might influence risk are host species richness, identity, and abundance (Daniels et al. 1993, Schmidt et al. 1999, LoGiudice et al. 2003, Kurtenbach et al.

Manuscript received 26 June 2007; revised 8 January 2008; accepted 28 January 2008. Corresponding Editor: M. F. Antolin.

[4] E-mail: logiudik@union.edu

2006, Perkins et al. 2006, Sinski et al. 2006), which in turn are potentially affected by abiotic conditions and interspecific interactions (Jones et al. 1998, Ostfeld et al. 2006*a*). In northeastern North America, high densities and infection prevalence in blacklegged ticks (*Ixodes scapularis*) have been associated with forest fragmentation and small fragment size (Allan et al. 2003, Brownstein et al. 2005). This is presumed to reflect the effect of fragmentation on the host community, as smaller fragments have generally been found to have lower species richness and have sometimes been found to support larger densities of the white-footed mouse (*Peromyscus leucopus*) (Nupp and Swihart 1996, 2000, Rosenblatt et al. 1999, Anderson et al. 2003, Wilder and Meikle 2006), which is a common host for ticks and the most competent reservoir for *B. burgdorferi*. Thus we would expect a negative relationship between species richness, especially of predators, and small mammal densities, particularly of the ubiquitous white-footed mouse ("host regulation" in Keesing et al. 2006).

In 2003, we published the "host community model" (LoGiudice et al. 2003) based on the dilution effect hypothesis (Van Buskirk and Ostfeld 1995, Norman et al. 1999, Ostfeld and Keesing 2000*a*, *b*). The model links host biodiversity to NIP in blacklegged ticks (*Ixodes scapularis*), the sole LD vector in eastern and central North America. A subsequent investigation of the model (Ostfeld and LoGiudice 2003) demonstrated that community composition (species identity and relative abundance) is a relevant metric in determining LD risk. Crucially important in a real world setting is the order in which species are lost from an intact community when it is disturbed. In some scenarios of biodiversity loss, a decline in species richness was accompanied by an increase in LD risk, while in others; the opposite effect was seen (Ostfeld and LoGiudice 2003). Thus we continue to investigate the impact of the composition of the host community on LD risk by applying the host community model across time in an extension of the original study conducted at the Cary Institute of Ecosystem Studies in New York (hereafter, Cary Institute) and across space by expanding the geographic reach of our work to three northeastern states (the tri-state study). We also report on the degree to which temporal and spatial variation in NIP is correlated with variation in host biodiversity as measured by more traditional metrics of diversity, species richness and the Shannon diversity index. This pursuit is analogous to the comparisons of species richness vs. species composition in affecting the performance of specified ecosystem functions (e.g., Loreau et al. 2001, Cardinale et al. 2006). Similar to the biodiversity–ecosystem function controversy, determining the relative importance of standard diversity metrics (e.g., species richness and evenness) vs. species composition has important implications for the biodiversity-disease risk debate (Ezenwa et al. 2006, Keesing et al. 2006, Begon 2008).

The model and parameterization

The 2003 host community model was based on the differential ability of various host species to feed and infect larval ticks. Parameters collected for the model confirmed that different host species have different capacities to infect ticks (termed realized reservoir competence [Schauber and Ostfeld 2002]) and demonstrated that as hosts are added to a virtual community under realistic scenarios of increasing biodiversity, the proportion of ticks feeding on the most competent and most common host, the white-footed mouse, declines. Thus, since mice are universally present (or nearly so) in all communities, the effect of mice is diluted and NIP declines, on average, as less competent hosts are taken into account (Fig. 1a, circles). Some species have fairly fixed roles in the community (i.e., mice always function as "reservoir hosts" and eastern grey squirrels (*Sciurus carolinensis*), which have low reservoir competence, act as "dilution hosts"), whereas the role played by other species depends on the composition of the host community. Species with moderate reservoir competence, such as short-tailed shrews (*Blarina brevicauda*) can act as dilution hosts in a depauperate community composed primarily of competent reservoirs, but become reservoir or "rescue hosts" in a more diverse community or when mice are scarce (Ostfeld and LoGiudice 2003).

The empirical parameters for the model were collected in 2000 and 2001 on the grounds of the Cary Institute, which contains ~750 ha of 75–150 year old second growth forest. Primary parameters are host species identity, body burden (the mean number of larval ticks feeding on an individual of a given host species), realized reservoir competence (the probability that larval ticks will acquire infection from a given host species under natural conditions), molting percentage (the probability that a larval tick will molt successfully into a nymph after having fed on a given host species), and the population density of each host species. Hosts included in the model, four small mammal species, two species of tree squirrel, three mesopredators, four songbirds, and white-tailed deer (*Odocoileus virginianus*), were common in our study site and are generally found in large enough numbers to be relevant tick hosts (for more detail, see LoGiudice et al. 2003). Species occurring at extremely low densities, like larger predators and rare small mammals, were considered to be insignificant sources of tick blood meals and were therefore excluded from the model. Observed NIP was expressed as the proportion of infected field-collected nymphal ticks. The model predicts NIP by calculating the number of infected nymphs produced by all species divided by the total number of nymphs produced by all species. The model was validated by comparing the model-predicted NIP at the rodent densities measured during the larval peak (from 1996 to 2001) to the corresponding field observed-NIP the following spring (Fig. 1b, circles).

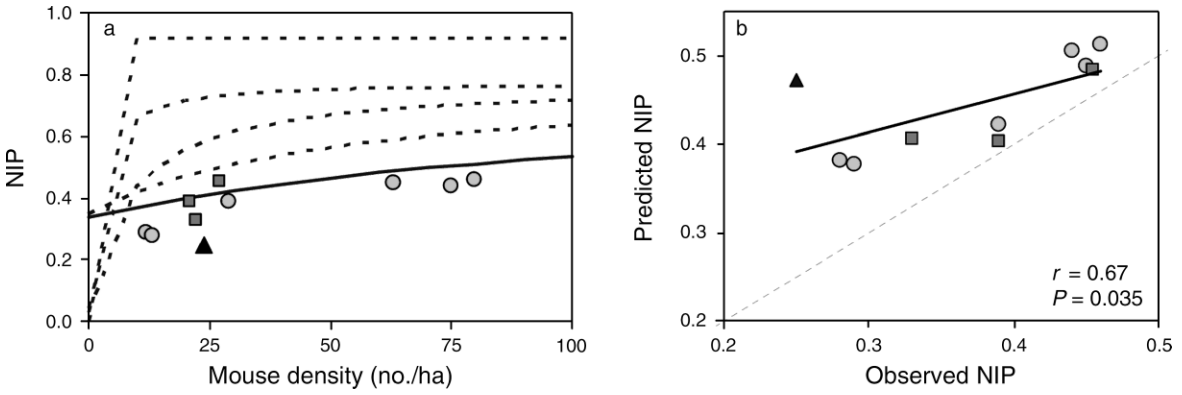

FIG. 1. (a) Predicted nymphal infection prevalence (NIP) of *Ixodes scapularis* across a range of white-footed mouse (*Peromyscus leucopus*) densities at the Cary Institute of Ecosystem Studies. Dashed lines show model-predicted NIP from host communities of varying diversity. Communities increase in diversity, with the top dashed line representing predictions from the least diverse community (mice only), and the lower dashed lines showing results from successively more speciose communities. The solid line shows NIP predicted by the highest diversity version of the 2003 dilution model. The markers show actual NIP observed in the spring with the corresponding mouse density from the previous summer when the nymphs were feeding as larvae. Years are: 1995–2001 (circles), 2002–2004 (squares), and 2005 (triangle). Figure revised from LoGiudice et al. 2003. (b) Model-predicted NIP vs. observed NIP. The solid line is best-fit linear regression line. The dashed line denotes the 1:1 ratio. Symbols are as in panel (a).

METHODS

Update of host community model (Cary Institute study)

Methods are the same as listed in LoGiudice et al. (2003). Briefly, host community parameters measured during the peak in larval abundance in late summer of 2000 were incorporated into the model to predict NIP, which was compared to the observed NIP measured during the nymphal peak the following spring. Small mammal, deer, and bird densities were determined by live-trapping, spotlight counts and point counts, respectively. All other densities were taken from the literature and considered typical moderate densities for our habitat type. See Appendix A for more complete methods. Observed NIP is defined as the proportion of ticks testing positive for *Borrelia burgdorferi* in the total sample for each trapping grid. NIP predicted by the host community model is defined as

$$\text{predicted\_NIP} = \frac{\sum(B_i S_i N_i C_i)}{\sum(B_i S_i N_i)}$$

when B_i is the average body burden of larval ticks feeding on species i; S_i is the molting percentage of ticks feeding on species i; N_i is the density of species i; and C_i is the reservoir competence of species i (LoGiudice et al. 2003).

Tri-state study

Forty forest fragments ranging in size from approximately 0.3 ha to 19.0 ha were chosen in three states with high numbers of LD cases, Connecticut ($n = 12$ fragments), New Jersey ($n = 11$), and New York ($n = 17$). Fragments were randomly selected from a group of candidates located in the counties in each state with the highest numbers of reported LD cases in 2001, dominated by non-oak deciduous trees (<40% oak canopy), and isolated by at least 80 m of non-forest matrix. Fragments sizes were determined using orthophotos and subsequently confirmed by GPS and all fragments used were separated from each other by at least 1 km.

Sampling of host communities was limited to measuring species richness and identities and estimating densities. Logistical considerations forced us to hold the other parameters in the model constant at the levels observed during the Cary Institute study in 2000–2001 (LoGiudice et al. 2003). As there is evidence that these two additional parameters, reservoir competence (Schauber and Ostfeld 2002) and body burdens (Goodwin et al. 2001), are likely to vary in space and time, a more robust test of the model would measure these parameters at each site.

Our goal was to determine a relative activity level (which we will call "activity density") of each species on a given fragment that would generate an estimate of the availability of each species to provide tick blood meals. Estimates of NIP in the spring of 2004 were generated from nymphs that fed on this host community as larvae in the late summer of 2003 but host community data were collected in 2003 and 2004. We use combined 2003–2004 frequency data for larger mammals (mesopredators and deer) to increase detection probabilities for these species, which we consider unlikely to fluctuate wildly interannually, and 2003 data only for small mammals (rodents and shrews), which can undergo large interannual changes in abundance. The small mammal community was sampled via trapping during peak larval abundance in August and early September 2003 and density was estimated directly for the most trappable species (white-footed mice and eastern chipmunks). Larger mammals (mesopredators and white-tailed deer)

were sampled via camera trapping in September and October of 2003 and 2004. For the less readily trapped small mammals (shrew and squirrel species) and the larger mammals, we created quasi-quantitative estimates by assigning observed densities to categories and using published values for similar habitats to create an activity density estimate (individuals/ha) for each category (see Appendix B).

Densities of forest bird species (including the four ground-dwelling birds that were used in the model, American Robin, Ovenbird, Veery, and Wood Thrush) were assessed during the 2003 and 2004 breeding seasons via point counts and transect counts, respectively. As point counts can overestimate occurrence on small fragments, we used the 2004 data with the assumption that densities of the four model species do not vary greatly interannually. See Appendix A for more complete methods. We also calculated the Shannon diversity index (H' [Magurran 1988]) for each fragment, using only the species in the model since we believe these to be the most important hosts in the system.

Nymphal ticks were collected via drag sampling in each fragment in June 2004. All ticks were maintained alive for later identification to species and nymphal *I. scapularis* were tested for *B. burgdorferi* as in the Cary Institute study. We attempted to test 40 ticks per fragment, but we were not always successful in collecting that number. We report findings using two correlation approaches comparing observed NIP to the model-predicted NIP: weighted regression where the dependent variable (observed NIP) is weighted by the tick sample size and simple or multiple linear regression using only those sites in which $\geq$30 ticks were tested. In addition, we maximized statistical power by using logistic regression when appropriate. These analyses compared the probability that a tick was infected to various landscape and community variables. All statistical analyses were conducted in JMP version 7 (SAS Institute Inc., Cary, North Carolina). Since population density of some hosts was coarsely estimated, we tested the model for sensitivity to inaccuracies in density estimation for each host species and found that the model is robust to considerable inaccuracy in host density estimation. See Appendix C for details about these tests. We also tested the observed NIP variables for spatial autocorrelation using Moran's I in ArcGIS 9.2 (Environmental Systems Research Institute, Redlands, California, USA).

Results

Update of host community model at the Cary Institute

The empirical data collected in the four years (2001–2002 through 2004–2005) since the 2003 host community model was published fit the predictions of the model well (Fig. 1a). For the entire 10 years, the model-predicted NIP is significantly correlated with the field-observed NIP ($P = 0.035$, $r = 0.67$). Model predictions were very highly correlated with observed NIP ($P = 0.0004$, $r = 0.92$) until the 2004–2005 season, in which the model substantially overestimated NIP (represented by triangle in Fig. 1a, b) and weakened the relationship between predicted NIP and observed NIP. The model consistently overestimated NIP by an average of seven percentage points (range 1–10 points for 1995–2004; 2004–2005 deviated by 22 points).

Tri-state study

Total mammalian and avian species richness in the forest fragments varied overall from 9 to 32 species (see Appendix D for a list of the species). We never captured an individual animal of any species on more than one fragment. Restricting our sampling to the 13 species (or groups of species) for which the model is parameterized, richness varied among fragments from 6 to 12 species. Complete data (host community composition and NIP) were collected for 26 of the 40 fragments sampled. Two fragments were developed or otherwise destroyed in the winter of 2003–2004, and we were unable to collect enough ticks ($\geq$30) to obtain a meaningful estimate of NIP in the remaining 12 fragments, including one in which no nymphs at all were found (although these data are incorporated into the weighted and logistic regressions). On average, these 12 fragments were smaller than the fragments with more ample tick populations (2.7 $\pm$ 3.1 ha [mean $\pm$ SD] and 5.9 $\pm$ 5.1 ha for insufficient and sufficient tick fragments, respectively; $P = 0.005$; two-tailed t test on log-transformed data) and they included six of the eight fragments under 1 ha (range 0.3–9.6 ha). Six fragments failed to produce any ticks testing positive for *B. burgdorferi*, although the sample size for four of these fragments was fewer than five ticks. The remaining two sites with NIP equal to zero had sample sizes of 16 and 25 ticks, indicating extremely low infection prevalence.

Observed NIP for sites with $\geq$30 ticks was significantly correlated with predicted NIP (Fig. 2, $P = 0.005$, $r = 0.53$, $n = 26$). Weighted regression produced similar results ($P = 0.0014$, $r = 0.40$), and allowed a larger sample size ($n = 37$). The correlation coefficient in the weighted regression was larger when sites in which no ticks tested positive were removed from the analysis ($P = 0.004$, $r = 0.50$, $n = 31$). NIP was generally highest in New York, intermediate in New Jersey, and lowest in Connecticut, but was not spatially autocorrelated ($n = 26$ fragments with $\geq$30 ticks; Moran's $I = 0.1$, $Z = 1.2$ standard deviations). There were no significant linear relationships between observed NIP and overall species richness ($n = 26$ fragments with $\geq$30 ticks) or between observed NIP and the richness of any taxonomic group (multiple regression, whole model, $P = 0.45$, Table 1). However, logistic regression (using all 1068 ticks collected on 37 fragments) revealed a significant negative relationship between richness and infection of nymphs driven by small mammals (multiple logistic regression: model likelihood ratio [LR] $\chi^2_1 = 9.37$, $P = 0.025$; Table 1). Both observed NIP and predicted NIP varied widely

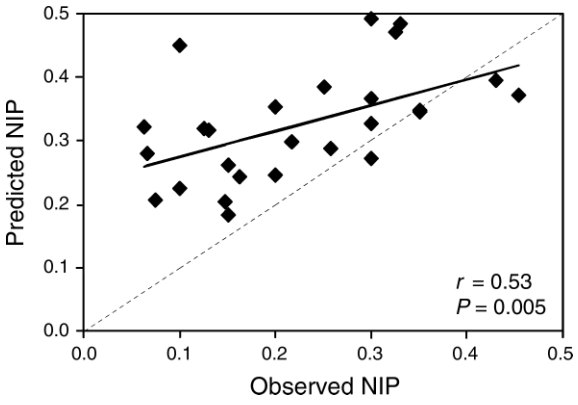

FIG. 2. Relationship between model-predicted nymphal infection prevalence and observed nymphal infection prevalence in 26 forest fragments (with ≥30 ticks) in Connecticut, New Jersey, and New York, USA. This model allows densities of all species to vary with empirical observations. The solid line denotes best-fit linear regression line. The dashed line is the 1:1 ratio. Each diamond represents a single forest fragment. Predicted NIP was based on the host communities described during the larval peak of 2003, and observed NIP was measured from nymphal ticks collected in the spring of 2004.

in different fragments with the same number of species but different identities and even in several cases when the exact same complement of species was present at different densities. Overall H' for the model species ranged from 0.48 to 1.68 and was unrelated to observed NIP using simple logistic regression ([LR] $\chi^2 = 3.5$, $P = 0.06$), multiple logistic regression taking each taxonomic group separately (model likelihood ratio [LR] $\chi^2_1 = 2.1$, $P = 0.55$; Table 1), and linear regression (multiple regression whole model, $P = 0.86$, Table 1).

White-footed mice were detected in every fragment, no other species showed such a ubiquitous distribution. Mouse population density varied from 3 to 50 individuals per ha (mean = 16 mice/ha). We observed consistently negative relationships between forest fragment size and white-footed mouse density in 2003 and 2004, but neither reached significance (Table 2). To test the hypothesis that mice are regulated more strongly by greater abundance of predatory species in more diverse communities ("host regulation" in Keesing et al. 2006), we also assessed correlations between *P. leucopus* density and the richness of predatory species. There was a negative relationship in both years, but the correlation reached statistical significance only in 2004 (2003 mouse density vs. predator richness, $P = 0.08$, $r = -0.28$; 2004 mouse density vs. predator richness, $P < 0.01$, $r = -0.40$; Table 2). In contrast to previous findings (Allan et al. 2003, Brownstein et al. 2005), we failed to detect a relationship between NIP and fragment size using the 27 fragments with at least 30 ticks sampled ($P = 0.95$) or using all ticks in a logistic regression ([LR] $\chi^2 = 0.02$, $P = 0.88$).

DISCUSSION

In this study, we assessed the ability of the host community model and simpler metrics of biodiversity (species richness and the Shannon diversity index) to predict entomological risk of Lyme disease across space and time. The host community model generated quantitative predictions of NIP across time (ten years at one site) and space (up to 37 forest fragments in three northeastern states) that were significantly correlated with the measured values. As there are many potential sources of error in estimating the model parameters, these consistently significant results suggest a robust ecological relationship.

Host species richness in a forest fragment was a significant, but weak predictor of the probability that nymphal ticks in the fragment would be infected (logistic regression). Species richness of small mammals was a better predictor than that of other groups of hosts or of total host species richness, and knowledge of the identities and relative abundances of the host species provided by the model added considerable predictive power. These results suggest that the relationship between LD risk and diversity depends on the identities and frequencies of the host species such that conventional metrics of biodiversity (i.e., richness and evenness)

TABLE 1. Results of multiple least-squares and logistic regressions regarding predictors of nymphal infection prevalence (NIP).

Predictors of NIP	Multiple least-squares regressions†				Logistic regressions: tick infection status (negative or positive) vs.			
	Richness (model species only) vs. NIP		H' (model species only) vs. NIP		Richness (multiple regression)		H' index (multiple regression)	
	P	R‡	P	R‡	P	χ^2	P	χ^2
Whole model	0.45	0.28	0.86	0.11	0.025	9.37	0.55	2.10
Small mammals	0.16	0.22	0.92	−0.05	0.01	6.19	0.24	1.38
Large mammals	0.81	−0.19	0.81	−0.09	0.09	2.87	0.95	0.00
Birds	0.43	−0.18	0.44	0.05	0.61	0.26	0.63	0.23

Notes: Neither species richness nor the Shannon index (H') is significantly correlated with NIP using a multiple least-squares regression ($n = 26$ fragments with ≥30 ticks). However, logistic regression ($n = 1068$ ticks collected on 37 fragments) revealed a significant negative relationship between richness and infection.

† There is no significant multicollinearity for any of the parameters in the multiple regression models (variance inflation factor [VIF] < 2.0) (Miles and Shevlin 2004).

‡ Bivariate correlation coefficients are reported for each independent variable.

TABLE 2. Potential correlates of white-footed mouse (*Peromyscus leucopus*)] density.

Correlate	ln (fragment size)	2003 mouse density	2004 mouse density	2003 predator richness	2004 predator richness
ln(fragment size)	1.00	−0.20	−0.30†	0.47**	0.35*
2003 mouse density		1.00	0.59***	−0.28†	−0.29†
2004 mouse density			1.00	−0.08	−0.40**
2003 predator richness				1.00	0.13
2004 predator richness					1.00

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

are less appropriate than are metrics that include species identity. The negative, albeit weak relationship between species richness and NIP occurred because all host communities included white-footed mice, which are the most competent reservoir for *B. burgdorferi*. Therefore, species-poor communities harbored mice and few other host species, whereas species-rich communities contained a higher relative abundance of non-mouse host species. However, because these non-mouse hosts vary strongly in their average population densities, tick burdens, and reservoir competences, the identity of the species occurring in each of these communities was important in determining the net effect on NIP (see also Ostfeld and LoGiudice 2003).

The importance of species identity and relative abundance in affecting LD risk is analogous to some results relating biodiversity to ecosystem functions such as primary production and rates of nutrient cycling (Loreau et al. 2001, Fargione and Tilman 2005, Cardinale et al. 2006). A positive correlation between richness and the performance of an ecosystem function can occur simply as a byproduct of the higher probability that more diverse communities will contain a particularly high-functioning species, a phenomenon called the "sampling effect" (Tilman et al. 1997). The LD system, however, appears to be characterized by a different type of sampling effect. In this case, low-diversity communities are more likely to be dominated by the ubiquitous white-footed mouse, which has a particularly strong positive effect on NIP. As communities become more diverse, the relative importance of white-footed mice decreases and that of other host species increases, generally reducing disease risk. The usefulness of species richness per se in predicting disease risk is limited simply because non-mouse species vary widely in the strength of their effects.

As expected, the model more accurately predicted NIP across time at the Cary Institute, the site in which it was originally parameterized, than across space in the tri-state (New York, New Jersey, and Connecticut) study. At the Cary Institute, the model predictions were highly correlated with observed NIP in eight of the nine years, although the model overestimated NIP, indicating that we may be missing an important dilution host. We suspect that flying squirrels (*Glaucomys volans*), which can reach high densities in mature forests, may be our missing dilution host. Unfortunately, this species is difficult to trap during midsummer (the peak larval period) and we have thus far been unable to collect sufficient data to evaluate this suspicion. The presence of an undetected dilution host may help to explain the tendency of the model to overestimate NIP more severely at low mouse densities. The relative contribution of an unknown, incompetent host would rise with a decline in mouse densities especially if the density of the unknown species was not positively correlated with mouse density.

In the longitudinal study, the only year in which the model substantially overestimated NIP was 2004–2005 (triangle in Fig. 1a). This could be caused by a number of possibilities including a decoupling of the densities of reservoir vs. dilution hosts or a change in larval tick feeding behavior. For example, we observed failure of the oak mast in 2003 concurrent with heavy hickory nut production (R. S. Ostfeld, *unpublished data*). This might have favored survival of squirrels over mice, which are less able to utilize these hard nuts. This would have produced an unusually high squirrel-to-mouse ratio in 2004, resulting in more larval blood meals being taken from relatively incompetent squirrels. Atypical abiotic conditions could have produced the same result. High humidity can cause ticks to quest higher on vegetation, potentially changing their encounter rates with various hosts (Randolph and Storey 1999, Vail and Smith 2002). This is consistent with the weather conditions during the peak larval period in 2004, which was the wettest in the 10 years of the Cary Institute study (data *available online*).[5] Larvae questing higher in the vegetation may be less likely to encounter white-footed mice, chipmunks and shrews, and more likely to parasitize larger, less reservoir-competent mammals, resulting in a lower NIP the following spring. This suggests that climate might indirectly affect NIP.

The model was somewhat less accurate in predicting NIP across space in the tri-state study. This is likely a consequence of a number of factors. First, because of logistical constraints, the only parameters estimated in every fragment were host identity and density. We relied on our Cary Institute values for host body burdens, molting percentages and realized reservoir competences. We expect that the predictions of the model would have been more accurate had we been able to measure these

[5] ⟨http://www.ecostudies.org/emp_data.html⟩

important parameters at each site and in each year. Second, the greater sampling effort in the Cary Institute study, three trapping grids of 2.25 ha sampled in each year, likely produced more accurate estimates of small mammal density than we were able to obtain in the many sites of the tri-state study. Finally, groups of forest fragments in the tri-state landscapes appear to function as meta-communities (Wilson 1992, Leibold et al. 2004) with varying rates of colonization and extinction of species important to LD ecology. For example, larval and nymphal tick densities varied dramatically across the sites, with one fragment failing to produce a single nymph and five more yielding fewer than five despite considerable sampling effort. Similarly, in several sites, *B. burgdorferi* appeared to be absent from the tick population. These sites (with low tick abundance and/or prevalence) appear to be in disequilibrium with respect to LD and would not be expected to conform to the model predictions. Not surprisingly, the weighted fit of the model improved when these disequilibrium sites were removed. In any case, it is notable that the model predictions were reasonably correlated with the empirical observations despite all the sources of error. This indicates not only the robustness of the host community model, but also that the relative, species-specific values for body burden and realized reservoir competence probably hold across large geographic areas.

Contrary to other studies (Allan et al. 2003, Brownstein et al. 2005), we did not find a negative correlation between tick infection prevalence and fragment size, and only a quarter of our very small fragments (<2 ha) yielded enough nymphs to estimate NIP, further suggesting that there are frequent tick extinctions in small fragments. These inconsistent observations concerning the relationship between fragment size and tick infection prevalence suggest an intriguing paradox. Small fragments that support high densities of reservoir hosts sometimes support high NIP, but these same fragments seem most likely to undergo stochastic extinction or near-extinction events for *B. burgdorferi* or *I. scapularis*, leading to extreme temporal variability in LD risk. Even in large, continuously forested areas, abundances of particular host species, ticks, and spirochetes can undergo dramatic fluctuations across years (Ostfeld et al. 2006*a*). Such disequilibrium situations, with species blinking on and off at every observable scale, complicate the application of simple models that assume stable values of key parameters. A major challenge is to incorporate stochastic processes into community or landscape models of disease risk.

An important area for further research is determining how community parameters of host richness and evenness directly or indirectly influence the abundance of nymphs, since NIP alone might be a poor indicator of entomological risk if the density of nymphs (DON) is extremely low (but see Connally et al. 2006). It is likely that certain combinations of host species and abundances increase DON by providing more feeding opportunities while others reduce DON by replacing preferred hosts with suboptimal ones ("vector regulation" in Keesing et al. 2006). If optimal community composition for tick feeding and survival is also optimal for spirochete transmission, then communities with the right profiles of richness, identity and relative abundance might be "super-infective," with very high NIP and very high DON. This could help explain the extreme variation in entomological risk that has been documented concurrently in otherwise similar forest patches (Allan et al. 2003, Brownstein et al. 2005).

Zoonotic disease systems, including LD, involve many host species, each of which can play multiple roles in disease transmission. For instance, chipmunks are a reasonably competent reservoir for *B. burgdorferi* and are a good host for the tick vector, but they also are likely to compete with white-footed mice (the most competent reservoir) and deflect tick meals away from mice (Ostfeld et al. 2006*b*, Brunner and Ostfeld 2008). Such an example illustrates both the contingent nature of individual species on LD and the importance of indirect effects of hosts on ticks and pathogens.

Our dearth of knowledge about the drivers of host community composition in the forests of eastern North America is yet another obstacle to understanding LD dynamics. Even the most intuitively obvious relationships are largely unconfirmed empirically. Thus, the negative correlations we observed between mouse densities and predator richness are important independently and for their implication that regulation of mice by predators may be occurring in these communities. Rarely will zoonotic disease risk be tied closely to the dynamics of a single host species; instead, progress in understanding ecological determinants of zoonotic disease risk will require attention to host community dynamics. Moreover, many of the species directly or indirectly involved in *B. burgdorferi* dynamics respond to anthropogenic stressors such as habitat fragmentation, but appear to do so individualistically. Ultimately, understanding variable LD risk to people will depend on constructing interaction webs for vertebrates and understanding how anthropogenic changes to the landscape affect individual species, both directly and indirectly.

Acknowledgments

This work was funded by a grant from the National Institutes of Health (R01 AI053109) to R. S. Ostfeld, K. LoGiudice, and K. A. Schmidt. We thank the many field assistants for their careful work in gathering the data for these studies. Earlier versions of this manuscript were greatly improved by the comments of J. Brunner and two anonymous reviewers. This paper is a contribution to the program of the Cary Institute of Ecosystem Studies.

Capture and handling of all animals conformed to Institutional Animal Care and Use Protocols issued annually.

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APPENDIX A

Detailed field methods (*Ecological Archives* E089-162-A1).

APPENDIX B

Host activity density categories (*Ecological Archives* E089-162-A2).

APPENDIX C

Sensitivity analysis (*Ecological Archives* E089-162-A3).

APPENDIX D

Tri-state study species list (*Ecological Archives* E089-162-A4).