LTREB: Acorn pulses and the dynamics of rodents, ticks, and Lyme-disease risk in oak forests

INTELLECTUAL MERIT
Traditionally, studies of the dynamics of infectious disease have been dominated by biomedical specialists with little input from the science of ecology. However, recent years have seen increasing recognition that infectious disease is the quintessential case of an ecological system. Infectious agents (usually viruses or bacteria) undergo population dynamics within hosts that influence virulence; they undertake habitat selection that results in invasion of specific host tissues; they experience competitive interactions with other pathogens and “predator-prey” interactions with the host’s immune system; they undergo dispersal events among hosts that influence dynamics of disease transmission. For any given vector-borne zoonotic disease, at least four species are intimately involved: the human victim, the pathogen, the vector, and at least one wildlife reservoir. Furthermore, each of these species is potentially regulated by both interspecific interactions (predation, competition) and abiotic conditions (temperature, rainfall). Our recent studies have used experimentation and monitoring to understand ecological causes of fluctuating Lyme disease risk. These studies avoid treating Lyme disease as an isolated biomedical condition. Instead, we explicitly treat Lyme disease as the culmination of a complex set of ecological interactions among the bacterial pathogen, tick vectors, their vertebrate hosts, and resources for those hosts (Ostfeld 1997, Ostfeld et al. 2006). We pursue general principles arising from this specific system and apply them to others.

We conceptualize the oak forest systems in which we have been working as a web of interacting taxa, including plants, animals, and microbes (Fig. 1). Our general approach has been to quantify the strength of interactions between pairs of taxa and to embed those pairwise interactions in a more comprehensive interaction web. This approach allows us to examine both top-down and bottom-up forces, e.g., effects of predation by rodents on seed survival; effects of acorn abundance on that of rodents. It also facilitates the integration of direct and indirect interactions, e.g., direct effects of mice on tick abundance; indirect effects of acorns on ticks. In addition, by focusing on dramatic pulses of resources (masting), we incorporate variation in the strength of interactions between species, including those that may switch on and off. Our conceptualizations of these communities incorporate parasites and pathogens together with producers and consumers because we think that a predictive understanding of disease dynamics is facilitated by an ecological perspective.

Below, we briefly describe the natural history of the Lyme disease system, focusing on the factors that affect ecological risk of human exposure. Next we describe the results of current support through the NSF LTREB program (this proposal is to extend our current LTREB research). These results lead to questions requiring further study followed by our central research plans for the next five- and ten-year periods, including plans for making data available. We then describe how the proposed LTREB research lays the foundation for related current and planned decadal research. Last we discuss the Broader Impacts of these studies.

NATURAL HISTORY
Lyme disease is by far the most common vector-borne disease in the United States, with >20,000 cases reported per year (CDC 2007). In the northeastern and northcentral U.S., where the vast majority of Lyme cases occur, the primary vector of the causative agent, Borrelia burgdorferi, is the blacklegged tick Ixodes scapularis. This tick has three active life stages: larva, nymph, and adult, each of which takes a single blood meal before molting to the next stage (in the case of larvae and nymphs) or reproducing and dying (adults). I. scapularis questing activity is highly seasonal, with larvae hatching and feeding in midsummer (year 1), molting into nymphs that feed the following early summer (year 2), and finally molting into adults that feed the following autumn or spring (year 2 or 3) (Fish 1993, Ostfeld 1997; Fig 2). Larvae and nymphs feed from dozens of different
mammalian and avian host species, whereas adults tend to prefer medium to large mammals, including white-tailed deer, raccoons, skunks, and opossums (Barbour and Fish 1993, LoGiudice 2003, 2008). Because transovarial transmission of the Lyme spirochete is highly inefficient (Piesman et al. 1986), larval *I. scapularis* typically hatch free of infection and transmit few if any cases of Lyme disease (Patrican 1997). If a tick acquires *B. burgdorferi* during its larval meal, it may then transmit the infection during its nymphal meal. At least 90% of human cases of Lyme disease are thought to be transmitted by nymphs, owing to their minute size, summer activity, and potentially high infection prevalence (Barbour and Fish 1993). The local abundance of infected nymphs, therefore, is the primary ecological risk factor for human populations (Barbour and Fish 1993).

**Population density** of nymphal *I. scapularis* and **infection prevalence** of those ticks with *B. burgdorferi* are likely to be determined by different processes. **Density of nymphs** (DON) is expected to be a function of density of larval-stage ticks in the prior generation and the proportion of those larvae that survive to the nymphal stage. Previous research has therefore focused on the abundance of white-tailed deer (thought to be the primary host for adult *I. scapularis*, which in turn produce the larval cohort) and on climatic patterns that might influence survival from larval to nymphal stage. On the other hand, **nymphal infection prevalence** (NIP) is expected to be a function of the proportion of larval meals that are taken from hosts that are competent at transmitting the pathogen to feeding ticks (i.e. the competent reservoirs). Within Lyme-disease endemic areas, white-footed mice are the most competent reservoir for Lyme bacteria, infecting up to 93% of the larval ticks that feed from them (Levine et al. 1985, Mather et al. 1989, LoGiudice et al. 2003, 2008). The high competence of mice as bacterial reservoirs leads to the prediction that the greater the abundance of mice in midsummer (peak larval activity), the greater the probability that larval ticks will acquire infection, and the greater the nymphal infection prevalence the following summer. These observations have led to the conclusion that the combined abundance of white-tailed deer and white-footed mice determine the density of infected nymphal ticks, with deer determining tick numbers and mice determining tick infection prevalence (Ostfeld et al. 2001).

Acorns (genus *Quercus*) are known to be a crucial overwinter food source for white-footed mice (Elkinton et al. 1996, Ostfeld et al. 1996a, Wolff 1996), and our experimental addition of acorns in a year of low natural acorn production significantly increased mouse density the following year (Jones et al. 1998). In addition, we have found a strong positive correlation between acorn
production in autumn and white-footed mouse density the following summer ($R^2 = 0.73$, df = 23, $P = 0.025$). Abundant acorns also attract white-tailed deer into oak-dominated forest stands in autumn (McShea and Schwede 1993), which appears to cause high local abundance of larval ticks in oak stands the following summer (Ostfeld 1997, Jones et al. 1998). Thus, until recently it was thought that large acorn crops cause concentrations of questing larval ticks and of reservoir hosts to coincide in time and space the following summer, culminating in high density of infected nymphs the second summer (Ostfeld et al. 2001). Our prior research has demonstrated that the (detrended) incidence of Lyme disease in humans is significantly correlated with regional acorn production two years previously and with mouse density one year previously (Schauber et al. 2005). Our LTREB-funded research over the past five years has demonstrated that **the effect of prior acorn production on Lyme disease risk and incidence remains robust, but the causal mechanisms are different from what was previously postulated.** These differences fundamentally change our understanding of the ways that community dynamics influence disease dynamics.

Continued monitoring, strategic experiments, and new statistical approaches conducted under our current LTREB funding have stimulated fundamental new insights into the ways that the dynamics of acorn production influence consumer communities and consequently Lyme disease risk. Our results demonstrate previously unrecognized complexities in oak forest ecosystems. Despite these complexities, however, the system remains highly predictable. We next describe these results and follow this description with the hypotheses we propose to evaluate with continued funding.
**Pulsed resources or resource crashes?** Synchronous production of large seed crops by a population of plants, punctuated by periods of little or no seed production (“masting”) is a well-documented phenomenon worldwide (Sork et al. 1993, Koenig and Knops 1998, 2000, Kelly & Sork 2002). Many studies have documented the strong, ramifying impacts of such pulsed resources on communities of consumers, decomposers, and ultimately producers (Ostfeld & Keesing 2000a, Yang et al. 2008, Holt 2008). Over the 17 years that we have been monitoring seed production on our Cary Institute sites, we have documented several episodes of heavy acorn production, supporting the pulsed-resource concept. However, similar to other temperate deciduous forests, the oak community at our sites is composed of several co-dominant species of *Quercus* belonging to two subgenera (*Erythrobalanus* and *Leucobalanus*: the red oaks and white oaks, respectively). Species in the red oak subgenus produce acorns 1.5 years after flowering, whereas those in the white oak subgenus mature acorns the same season that they flower. Because of these distinct life-histories and perhaps the use of different abiotic cues for masting, the red oaks (predominantly *Q. rubra*) and white oaks (predominantly *Q. prinus* and *Q. alba*) at our sites produce acorn crops asynchronously (Fig. 3). Consequently, in most years, one or the other group is producing acorns such that total acorn production is moderate to high. Periodically, neither group produces an acorn crop, leading to an episodic resource crash for mast-consuming wildlife. Episodic resource failure might be as important as episodic pulses for the dynamics of the system.

**Rodent dynamics.** Sixteen years of monitoring rodent populations at these same sites reveals that densities of the numerically dominant rodent – the white-footed mouse – are moderate to high during most years, probably as a result of moderate to high acorn production. This ecologically important species undergoes periodic crashes that tend to follow acorn failures. Of the six acorn failures during the past 17 years (defined as < 3 acorns/m² of forest floor), five were followed by a sharp decline in mouse density to below 10 individuals per 2.25-ha plot (<4 per ha) (Fig. 4). In only one case was an acorn failure not followed by a mouse crash; this year (2003) saw high production of red maple (*Acer rubrum*) seeds in late spring and enormous production of sugar maple seeds (*A. saccharum*) in autumn. The importance of crashes in rodent populations will be described below.

**Dynamics of nymphal Ixodes scapularis.** The early years of our long-term monitoring suggested that population densities of blacklegged ticks were determined by two acorn-driven

![Figure 3. Acorn production (acorns/m²) for the four major species of *Quercus* at our study sites between 1992, when seed traps were installed, until last year. Mast production by either red-oak-group or white-oak-group acorns tends to occur in most years, resulting in episodic masting and occasional mast failures.](image-url)
pathways, one mediated by deer and the other by mice. Data from our first decade of monitoring suggested that acorns attracted white-tailed deer and their burdens of adult ticks to oak-dominated stands, resulting in high densities of newly hatched larval ticks the following summer. Abundant acorns also boosted densities of white-footed mice, providing ample, high-quality blood meals for those larval ticks. The coincidence in time and space of abundant larvae and abundant mice appeared to cause outbreaks of nymphal ticks one year later. The results of our current LTREB research support one of these pathways and weaken the other. Analysis of our long-term data indicate no support for an effect of deer abundance or space use on abundance of nymphs two years later (R² = 0.01; Ostfeld et al. 2006). In fact, the abundance of larval ticks in one year does not predict the abundance of nymphs the following year (R² = 0.02) indicating a lack of demographic forcing in our tick populations and a decoupling of across-generation dynamics (Ostfeld et al. 2006). However, our data consistently support a powerful effect of mouse abundance on abundance of nymphs in the following year (Figure 5). Regardless of the size of the larval tick cohort, an abundance of mice leads to an abundance of nymphs the next year. The data best support a non-linear relationship between rodent abundance in year t-1 and nymph abundance in year t, though a linear relationship is also statistically significant. In the non-linear model, a threshold mouse density of about 80

![Graph showing data and analysis](image1)

Figure 4. Population density of white-footed mice (numbers per 2.25-ha plot) on the two original grids from their establishment in 1991 through 2008 (four other grids were phased in during this period). Mast failures, defined as <3 acorns/m², were almost always followed by mouse crashes.

![Graph showing data and analysis](image2)

Figure 5. Relationship between mouse density (numbers per 2.25-ha plot) in the prior year and nymphal tick density in the current year. Both linear and step-function curves provide a statistically significant fit to the data.
individuals per 2.25-ha plot (~35 per hectare) exists, below which nymph density is low and above which it is high. The years with lowest mouse density always follow years of acorn failure. The likely mechanism underlying the positive relationship between mouse density in year t-1 and nymph density in year t was recently revealed. Experimental work supported in part by LTREB funds revealed that white-footed mice are by far the most permissive vertebrate host for larval blacklegged ticks. By inoculating individual mammal and bird hosts with 100 larval ticks, we determined that tick feeding success on mice was dramatically higher than it was on any other host species (Fig. 6; Keesing et al., in review). Non-mouse hosts tend to kill high percentages of the larval ticks that attempt to feed on them. When mice are abundant (in post-mast years), many larval ticks feed on mice, resulting in high survival to the nymphal stage and high nymph density the next year (Keesing et al., in review).

Figure 6. Proportion of (100) larval ticks that successfully fed to repletion after being placed on field-caught hosts of different species. The vast majority of ticks not feeding successfully were consumed by hosts and killed. Letters indicate means that are significantly different based on post hoc comparisons.

For many years, researchers have proposed that blacklegged tick populations are strongly influenced by climatic conditions. High mortality of ticks subjected in the lab to low humidity and extreme temperatures has generated the expectation that the abundance of ticks will be predictable from specific climatic variables, although no two studies have proposed a similar set of variables. For example, a warmer overall climate is expected to cause an increase in tick abundance because warmer temperatures should lead to faster tick development and therefore fewer days ticks are exposed to an assumed constant daily mortality rate; thus, warmer temperatures should lead to higher tick survival and density (Rand et al. 2004, Ogden et al. 2005). Warm autumns have been proposed to cause a high percentage of fed larval ticks to molt into nymphs before the onset of cold weather. Because newly molted nymphs survive winter better than do engorged larvae, warm autumns should lead to high survival and subsequent population densities (Lindgren & Jaenson 2006). Warm winters are postulated to protect ticks from periods of extreme cold, which are expected to cause high mortality (Lindgren et al. 2000, Estrada-Peña 2002). Finally, periods of drought during either year of the tick’s 2-year life cycle have been proposed to cause high mortality and therefore low density (Jones and Kitron 2000). To our knowledge, our long-term studies of the abundances of all three active life-stages of *I. scapularis* provide the first opportunity to confront this group of mechanistic models of tick population dynamics with adequate data. We have found no support in our data for any of the climatic models. Models with total degree-days >0 C (overall climate warmth), average autumn temperature, average winter temperature, and growing season precipitation in current or prior year all fail to produce $R^2$ values >0.20 (compared to $R^2 = 0.90$ for prior density of mice) and are not supported by likelihood-based statistics ($\Delta$AIC > 20 compared to a mouse model).

**Dynamics of Borrelia burgdorferi**. The initial years of our long-term monitoring supported a relationship between abundance of white-footed mice in year t-1 and infection prevalence of
nymphal ticks with *B. burgdorferi* in year t (Ostfeld et al. 2001). This was expected because mice are the most competent reservoir for the Lyme disease spirochete. However, although this relationship remains positive, the accumulation of long-term data has weakened it ($R^2 = 0.16$; Ostfeld et al. 2006). Our research over the past five years has suggested that the proportion of nymphal ticks infected with *B. burgdorferi* is a consequence of the community of hosts upon which larval ticks feed, rather than being determined by the abundance of any single host species. As described above, blacklegged ticks are extreme host generalists, feeding abundantly from dozens of mammal and bird hosts. We have estimated that two cryptic but abundant species of shrew – the short-tailed shrew (*Blarina brevicauda*) and the masked shrew (*Sorex cinereus*) – each produce between 20 and 25% of all infected nymphs at our sites, values roughly equivalent to that of white-footed mice (25-28%; Brisson et al. 2008). Although their reservoir competencies (42% and 50%, respectively) are lower than that of mice (92%), their average population densities and tick burdens are large enough to produce such a high percentage of infected nymphs. Because eastern chipmunks produce about 10% of infected nymphs, these four species combined are responsible for producing about 90% of the infected nymphs at our sites (Brisson et al. 2008). Our methods of monitoring small-mammal populations – mark-recapture techniques using Sherman traps – are effective for mice and chipmunks but not for shrews (see below). Therefore, we have not yet assessed the interannual dynamics of the entire small-mammal community and how they might influence dynamics of tick infection prevalence.

**ISSUES REQUIRING FURTHER STUDY**

Knowledge acquired over sixteen years of monitoring acorns, mice, ticks, and spirochetes in oak forests, supplemented with experimental manipulations of acorns and mice, has revealed the importance of sustained ecological research for understanding the dynamics of Lyme-disease risk for humans. Indeed, the predictive power of acorns and mice extends to actual incidence of Lyme disease in human populations locally and regionally (Schauber et al. 2005). The ability of ecologists to predict disease risk facilitates efforts to educate the public about when and where they are most at risk and how to avoid exposure to this and other tick-borne diseases. Avoidance is particularly important in light of the lack of a Lyme disease vaccine, poor diagnostic accuracy, and problems with post-exposure treatment. Our studies also help demonstrate the usefulness of ecological research to public health. Nevertheless, given the slow acquisition of data (one landscape-level data point per parameter per year), lags of up to 2 yrs between the independent variable (acorns or mice) and dependent variables (tick or LD parameters), and the relatively long period between masting events, a long-term record is necessary to assess confidence in the predictive power of acorns.

The following key issues require further study:

1. **Direct and indirect effects of climate change on Lyme-disease risk.** Our long-term monitoring has established a strong link between acorn production and the density of infected nymphal ticks and, consequently, Lyme-disease risk two summers later. This relationship appears to be mediated by acorn-induced changes in population density of white-footed mice. Our data do not support several climatic (seasonal or overall mean temperature, seasonal precipitation) variables as predictors of Lyme-disease risk. However, we cannot conclude that climate has no impact on Lyme-disease risk, because only very few of the almost infinite number of potentially important climatic variables have been assessed. We selected climatic variables based on published reports of possible or realized associations between climate and tick vital rates. We have avoided using our data to “fish” for predictor variables that do not arise from known, biological effects of climate. Under separate funding from the NIH, we have begun to assess climatic correlates of tick mortality schedules in the
field (see below). We are determining field mortality of ticks at regular intervals throughout the year in order to assess for the first time whether mortality is associated with specific climatic conditions or events. We expect these experiments to provide a new set of biologically supported climate variables with which to create specific models.

The climate at the Cary Institute appears to have been steadily warming during the nearly 2 decades we have been monitoring ticks and their hosts (Fig. 7). Whether a sustained period of warming will cause a linear or nonlinear change in tick abundance or infection prevalence (or no change) is important to current debates on links between climate warming and infectious disease dynamics (Harvell et al. 2002, Dobson 2009, Lafferty 2009, Ostfeld 2009, Pascual and Bouma 2009). Any effects of climate warming on ticks need not be direct. Both warming and increased atmospheric concentrations of CO$_2$ can increase growth rates and reproductive allocation of many trees (Korner 2000, Kirschbaum 2004, McMahon et al. 2009, Zhao and Liu 2009). Consequently, climate warming could cause an increase in average tick density, or longer intervals between low-tick-density years, by stimulating heavier acorn production, which would result in higher average rodent abundances. However, superimposed on any possible long-term increase in seed production by individual forest trees is a decline in abundance of oak trees locally and regionally (Abrams 2003, Schuster et al. 2008). Deciduous forests throughout eastern North America are simultaneously experiencing successional changes in community dominance (e.g., oak decline) and stimulated growth through warming, CO$_2$ fertilization, and nitrogen deposition. The long-term net effects on seed production, wildlife abundances, and zoonotic disease risk remain to be determined.

Analyses of long-term trends in acorn production must account for patterns driven by both individual allocation strategies and population dynamics. In addition, masting behavior, which consists of occasional heavy seed production interspersed with years of seed failure, is a process that is inherently characterized by temporal autocorrelation. This issue has both statistical and biological ramifications. Interdependence among sequential data points violates assumptions in standard linear regression and correlation analyses, weakening our power to detect significant relationships using traditional statistical modeling procedures. In a more biological sense, the dynamics of acorn production, mouse and tick abundance, and disease incidence, are cyclic (or quasi-cyclic) phenomena, and we have only observed essentially 3–4 masting cycles. Increasing the duration of our dataset will allow us to explicitly address autocorrelation more rigorously in significance tests and hypothesis testing, and will dramatically increase our ability to assess the importance of masting cycles for the dynamics of Lyme disease risk.

(2) Linear vs. nonlinear relationships. Based on preliminary knowledge of the ecological interactions between acorns and the demography of mice, and between mouse density and parasitism
by ticks, we began assessing relationships using linear models. Although some of our regression models demonstrate statistically significant linear relationships (Ostfeld et al. 2006), we have also found support for nonlinear relationships. For instance, a step function relating nymph density to prior mouse density is more strongly supported than is a linear function (Fig 5). Similarly, the relationship between acorn and subsequent mouse density is best fit by an asymptotic curve (Fig. 8). Our ability to distinguish between the explanatory power of linear vs. nonlinear relationships is barely adequate with the current sample size. Distinguishing between linear and nonlinear relationships is important for detecting whether dependent variables (e.g., density of infected nymphs) are equally sensitive to incremental changes in independent variables (e.g., acorn production, mouse density) over all regions of parameter space, or are especially sensitive to incremental changes that occur in particular regions. Threshold effects and other forms of variability in predictive power of independent variables across parameter space have potentially high epidemiological relevance.

(3) **High leverage of few mast years.** An issue related to issue (2) above is whether regression coefficients are driven largely by one or a few data points resulting from occasional high values in the independent variable (i.e. from mast years). This issue has both statistical and biological components. Within a traditional statistical paradigm, it may be necessary to treat extreme data points as outliers, weakening statistical interpretations of significant regression coefficients. Biologically, high leverage of mast years in causing particularly high values in tick density or infection prevalence is to be expected due to extreme variability in acorn production among years. The predictive power of acorns may be low in years when production is very low or very high. Time series that include additional masting cycles combined with less traditional statistical approaches are necessary to resolve these issues.

(4) **Explaining the variation not caused by acorn production and mouse densities.** Although $R^2$ values in many of our regressions are relatively high (Figs.5,8), the predictive power of our models for other key relationships is still fairly weak. Neither acorn production nor mouse density significantly predicted subsequent values of nymphal infection prevalence (NIP), an important metric of disease risk for humans (LoGiudice et al. 2003, 2008, Ostfeld and LoGiudice 2003). This is surprising given that white-footed mice are by far the most competent reservoir of *B. burgdorferi* infection. However, a recent comprehensive analysis of the roles of all native vertebrate hosts for ticks in contributing to NIP revealed that tree squirrels (gray – *Sciurus carolinensis*, red – *Tamiasciurus hudsonicus*, and southern flying – *Glaucomys volans*) play an enormously important role as “dilution hosts” (LoGiudice et al. 2003, 2008). By feeding a large proportion of each cohort of larval ticks, but infecting very few of them (low reservoir competence), these squirrels strongly...
reduce NIP and therefore Lyme disease risk. Similarly, as described above, short-tailed shrews and masked shrews are moderately efficient reservoirs for B. burgdorferi, and are capable of hosting a high percentage of total larval-tick meals. Therefore, these shrews together are responsible for infecting almost half of the larvae that molt into infected nymphs (Brisson et al. 2008). Models to predict interannual variation in NIP might need to incorporate interannual variation in abundance of shrews and squirrels as well as mice to provide predictive power. Similarly, the causes of the apparently nonlinear relationship between mouse density and subsequent nymph density are not evident. Variation in other small-mammal hosts that is independent of acorn production could potentially produce this relationship.

**PROPOSED RESEARCH**

We propose to conduct intensive research on our 6 oak-forest plots for an additional 5-10 years. We will collect long-term data on: (1) acorn production; (2) population dynamics of white-footed mice and eastern chipmunks; (3) population dynamics of tree squirrels and shrews; (4) population dynamics of larval, nymphal, and adult blacklegged ticks; and (5) infection prevalence of ticks with Lyme disease bacteria. These data will be used to test four specific hypotheses:

**Hypothesis 1: Ecologically vetted climate variables, combined with acorn production and mouse density, influence interannual variation in the density of nymphal blacklegged ticks.**

Under separate funding from NIH, we have begun experiments in which we will place live nymphal ticks (deployed in mesh-encased soil cores) in the forest floor along a climatic gradient in New York State, from more benign (Hudson River Valley) to more harsh environments (near Syracuse). In the first year of this study, we will remove samples biweekly from autumn through early summer to produce survivorship curves for each site and assess whether extreme events (e.g., icing, subzero temperatures, drought), chronic conditions (e.g., number of days below zero or with no precipitation), or statistical metrics (mean and variance in specific seasons) affect tick survival. In the second year, we will deploy ticks in soil cores inside and outside plots that will be warmed (2 or 4 deg C) and sheltered from precipitation. If necessary, we will incorporate annual deployment of ticks in soil cores as part of our LTREB monitoring. We expect these field studies to provide a new set of ecologically vetted candidate variables for inclusion, together with prior acorn production and rodent density, in models to explain our long-term data on dynamics of nymphal tick populations. We expect the candidate variables to be quantifiable from the Cary Institute weather data beginning in 1991/2 when our current monitoring commenced. Candidate models comprising different sets of biotic and abiotic independent variables will be compared using the Akaike Information Criterion (AIC) to assess direct effects of climate, acorns, and rodents on density of nymphal ticks.

During the next 5 years under LTREB funding, we propose to gather 5 more grid-specific data points on each of the following variables: acorn production in year t; population density of white-footed mice the summer of year t + 1; and population density and infection prevalence of nymphal ticks in year t + 2. After 5 more years, we will be able to analyze effects of variable acorn production and climate on subsequent Lyme disease risk with a sample of ≥ 22 annual data points for each likelihood model. With continued funding, we expect that at least one and probably two additional masting (or mast-failure) cycles will be included by the end of the period (2015), resulting in the monitoring of ca 6 complete cycles. We will examine alternative models that explicitly factor temporal autocorrelation into both the underlying processes and the error terms of the models. The hypothesis will be rejected if: (a) no models containing climate variables are supported; or (b) climatic variables fail to explain patterns of acorn production by the dominant mast-producing trees. The hypothesis that acorn production and mouse density influence Lyme disease risk will be rejected if continued study of these variables undermines the correlations that we have established.
Hypothesis 2: Relationships between acorn production and tick density, and between mouse density and tick density, are best described by curvilinear rather than linear regressions. Testing this hypothesis will rely on the same data that will be used for Hypothesis 1. We will use likelihood- and information theory- based approaches to model selection (Burnham and Anderson 2002, Johnson and Omland 2004) to compare alternative linear vs. non-linear models. Using AIC and the principles of parsimony, we will accept the linear relationship as the inherently “simpler” model if alternative, nonlinear models do not produce a significantly more likely fit to the data, given any differences in the numbers of parameters in the alternative models. Analyses based on our initial 11 years of data supported linear relationships, but more recent analyses based on 16 years supported a step function to describe the relationship between prior year mouse density and current year nymph density, and an asymptotic curve for the relationship between prior year acorn production and current year mouse density. As the dataset lengthens, we expect increasingly robust estimates of the shapes of these relationships.

Hypothesis 3: Interannual variation in nymphal infection prevalence (NIP) with *B. burgdorferi* is caused by prior year’s abundance of the entire set of reservoir-competent hosts (mice, chipmunks, and shrews) and prior year’s density of tree squirrels (the most potent dilution hosts). Testing this hypothesis will require a modification to the live-trapping protocols to include mark-recapture studies of short-tailed shrews and masked shrews, as well as continued monitoring of gray and red squirrels, which commenced in 2005. Ultimately, our goal is to use AIC to select the most parsimonious model (e.g., mice only, mice plus shrews, squirrels only, mice plus shrews plus squirrels) that explains interannual variation in NIP. To increase our ability to undertake these analyses, we intend to commence the shrew census in the last full year of current funding (2009) so that six years of data will be in hand by the end of the proposed funding period.

Hypothesis 4: Our sites, which are representative of many areas of the Mid-Atlantic and southern New England, are experiencing long-term trends in acorn production as a consequence of changes in age structure and relative dominance of oaks. Stand-level seed production is a function of both the per-capita fecundity of individuals, and the abundance and population size structure of the tree species that produce seeds in any given year. All canopy trees in each of our 6 study sites are mapped and will be re-censused during the 2010 and 2015 field seasons. We only census canopy trees (stems ≥ 10 cm DBH), so a census can be completed by two project assistants over the course of a field season. There have been significant changes in tree species abundance at our sites over the past 25 years, including a significant decline in the abundance of chestnut oak (*Q. prinus*), and an increase in the abundance of sugar maple (*Acer saccharum*) and to a lesser degree, red oak (*Q. rubra*) [manuscript in review, with Dan Katz (a former REU student) as lead author]. Under the proposed research, at the end of the next 5 year cycle we will have 24 years of seed rain data and corresponding changes in tree populations. We will then use inverse modeling (Clark et al. 1998, Greene et al. 2004, Canham and Uriarte 2006) to estimate year-to-year variation in average per-capita seed production (i.e. seed production relative to individual tree size) for each of the major tree species at our sites. This will allow us to decompose the temporal variation in both the magnitude and timing of masting events by individual species into patterns due to (1) variation in individual-level average per-capita seed production versus (2) changes in overall abundance and population-level size structure.

**General methods:** Our field studies are conducted at the Cary Institute of Ecosystem Studies in Dutchess County, southeastern New York, in a post-agricultural landscape consisting of an oak forest matrix (57 to 70% oak relative basal area [Ostfeld et al. 1998]). Three control plots (two 150 by 150m, one 165 by 120m), separated by >500m, were established in the oak sites (2 in 1991, 1 in 1994). Three
additional experimental sites were established in oak forest in 1994 for the performance of acorn addition or rodent removal studies. No whole-grid experiments have been performed on the 3 “experimental” plots since the late 1990s. In our two original long-term plots, established in 1991, one 0.5m² seed trap is deployed beneath each of 10 mature specimens of each of the 4 dominant canopy trees, *Quercus rubra*, *Q. prinus, Q. alba*, and *Carya glabra*, for a total of 40 traps per plot. In addition, on all plots 25 additional 1m² seed traps are spaced uniformly in a 30m by 30m grid (every other trap station) to sample all canopy trees. Weekly counts of seeds are made each year between May and July and again between September and December, and totals per plot are divided by the total area of traps to create a mast index (acorns m⁻²).

On each of the 6 oak forest plots, either an 11 by 11 or a 12 by 10 grid of Sherman live traps was established, with 2 traps per station and 15 m between stations (240-242 traps per grid). Traps are baited with whole oats, supplied with cotton batting during cold weather, and covered by plywood boards for protection from rain and sun. Trapping is conducted each year for 2-3 consecutive nights every 3 weeks from April or May to November. In 2005 we added one squirrel-sized Tomahawk live trap to every other trap station for monitoring tree squirrels. Here we propose to add two 10-trap arrays of dry pitfall traps to each grid, centered on randomly selected coordinates. Dry pitfall traps will be opened for three consecutive days once each week during midsummer (the larval tick activity peak) and checked every three hours from dawn to dusk. Shrews are active throughout daylight hours but can die from dehydration or starvation within three hours of capture in traditional live traps. Pitfall trapping avoids generally poor capture probabilities and trap mortality associated with Sherman traps. Each white-footed mouse, chipmunk, and squirrel is given a uniquely numbered metal ear tag for identification at first capture. Shrews are tagged with passive integrated transponder (PIT) tags, owing to their small size (masked shrews) or lack of external ears (short-tailed shrews). Because each trap session is too short for closed population estimators (e.g., Program CAPTURE; McKelvey and Pearson 2001), mouse, squirrel, and shrew abundances are estimated using the Jolly-Seber open population model in program POPAN5 (Arnason and Schwartz 1999). Larval ticks in the northeastern U.S. feed primarily in late summer (Fish 1993, Ostfeld 1996b), so we use linear interpolation between trap sessions to estimate host abundances on August 15 of each year. Care of live-trapped animals is in accordance with institutional guidelines.

Based on sampling every third week from April through November for several years in the 1990s, we established that nymphal ticks at our sites reach their annual activity peak in late June to early July (Ostfeld et al. 1996b). We estimate peak abundance of nymphs in June-July of each year by dragging 1m² white corduroy drag cloths along 450m of transects in each of the 6 forest plots. Drag cloths are examined and all ticks counted and removed every 30m. Ticks sampled by this method are those that are seeking hosts, which is the subset of the population relevant to infestation of both small-mammal hosts and human victims. Infection of individual ticks with *Borrelia burgdorferi* is determined using polymerase chain reaction (PCR) using a primer for the outer surface protein C (OspC) gene (Brisson et al. 2008). Typically, at least 100 nymphs plot⁻¹ year⁻¹ are tested for their infection with *B. burgdorferi*. With NIP values of ~0.35, 100 ticks provides a robust sample size for estimating infection prevalence.

The Cary Institute runs an environmental monitoring program, including a weather station that is part of the U.S. Climate Reference Network. The program began operation 1984 with collection and chemical analysis of precipitation and stream samples. Temperature, relative humidity, wind and radiation instruments were added to the program in 1987. The first complete year of data from those instruments is 1988. The program continues to be updated and revised as new technology develops and as funding allows. Temperature and precipitation data are posted on the Cary internet site (www.caryinstitute.org/research/emp) daily and are summarized at regular intervals.

One of the basic feedback loops in the web of interactions in our study system is the link between tree seed production, rodent density and distribution, and subsequent predation by rodents on tree seeds and seedlings (Fig. 1; Schnurr et al. 2002, 2004). The seed traps at each of our study sites are paired with a 1 m² quadrat in which we monitor seedling emergence each spring and subsequent seedling survival. These data allow us to track the implications of spatial and temporal variation in tree seed production for understory development and tree population dynamics.
Our general statistical approach is to use techniques based on likelihood and information theory (Burnham and Anderson 2002, Johnson and Omland 2004). Under this approach, hypothesis testing is accomplished through comparison of alternative models, using likelihood to assess the strength of evidence for alternative models (Royall 1997). This provides a powerful and intuitive approach that eliminates the use of p-values for arbitrary test statistics.

**RELATED RESEARCH**

Extension of our monitoring efforts for five additional years will allow us to continue investigating a number of additional questions currently under study with separate funding sources.

**First**, our long-term data on acorn production and rodent populations provide the foundation for understanding interactions webs in oak forest ecosystems (Fig. 1). Data supported by the current LTREB funding are fundamental in other NSF-funded projects concerning regulation of gypsy moth populations (Schauber, Jones, Goodwin, Ostfeld, PIs), and songbird demography and populations dynamics (Schmidt PI). The gypsy moth project is currently using mouse-removal trapping to create “cold spots” to contrast with naturally occurring rodent “hot spots”, in terms of gypsy moth survival. Our LTREB project provides the critical controls for these manipulative experiments. In the songbird study, we have found that summer rodent (mouse and secondarily chipmunk) density and space use (total amount of space used by the mouse population) strongly influence songbird nesting success. Nest predation rates, the principal cause of nest failure in these populations, increased with rodent density for a ground-nesting (Veery, *Catharus fusciscens*) but not a shrub-nesting (Wood Thrush, *Hylocichla mustelina*) thrush. During rodent population booms, over 90% of Veery nests may fail and our maximum estimate of the finite rate of increase is < 0.88, whereas during rodent crashes our minimum estimate is > 1.06 (Schmidt 2003). Thus, rodent population fluctuations, driven through variable acorn crops, can exert a large influence on local population growth rates. Moreover, because acorn production can be synchronized across large spatial scales (Koenig et al. 1999, Koenig and Knops 2000), oaks might influence some songbird populations at larger spatial scales (Schmidt 2003). Analysis of Breeding Bird Survey data from surrounding counties demonstrated that the relationship between rodent density and adult Veery abundance measured at the Cary Institute extends regionally (~100 km, the limit of the analyses). Indeed, analyses showed synchronized dynamics when regressed against the prior year’s rodent abundance despite the fact that these thrushes have unique migratory strategies and non-overlapping over-wintering sites (Schmidt and Ostfeld 2003a,b). This strongly implicates breeding season events as essential in affecting their population trends (also see Blomqvist et al. 2002 for a similar example).

Under separate funding, our collaborator Kenneth Schmidt will continue monitoring nest predation, nesting success, and population density (number of breeding pairs) of selected songbird species (mainly Veery and Wood Thrush), which was begun in 1998. Continued LTREB funding will provide essential data on rodent population dynamics. Our goals are to assess the explanatory power of prior summer rodent abundance on current songbird populations (locally and regionally) and the shapes of the relationships between rodents, nesting predation, and songbird abundance. At the end of the funding cycle we would have 17 years of data on rodent-songbird interactions.

**Second**, we are undertaking separately-funded projects to understand variation in risk of human exposure to two other tick-borne pathogens, *Anaplasma phagocytophilum* and *Babesia microti* (F. Keesing, PI, Ostfeld and Schmidt coPIs). Both pathogens, vectored by the same species of tick, cause serious human illness and even death, and both diseases are increasing in the northeastern and Midwestern United States. Our studies focus on host-pathogen-vector interactions that determine tick infection prevalence. The LTREB project provides essential long-term data on interannual population dynamics of all three active life stages of the tick vector and on determinants of those dynamics.
Third, with support from the CDC, we (Gomes-Solecki, Brisson, Ostfeld) are field testing a bait vaccine that appears to protect white-footed mice from infection with *B. burgdorferi*. By providing mice with an edible vaccine consisting of an antigenically active protein, we are stimulating production of neutralizing antibody which prevents infected ticks from infecting mice. The vaccine is effective in laboratory trials, and we are assessing its efficacy in the field by testing both reservoir competence of vaccinated and unvaccinated mice and also infection prevalence of nymphal ticks in both vaccinated and unvaccinated field plots. Our LTREB sites provide the unvaccinated controls.

**Decadal Plan**

The proposed research constitutes the core monitoring component of our multifaceted program on community dynamics of forested landscapes. Current and prior studies have identified acorn production and population dynamics of white-footed mice as critical variables that have far-reaching impacts on community dynamics and that link issues of ecological and epidemiological importance. Acorn masting and acorn failures comprise enormous signals in these forested landscapes; acorns are large, nutritious, cacheable, and produced in large numbers (by any one species) only sporadically. Fluctuating abundance of acorns affects many species of vertebrate and invertebrate consumers and may influence rates of survival and reproduction, foraging behavior, space use, and dispersal (Ostfeld and Keesing, 2000). White-footed mice can be considered a “hub species” (P. Turchin, pers. comm.) because they are at the center of a web of interactions with predators (e.g., carnivores and raptors; McShea, 2000, Schmidt and Ostfeld 2003a, 2008), alternative prey (e.g., insects and bird eggs; Elkinton et al. 1996, Goodwin et al. 2005, Schauber et al. 2009, Ketterson et al. 1996, Schmidt and Ostfeld 2003b, 2008), competitors (e.g., chipmunks; RSO, unpublished), parasites (e.g., ticks), and zoonotic pathogens (Fig. 1). We propose to continue these studies in the context of anthropogenic climate change. Climate warming and increased average or variability in precipitation are now known to exacerbate several infectious diseases of humans and wildlife (Harvell et al. 2002, Dobson 2009, Pascual 2009, Ostfeld 2009), although the nature of climate-disease linkages remain highly controversial (Lafferty 2009). Although Lyme disease is often suggested as one of the zoonotic diseases likely to be exacerbated by anthropogenic climate change (Benson et al. 2000, Gubler et al. 2001, Bunyanavich et al. 2003), data that support this suggestion are provisional (Ogden et al. 2006, 2008) or lacking (Ostfeld et al. 2006, Eisen 2008). In fact, our studies have found no support for several specific climatic variables as determinants of Lyme-disease risk. However, these studies are hampered by a limited and somewhat arbitrary set of climatic variables under study, and more importantly by an inadequate time series. At the end of an additional decade of support, year 2020, we will have been monitoring climate, acorns, vertebrates, ticks, and pathogens for almost 30 years. Our ability to fully characterize the potential interactive effects of climate and masting cycles on Lyme disease risk will require such a dataset.

**Timetable, Personnel, and Data Availability**

In each of the five years of the project, the schedule will be as follows. In February, we will solicit applications for Project Assistants, transmitting job announcements to colleges and universities listed in the large database maintained by the Human Resources Department at the Cary Institute, which includes many historically black institutions. Phone interviews will occur in March and successful applicants will begin in May. The PIs, our long-term Research Specialist, serve as direct supervisors for student PAs. When all PAs have arrived, an orientation into the science behind the field work is provided, and from June through November, weekly lab meetings are held during which scientific issues from the overall research program are discussed. Themes include: rodent population dynamics; ecology and evolution of mast seeding; infectious diseases; and the relationship between applied and basic ecology. The purpose of the orientation and weekly meetings is to provide an
educational experience for students. We target as PAs students who are between their junior and senior years in college, or who have recently finished their bachelors degree and are considering graduate school. From May through October we will maintain a rotation of small-mammal trapping, drag-sampling for ticks, and PCR sampling of ticks. All plots are sampled for small mammals and ticks every 4 weeks. In May and June (for red maple) and September through December (for other tree species), we deploy and monitor seed traps.

As indicated above, data from the Cary Institute weather station are updated regularly on the Cary website (http://caryinstitute.org). If funded, we will make other data available through our project webpage, linked to Ostfeld’s home page (http://caryinstitute.org/people_sci_ostfeld.html) no more than two years after data have been collected. Included with the data will be metadata covering protocols for mammal sampling, tick sampling, PCR and immunofluorescence microscopy, and tree seed and tree population sampling. This webpage will encourage visitors to discuss specific data needs, together with linked meta-data, for specific research purposes.

**Broader Impacts**

Broader impacts of this research fall into three general categories: professional education; public education; and human health protection. **Professional education:** during the 2005-2009 funding period, we have mentored the following Research Experiences for Undergraduates (REU) students on projects related to this research: Justin Halsey (2005), Rachel Rollins (2006), Eunice Lee (2006), Melissa Colon (Hispanic) (2007), Erica Dolven-Kolle (2007), Jeffrey Grabowski (2007), Elizabeth Hinman (2007), Aiko Weverka (2008), Andrea Egizi (2008), David Fraker (2008), Sarah Moreno (Hispanic) (2008), and Jacob Malcomb (2009). Although these students are supported largely by the Cary Institute REU site grant (A.R. Berkowitz, PI), the current LTREB grant has supplemented student needs for equipment and personnel. Thirteen female and 8 male Project Assistants (PAs) have been supported, many of whom are now in graduate school. Postdoctoral training of Jesse Brunner, Mary Killilea, Lisa Schwanz, and Andrea Previtali (Hispanic) was facilitated by current LTREB support. Although we do not request funds for postdoctoral researchers, we anticipate similar support for student PAs and REU researchers. We will continue to assist PAs in professional training and development through journal clubs and research presentations. Results of our previous and ongoing research are described in textbooks in ecology (e.g., Morin 1999, Botkin and Keller 2009), conservation biology (Primack 2006) and mammalogy (Feldhamer et al. 2007). **Public education:** We continue to present our results in public forums. Each spring we are contacted by science reporters from our local newspapers (e.g., Poughkeepsie Journal) to increase awareness of tick-borne disease. Over the past five years our research has been covered by ABC News Radio, AMC Outdoors, Associated Press, Bloomberg News Radio, Boston Globe, Discovery Channel, Time, Newsweek, Environmental Health Perspectives, PLoS Biology, Los Angeles Times, BBC, Radio Free Europe, Science Times, Discover, ENN (Environmental News Network), Family Circle, Parents, Mother Earth News, Oregon Public Broadcasting, MSNBC, National Public Radio, Business Week, National Wildlife Federation, New York Times, Washington Post, Wall Street Journal, Newsday, Reuters, Science News, Backpacker, BioScience, Chronicle of Higher Education, Congressional Quarterly Weekly and others. Our research provides the basis for a question on the ACT college admissions test and for high school curriculum via NOVA Online (http://www.pbs.org/wgbh/nova/teachers/activities/3603_rats.html). We will continue to invest in public dissemination of our results. **Health protection:** Our research has strong practical implications because it allows prediction of times and places of high disease risk. As such, it provides a concrete example of the utility of ecology for human welfare.