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A MODEST MODEL EXPLAINS THE DISTRIBUTION AND ABUNDANCE OF *BORRELIA BURGDORFERI* STRAINS

DUSTIN BRISSON* AND DANIEL E. DYKHUIZEN
Department of Ecology and Evolution, Stony Brook University, Stony Brook, New York

Abstract. The distribution and abundance of *Borrelia burgdorferi*, including human Lyme disease strains, is a function of its interactions with vertebrate species. We present a mathematical model describing important ecologic interactions affecting the distribution and abundance of *B. burgdorferi* strains, marked by the allele at the outer surface protein C locus, in *Ixodes scapularis* ticks, the principal vector. The frequency of each strain in ticks can be explained by the vertebrate species composition, the density of each vertebrate species, the number of ticks that feed on individuals of each species, and the rate at which those ticks acquire different strains. The model results are consistent with empirical data collected in a major Lyme disease focus in New England. An applicable extension of these results would be to predict the proportion of ticks carrying human infectious strains of *B. burgdorferi* from disease host densities and thus predict the local risk of contracting Lyme disease.

INTRODUCTION

Understanding natural ecology has become an essential part of applied sciences such as conservation biology, fisheries management, and forestry.[1] It is becoming increasingly evident that understanding the interactions that affect the distribution and abundance of disease-causing organisms in natural ecosystems is critical for infectious disease control and prevention. Given the complexity of these ecological interactions, mathematical models can be used to identify the main factors involved in human disease risk in both time and space and to identify factors that can be manipulated for disease control. Additionally, these models can identify important interactions, as well as those that can be disregarded, for future empirical investigation. Here, we present a mathematical model characterizing the ecological interactions that affect the distribution and abundance of all genotypes of *Borrelia burgdorferi* sensu stricto, including those that cause Lyme disease.[2,3]

Lyme disease is the most prevalent vector-borne disease in the United States, with ~20,000 cases reported annually, and is even more abundant in Europe and Asia.[4–6] In the northeastern United States, *B. burgdorferi* is carried between vertebrates by the black-legged tick, *Ixodes scapularis*.[7–12] Ticks take a single blood meal at each of the three post-egg life stages: larva, nymph, and adult.[11,13,14] Larval ticks typically hatch without *B. burgdorferi*[10,15,16] and must acquire the bacteria through a blood meal from an infected vertebrate.[10] *B. burgdorferi* persists to successive life stages, permitting nymphal and adult ticks to transmit *B. burgdorferi* to susceptible vertebrates, including humans.[11,14] Humans typically contract Lyme disease when fed upon by infected nymphs, although adult ticks are responsible for some disease transmission.[17] Adult ticks typically feed on a different suite of animal species than the immature stages, effectively removing adults from the enzootic cycle.[14] Thus, the natural ecology of *B. burgdorferi* is dominated by interactions with immature ticks and the vertebrate species on which they parasitize.

Larvae and nymphs are blood meal–host generalists, capable of parasitizing ~80 vertebrate species including many mammalian, avian, and reptilian hosts.[16,18–21] However, not all vertebrate species are equally competent as blood meal hosts for ticks or disease hosts for *B. burgdorferi*.[22–24] The larval body burden, the average number of larval ticks feeding on an individual, differs significantly among species, although little variation exists among conspecifics regardless of population densities.[22] Consequently, it can be assumed that the proportion of nymphs that received their larval blood meal from each vertebrate species can be determined from the relative densities of species and the average body burden for each species. Additionally, the proportion of those larval ticks that are infected with *B. burgdorferi* post-feeding, the competence of that species as a reservoir for *B. burgdorferi*, depends on the vertebrate species from which the blood meal was taken, again with little variation among conspecifics.[22,25] Taken together, these data suggest that the vertebrate community composition strongly affects the proportion of infected ticks, which has been the standard measure of human disease risk.[22,23,26,27] However, humans can only be infected by *B. burgdorferi* strains that carry an allele from one of four major allelic groups at the outer surface protein C (*ospC*) locus,[28] although 15 groups coexist in most natural populations in the northeastern United States.[29,30] Thus, the proportion of ticks infected with at least one of these strains is a better proxy for the risk of human Lyme disease than the proportion of ticks infected with any strain.

The *ospC* locus is the most variable gene in the *B. burgdorferi* genome.[31,32] Pairs of *ospC* alleles are either very similar (< 2% sequence difference, average = < 1%) or very divergent (> 8% sequence difference, average = ~20%)[30]. Similar alleles are sorted into *ospC* major groups (oMGs) denoted A through V,[28,33] some of which are present only in Europe or the southern United States.[33,34] Throughout this work, we will refer to strains of *B. burgdorferi* simply by the oMG the strain encodes[25] because the *ospC* locus is in near-complete linkage disequilibrium with all other loci examined.[35] For example, a strain with an allele from *ospC* major group (oMG) A will be referred to as oMG A.

Other vertebrate species, similar to the case for humans, can serve as disease hosts for only a subset of these 15 oMGs.[25] For example, the white-footed mouse is infected by only oMGs A, B, D, F, G, I, and K, while the short-tailed shrew is infected by A, D, E, F, K, and T.[25] In many northeastern oak-maple forests, similar to the one from which the empirical data for this study were collected, the number ticks infected with each oMG is large enough that every individual

* Address correspondence to Dustin Brisson, Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, 11794-5245. E-mail: brisson@life.bio.sunysb.edu

is exposed to all 15 oMGs, and is therefore infected by all oMGs that infect that species.[25] The proportion of larvae infected with each oMG after feeding differs among oMGs as well as among species from which the blood meal was taken. For example, 53% and 36% of the larval ticks become infected with oMG K after feeding on mice and chipmunks, respectively, while 21% and 15% become infected with oMG I.[25] These proportions, which we call transmission probabilities, vary little among conspecifics.[25] We calculate the transmission probability as the proportion of larvae that become infected with a particular oMG after feeding on a particular species. If one considers both the consistency of transmission probabilities among conspecifics, as well as the consistency in the number of larvae that feed on each species, it is likely that the distribution and abundance of oMGs in ticks changes as a function of the densities of vertebrate species.

In this report, we develop an empirically based model to predict the proportion of host-seeking nymphs infected with each oMG as a response to the relative abundance of four species of mammalian hosts thought to be important reservoirs for *B. burgdorferi*.[22] We used empirical measures of the transmission probabilities and the frequency of oMGs in host-seeking nymphs to estimate the proportion of larval blood meals taken from each vertebrate species in an oak-maple forest using inverse modeling. We compared these estimates to empirical measures of the proportion of larval blood meals taken from vertebrate species at the same geographic location to validate the model and its assumptions. Using this model, we can measure the relative contribution of each vertebrate species to the total larval blood meals and the proportion of those larvae that become infected with *B. burgdorferi*. Additionally, the model can be used to determine if additional vertebrate species should be considered important hosts for *B. burgdorferi*. An extension of this model can be used to predict the proportion of ticks carrying at least one human infectious strain across a range of hypothetical vertebrate communities.

METHODS

We developed a mathematical model designed to represent the ecological outcome of the interactions between *B. burgdorferi*, *I. scapularis*, and several species of vertebrate hosts. The principal objective of this study was to verify that the model, and the included assumptions, accurately characterizes the ecology of the system. That is, are the interactions that affect the distribution and abundance of oMGs included in the model? To this end, we estimated the explanatory variables of the mathematical model using inverse modeling and compared these values to empirically derived values where available.

Mathematical model. The mathematical model contains one class of parameters and two classes of explanatory variables. The transmission probability parameters (T_{ij}), the probability oMG i is transmitted from species j to a larval tick, were measured from four natural species: the white-footed mouse (*Peromyscus leucopus*), the eastern chipmunk (*Tamias striatus*), the short-tailed shrew (*Blarina brevicauda*), and the gray squirrel (*Sciurus carolinensis*) (Table 1).[22,25] Additionally, we included a fifth category, called category X, which was designed to represent vertebrate species that feed larvae for which we have no empirical transmission probability measurements. The transmission probabilities of category X (T_{iX}), along with the proportion of nymphs that took their larval blood meal from each vertebrate species (P_j), are the two classes explanatory variables. The model is a series of linear equations, one for each oMG found in New England, that calculates the frequency of each oMG in host-seeking nymphs (F_i), the response variable, from the parameters (T_{ij}), and explanatory variables (P_j and $T_{i(X)}$). Each equation has the form

$$F_i = \sum_0^j (P_j \times T_{ij}) \quad (1)$$

where F_i is the frequency of oMG i in host-seeking nymphs, P_j is the proportion of larval meals taken from species j, and T_{ij} is the transmission probability of oMG i from species j. Thus, the proportion of nymphs that carry oMG i (F_i) is the arithmetic average of the proportion of blood meals taken from each vertebrate species (P_j) and the probability that a blood meal on each host species infects the tick (T_{ij}). This model assumes that oMGs have independent population dynamics and no population level epistasis.

Empirical data. Three classes of empirical data were collected from oak-maple forests at the Institute for Ecosystem Studies (IES) in Dutchess County, New York: 1) the frequency of each oMG in host-seeking nymphs ($F_{i(measured)}$), 2) the transmission probability of each oMG from each species included in the model (T_{ij}), and 3) the proportion of larval *I. scapularis* that fed on each vertebrate species ($P_{j(measured)}$). The frequency of each oMG in host-seeking nymphs at the IES reported by Brisson and Dykhuizen[25] (Figure 1) were collected using the polymerase chain reaction/reverse line blot hybridization method previously described.[25,29] We measured the transmission probability of each oMG (T_{ij}), the proportion of larvae infected after feeding on a vertebrate species, from the 55 animals drawn from four vertebrate species[25] (Table 1). Although transmission probabilities differ

TABLE 1
Measured transmission probability of each oMG from each species*

	oMGs														
	A	B	D	E	F	G	H	I	J	K	L	M	N	T	U
Mouse	0.33	0.28	0.45	0	0.27	0.32	0	0.21	0	0.53	0	0	0	0	0
Chipmunk	0.21	0	0.35	0	0.23	0.23	0	0.15	0	0.36	0	0	0	0.24	0.13
Shrew	0.14	0	0.20	0.17	0.11	0	0	0	0	0.36	0	0	0	0.13	0
Squirrel	0.08	0	0.03	0.04	0.04	0.02	0	0	0	0.10	0	0	0	0	0
Category X†	0.25	0.17	0.07	0.03	0.12	0.12	0.21	0.10	0.13	0.19	0.04	0.34	0.09	0.03	0.04

* Transmission probabilities are assumed to be 0 if < 5% of the individuals from a species examined tested positive for that oMG.[25]
† Estimated by inverse modeling.

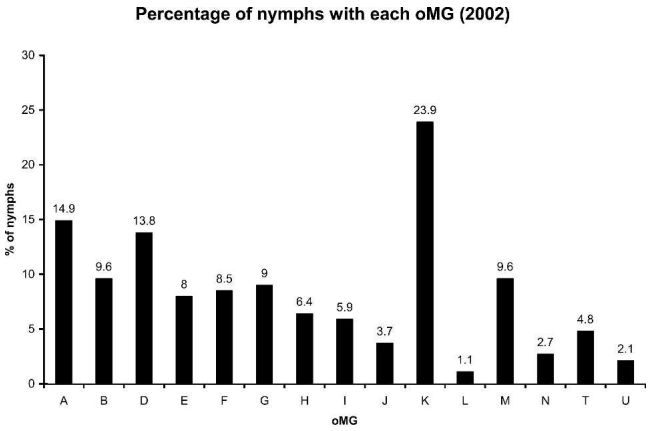

FIGURE 1. Distribution (%) of outer surface protein c major groups (oMGs) in host-seeking nymphs caught at the Institute for Ecosystem Studies in Dutchess County, New York in 2002.[25]

significantly among the four species and among oMGs within a species ($P < 0.001$), there are no differences in the transmission probabilities among conspecifics ($P > 0.7$). It should be noted that with few exceptions, all conspecifics were infected by the same oMGs.[25] Therefore, we used the pooled data for each oMG from conspecifics as the species-specific transmission probability parameter. The proportion of larval *I. scapularis* that fed on each vertebrate species was calculated from the vertebrate density (D_j) and larval burden (B_j) data reported by LoGiudice and others.[22] Assuming that all vertebrate species that host a substantial number of larval ticks were assessed, the approximate proportion of larvae that fed on each vertebrate species is the product of the vertebrate density (D_j) and the larval burden (B_j) divided by the total number of larvae that fed on any species (Table 2):

$$P_j = D_j \times B_j / \sum_0^j (D_j \times B_j).$$

This estimation assumes that the larval burdens are constant among years[36] and that a uniform percentage of larvae successfully molt to nymphs from each species,[22] both of which appear valid. The densities of short-tailed shrews or squirrels have not been measured at IES. Density estimates from literature sources vary dramatically for these species such that an empirical estimate should not be used directly in the model. Thus, we estimated P_j for all species using inverse modeling. The measured P_j for mice and chipmunks are later compared with the model estimates to validate this approach and the model assumptions.

Inverse modeling. We estimated P_j and T_{iX} using a computer simulation. We found the combination of P_j and T_{iX} resulting in the distribution of F_is that best fit the measured oMG frequencies by calculating the frequency of each oMG (F_i) from every T_{iX} (0–50%, 1% increments) in each of 400,000 combinations of P_j, where the $\sum_0^j P_j = 1$. The measured transmission probabilities for the four natural species were used as parameters for all permutations (Table 1). Each permutation of P_j and T_{iX} resulted in a goodness-of-fit score (G), calculated as sum of the deviations of $F_{i(\text{simulated})}$ from $F_{i(\text{measured})}$ across all oMGs ($G = \sum_0^i |F_{i(\text{measured})} - F_{i(\text{simulation})}|$).

The estimates of P_j were nearly identical for combinations with goodness-of-fit scores less than 0.025 (the 2,450 best-fitting combinations). These data indicate that one area of the variable space, not several disparate but equally likely combinations, resulted in the best fit to the empirical F_i data. Therefore, the model estimates P_j precisely. To visualize this, we ranked $P_j - T_{iX}$ combinations according to their goodness-of-fit score and calculated the variance for each P_j for the 25, 50, 75 . . . 32,000 best-fitting combinations (combinations with scores worse than the 32,000 best-fitting models were not saved because of computer space limitations). The variance for P_j increased slowly as combinations with greater G scores (worse fit) were added (in groups of 25) to the best-fitting combinations up to $G = 0.025$ (Figure 2). When combinations with $G > 0.025$ were included in the variance calculations, the variance for all P_j increased markedly. Therefore, we report the average P_j from the variable combinations with goodness-of-fit scores < 0.025 as our estimates for P_j, as opposed to the values from the single best-fitting combination, although these two values are nearly identical.

Model validation. We validated the model and its assumptions by comparing $P_{j(\text{simulation})}$ averaged across the variable combinations with the best goodness-of-fit scores ($G < 0.025$), to $P_{j(\text{observed})}$ measured at the IES (Table 2).

Sensitivity analysis. This model explicitly includes only four vertebrate species, although several other vertebrate species are parasitized by *I. scapularis*[16] that may substantially affect

TABLE 2

Summary statistics of vertebrate species known to be reservoirs of *Borrelia burgdorferi* in forests in the northeastern United States*

Species	A	B	C	D	E
Peromyscus leucopus (mouse)	27.8	20	556	10.2%	10.2%
Tamias striatus (chipmunk)	36.0	10	360	6.6%	7.2%
Blarina brevicauda (short-tail shrew)	62.9	25†	1,150.2	29.0%	18.9%
Sciurus carolinensis (squirrel)	142	8.1†	1,572.5	21.2%	34.5%
Sorex sp.	55.5	25	1,387.5	25.6%	
Birds (several species)	1.7	31.6	53.7	1.0%	
Mephitis mephitis (skunk)	66.8	0.05†	3.34	0.06%	29.4%
Odocoileus virginianus (deer)	239	0.25	59.8	1.1%	
Didelphis marsupialis (opossum)	254	1†	254	4.7%	
Procyon loto (raccoon)	127	0.2†	25.4	0.5%	
Total			5,422.4		

* A = Average number of larvae per individual per 48 hours during peak larval season; B = Density per hectare at the Institute for Excosystem Studies. † Data from literature sources; C = Larvae per species per hectare (A × B); D = % larvae per species per hectare (C/Total); E = larvae per species per hectare (P_j) estimated by the simulation. A–D. 122

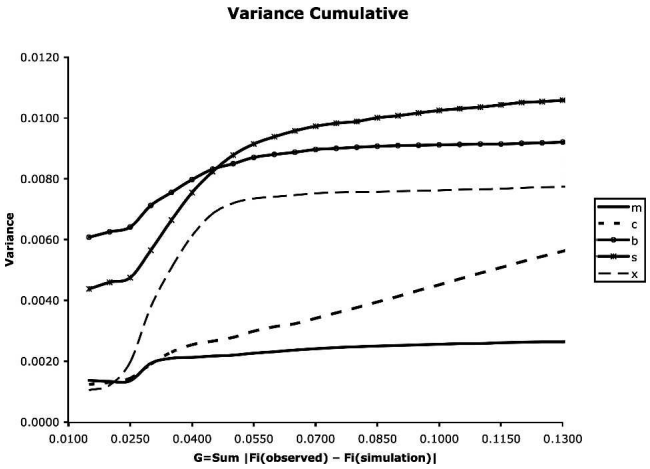

FIGURE 2. Variance of the estimates of $P_{species}$ plotted against G, the measure of the fit of the simulated oMG frequencies to the observed data. The variance for all parameters remain relatively constant when G is less than 0.025. When values of G greater than 0.025 are included, the variance in the estimates of $P_{species}$ increases sharply for all species. The variance continued to increase for all $P_{species}$ through G = 0.3, the largest value retained during the simulation. m = mouse; c = chipmunk; b = shrew; s = squirrel; x = category X.

the frequency of oMGs. Thus, the accuracy of our estimates of P_j may be sensitive to the inclusion of these species in category X, as opposed to explicitly including each separately. To examine the potential for this bias, we removed each of the four species individually, forcing them into category X, such that the model contained three species plus category X. We then ran a simulation (four times, once for the removal of each species) to estimate the proportion of nymphs that took their larval blood meal from each species (P_j). We then compared the estimates of P_j from each of these simulations to the parent simulation, which included all four species, to determine how P_js are affected when vertebrate species that effect oMG frequencies are excluded.

Additionally, we tested the sensitivity of this model to empirical errors in transmission probability estimation by 1) increasing individual transmission probabilities in a focal species by 20% in 27 simulations (15 transmission probabilities × 4 species – (transmission probabilities that equal 0) = 60 – 33 = 27 simulations) each with 400,000 permutations of P_j, 2) increasing all transmission probabilities in a focal species by 20% (four simulations, one for each species) each with 400,000 permutations of P_j, and 3) increasing all transmission probabilities in all species by 20% (one simulation) in a simulation with 400,000 permutations of P_j.

RESULTS

We used the empirically determined transmission probabilities (T_{ij}) and the frequency distribution of oMGs in host-seeking nymphs ($F_{i(measured)}$) to estimate the percentage of host-seeking nymphs that took their larval blood meal from each vertebrate species (P_j) using inverse modeling. The values of P_j ranged from zero and one, with the condition the sum of all P_js must equal unity, to explore possible combinations. Of the 400,000 combinations considered, we chose the combinations with goodness-of-fit scores less than 0.025 ($G = \sum_0^i |F_{i(measured)} - F_{i(simulation)}| \leq 0.025$) as containing the most likely values of P_j and used the average of these 2,450 (of 400,000) best-fitting simulations as our estimate of each P_j. This cutoff was chosen *a posteriori* because the variance in P_j was low for simulations where $G \leq 0.025$ for all P_j and increased substantially thereafter (Figure 2). That is, the values for P_j differ only slightly among the best-fitting simulations. The consistency of the estimates of P_j among the best fitting simulations ($G \leq 0.025$) (Figure 2) suggests that there is a single optimal set of variable values that match the observed values. Additionally, the combination of P_j that resulted in the lowest goodness-of-fit score (the best combination) is indistinguishable from the average of each P_j from simulations where $G \leq 0.025$ to the thousandth decimal place.

The estimates for P_{mouse} and $P_{chipmunk}$ from the simulation are nearly equivalent to the measured values (Table 1, columns D and E). However, the simulated estimate of P_{shrew} is less than the average value from the data in LoGiudice and others,[22] while the estimate of $P_{squirrel}$ is greater. We will discuss these data in greater detail below. Our estimate of the proportion of the larvae that feed on category X (29.4%) is similar to that calculated by LoGiudice and others[22] at the IES (32.9%) (Table 1). Both data sets suggest that species other than those included in this model account for a substantial proportion of the larval blood meals. Additionally, the estimated transmission probabilities from category X are greater than those measured for gray squirrels and are nearly equivalent to those of short-tailed shrews (Figure 3), suggesting that at least one species represented by category X both feeds many larvae and infects a large proportion of those with *B. burgdorferi*. The transmission probabilities estimated by the simulation for category X (Table 1) provide a testable prediction of the transmission probabilities of the vertebrate species not explicitly included in the model.

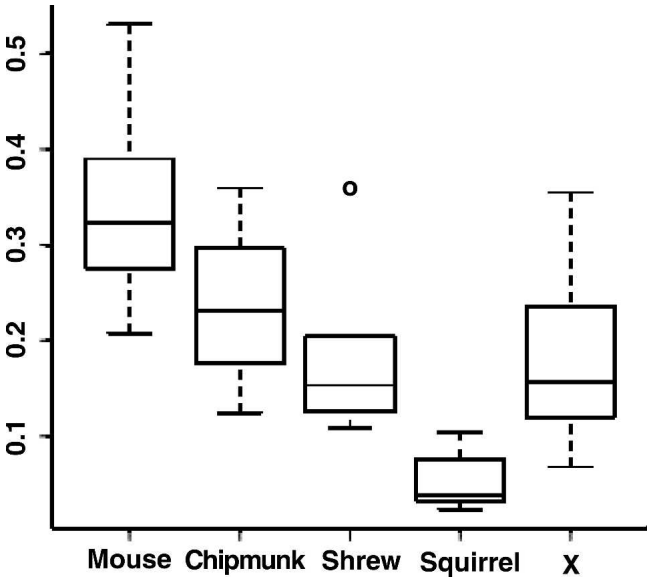

FIGURE 3. Box-and-whisker plot of the measured transmission probabilities[25] as well as those estimated by the simulation (category X). The box represents the median (line) and upper/lower quartiles and the whiskers include all values (the small circle is an outlier). The transmission probabilities for category X are quantitatively similar to those of short-tailed shrews and are much greater than those of squirrels, suggesting that category X does not represent species with low transmission probabilities.

Sensitivity analysis. *Excluding species*. To determine the affect of excluding a vertebrate that is an important disease host of *B. burgdorferi* on the estimates of P_j, we estimated P_j after removing one of the four species originally included in the simulation while retaining category X. If category X truly represents all species not explicitly included in the model, removing a species would increase only $P_{\text{category X}}$, while all other P_js remain constant. This result would suggest that the model is robust to missing vertebrate species that are important reservoirs of *B. burgdorferi*. We found that P_j varied only slightly, except for P_{shrew} when chipmunks were eliminated and for P_{squirrel} when mice or shrews were eliminated. This suggests that our estimates of P_j are generally robust to the exclusion of important *B. burgdorferi* reservoirs. Most of the other variations were minor increases in P_j for species sharing comparable transmission probabilities with the removed species (Table 3). For example, the transmission probabilities of chipmunks resemble both mice and shrews to a similar degree (Table 2), and both P_{mouse} and P_{shrew} increase slightly when chipmunks are excluded (Table 3). Additionally, the transmission probabilities of category X changed to better resemble the removed species, indicating inclusion of that species in category X. The transmission probabilities of category X change most dramatically to resemble the removed species when the removed species has transmission probabilities that differed from all other species. For example, removing mice from the simulation, which have very high transmission probabilities compared with the other species, results in ample increases in the transmission probabilities from category X. The concomitant increase in P_{squirrel} is caused by its increased importance in supplying uninfected larvae to balance the increase of infected ticks supplied by category X. Likewise, when squirrels are not included, which have very low transmission probabilities compared with the other species, category X completely incorporates P_{squirrel} and its transmission probabilities are concomitantly reduced. We attribute the minor reductions in P_{mouse} and P_{chipmunk} to the lack of a category supplying uninfected ticks to balance these species with high reservoir competence. The estimates of P_j will likely correspond to measured values best when all species that are important disease hosts for *B. burgdorferi* are explicitly included. However, with the exception of P_{squirrel}, our estimates of P_j appear remarkably robust to this perturbation, giving support to the accuracy of the estimates of P_{mouse}, P_{chipmunk}, and P_{shrew}.

Transmission probabilities. In this model, each species affects the frequency of an oMG in proportion to its oMG transmission probability. Thus, the accuracy of the results might be sensitive to errors in transmission probability measurements. We examined the sensitivity of the model to errors in the transmission probability parameters in three ways: by estimating P_j when 1) one transmission probability from a focal species was increased by 20%, 2) all transmission probabilities from a focal species were increased by 20%, and 3) all transmission probabilities from all species were increased by 20%. In the first test, $P_{\text{focal species}}$ decreased (Table 4, A) while P from all other species increased, although trivially (Table 4, B). The exception is P_{squirrel}, which increased, although slightly, when individual transmission probabilities from squirrels were increased by 20%. When all transmission probabilities from a focal species were increased, $P_{\text{focal species}}$ decreased to a slightly greater extent than when individual transmission probabilities were increased, again with the exception of squirrels. However, simultaneously increasing all transmission probabilities substantially decreased P_j for all species except squirrels, where there is a notable increase (Table 4, C). These data suggest that the accuracy of this model is insulated from a measurement error in a transmission probability by the other transmission probability parameters and that errors affect species with high transmission probabilities differently than species with low transmission probabilities.

DISCUSSION

The accuracy and precision of the model described in this report suggests that the ecological interactions that most affect the distribution and abundance of *B. burgdorferi* oMGs in host-seeking nymphs can be summarized by two classes of parameters: the proportion of larval ticks that feed on each competent vertebrate species, P_j, and the transmission probabilities of the oMGs from those species, T_{ij}. This model, which includes parameters for four vertebrate species[22] accounts for most of the variation in the distribution of the oMG frequencies. Although the molecular mechanisms for these ecological interactions are not known, this model connects the natural ecology of the forest ecosystem in New England with the life history of *B. burgdorferi*.

Although the estimates from the simulation match those found by LoGiudice and others[22] remarkably well, there are two points of discrepancy: the model results underestimate P_{shrew} and overestimate P_{squirrel}. There are at least two possible explanations for these discrepancies. First, the densities of both the short-tailed shrew and gray squirrel reported by LoGiudice and others[22] were taken from the literature, and not quantified directly at the IES, as were the other species. Since densities of all vertebrates are known to vary geographically,[36–40] literature values should be used with caution. In addition, short-tailed shrew densities are notoriously difficult to measure and vary dramatically both intra-annually and inter-annually.[39,40] We believe that our simulated estimate of P_{shrew} more likely represents the average shrew density during the larval feeding period in the late summer of 2001 than the density reported by LoGiudice and others.[22] A similar

TABLE 3
Estimates of P_j when individual species are excluded

	P_{mouse}	P_{chipmunk}	P_{shrew}	P_{squirrel}	$P_{\text{category X}}$
Model estimates	10.2%	7.2%	18.9%	34.5%	29.4%
Without mice	0%	6.9%	21.6%	45.6%	25.9%
Without chipmunks	12.1%	0%	24.2%	35.2%	28.5%
Without shrews	11.9%	11.0%	0%	46.9%	30.2%
Without squirrels	8.4%	4.9%	22.2%	0%	64.6%

TABLE 4
Sensitivity to perturbations in transmission probabilities*

	P_{mouse}	$P_{chipmunk}$	P_{shrew}	$P_{squirrel}$	$P_{category\ X}$
A	8.8 ± 0.009%	6.5 ± 0.002%	17.3 ± 0.01%	35.5 ± 1%	X
B	9.7 ± 0.31%	7.3 ± 0.14%	19.0 ± 0.26%	36.2 ± 0.71%	28.8 ± 0.16%
C	7.5 ± 0.09%	6.2 ± 0.09%	15.7 ± 0.6%	41.0 ± 0.6%	29.0 ± 0.3%

* A = Average value of $P_{focal\ species}$ when individual transmission probabilities of the focal species are increased by 20%; B = average of $P_{non\text{-}focal\ species}$ across all simulations when transmission probabilities were increased in focal species by 20%. The difference between P_j in the original simulation and $P_{non\text{-}focal\ species}$ was nearly identical for all species; C = all transmission probabilities in all species increased simultaneously by 20%.

argument could be made for the squirrels, but we believe that there is a more likely explanation. Since many other species not explicitly included in the simulation have low reservoir competence and consequently low transmission probabilities, similar to the reservoir competence and transmission probabilities of squirrels,[22] the squirrel category actually represents squirrels and the other low reservoir-competence species such as deer, raccoons, opossums, and birds, that category X was designed to represent. The transmission probabilities derived for category X by the simulation were greater than the reservoir competence of squirrels (Figure 3), suggesting that category X does not represent species with low reservoir competence. Instead category X represents at least one species with high reservoir competence and high transmission probabilities that was not included in the study. Thus, the inclusion of the larvae that had fed on these species with low reservoir competence into the squirrel category caused an increase in, and thus an overestimation of, $P_{squirrel}$. It is likely that our estimation of $P_{squirrel}$ will decrease if all species with high reservoir competence are included in the model and category X functions as it was designed. One potential application of this model, when all important disease hosts are included, is to estimate the densities of species for which density data are notoriously difficult to collect,[39,40]

Category X represents at least one species that both feeds and infects a substantial proportion of larval ticks that is not explicitly included in the model (Figure 3). This was not surprising because five oMGs found in host-seeking nymphs, some in relatively high frequency, do not infect any of the four vertebrate species that were included in this study. The species that are the hosts for these five oMGs must have high transmission probabilities to infect sufficient numbers of larvae to result in the observed frequencies found in host-seeking nymphs (Figure 1, oMG H, J, and L–N). The simulated estimates of the transmission probabilities for category X are relatively high, some as great as 35%, suggesting that this/these species can significantly affect the distribution and abundance of *B. burgdorferi* oMGs. Most species in New England forest communities have a reservoir competence below these estimated transmission probabilities, suggesting these species are not included in category X.[22] However, shrews of the genus *Sorex* have a measured reservoir competence of 51.2%[22] and are thus the most likely species represented by category X. Additionally, *Sorex* shrews are believed to provide 25.6% of the larval blood meals at the IES[22], which is slightly below the estimate of $P_{category\ X}$ (29.4%), suggesting that *Sorex* shrews may substantially affect the relative distribution and abundance of *B. burgdorferi* oMGs. Work is currently underway to empirically determine the transmission probabilities from *Sorex* species and will be used to test the validity of this model and our predictions.

There are two results that warrant further discussion. First, these data suggest that the white-footed mouse, *P. leucopus,* is not the primary disease reservoir for *B. burgdorferi* in diverse forest communities, as has been postulated.[10,26,41,42] *P. leucopus* contribute only 10.2% of all larval blood meals, and only 22.3% of the infected host-seeking nymphs fed on mice as larvae. *B. brevicauda*, the short-tailed shrew, feed more larvae (18.9%) and infect more of the nymphal population (23.2%), although it is still not a majority for either metric. The concept of one "primary" disease reservoir needs to be challenged in this and other multi-host parasite systems; this type of typologic thinking can lead to research programs that are irrelevant and can possibly derail control measures. Second, *S. carolinensis*, the eastern gray squirrel, contributes relatively few infected ticks to the host-seeking nymphal pool and thus does not greatly effect the distribution of oMGs. However, squirrels do contribute a large proportion of uninfected ticks because of their large larval body burdens and low reservoir competence. Thus, squirrels are important in reducing the proportion of host-seeking nymphs that are infected. This supports the claim of previous researchers that squirrels are a key diluting species.[22] We will be able to test this assertion when the model is developed sufficiently that category X includes species with low reservoir competence.

In addition to increasing our understanding of the ecology of *B. burgdorferi*, this model can be applied to predict the risk of human Lyme disease. The probability a human will be exposed to a human infectious oMG of *B. burgdorferi* is proportional to the fraction of *I. scapularis* nymphs that carry a human infectious oMG. The density of nymphs and human behavior are also important factors contributing to the probability of infection but are outside the scope of this model and will thus be neglected. We calculated the proportion of nymphs expected to carry at least one human infectious oMG, which we are using as a metric for human Lyme disease risk, from P_j and the frequency with which larvae acquire a human infectious oMG from each vertebrate species. This prediction cannot be calculated directly from the transmission probabilities of the four infectious oMGs because they are not independently acquired by ticks, but must be computed from the proportion of larvae that tested positive for at least one infectious oMG. As previously predicted, the risk of human Lyme disease is very high in regions where mice are the sole blood meal host for *I. scapularis*; approximately 75% of nymphs will carry at least one human infectious strain (Figure 4). The various hypothetical communities represented in Figure 4 demonstrate the utility of biologic models in human health. For example, this model suggests that the human Lyme disease risk decreases as the vertebrate diversity increases. In areas with a vertebrate community assemblage

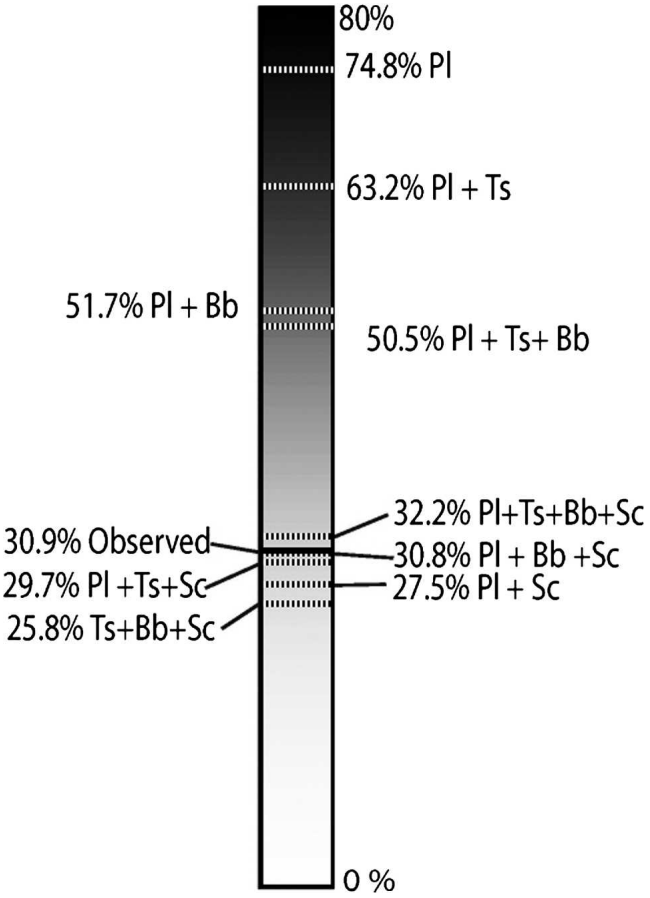

FIGURE 4. Percentage of nymphs predicted to carry a human infectious strain of *Borrelia burgdorferi* in areas with different vertebrate community structures. Pl = *Peromyscus leucopus*; Ts = *Tamias striatus*; Bb = *Blarina brevicauda*; Sc = *Sciurus carolinensis*.

consisting of only the four species from our model at the relative densities reported by LoGiudice and others,[22] 32.2% of the ticks will carry a human infectious strain, very near the observed percentage. Interestingly, regions that do not have white-footed mice should have only modest decreases in the risk of human infection, which is similar to the results of a recent vaccination experiment,[42] again suggesting that concentrating on a single disease reservoir is inappropriate in this multi-host zoonotic disease.

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Authors' address: Dustin Brisson and Daniel E. Dykhuizen, Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, 11794-5245, E-mails: brisson@life.bio.sunysb.edu and dandyk@life.bio.sunysb.edu.

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