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Research Article

Landscape structure influences continental distribution of hantavirus in deer mice

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Abstract

We hypothesized that landscape structure affects movement of individuals through the landscape, which affects the rate and pattern of disease transmission. Based on this hypothesis, we predicted a relationship between landscape structure and disease incidence in spatially structured populations. We tested this prediction for hantavirus incidence in deer mice (*Penomysens moniculatus*), using a novel index of habitat fragmentation for transect data. A series of four stepwise logistic regression analyses were conducted on serological and ecological data from 2837 mice from 101 sites across Canada. The significant variables, ranked in decreasing order of size of their effect on virus incidence were: human buildings, landscape composition (amount of deer mouse habitat in the 1-km radius landscape surrounding each site), landscape configuration (fragmentation of deer mouse habitat in the 1-km radius landscape surrounding each site), mean annual temperature, and seasonal variation. Our results suggest that epidemiological models should consider not only the demographic structure of the host population, but its spatial structure as well, as inferred from landscape structure. Landscape structure can have a greater effect on the pattern of distribution of a virus in its host population than other ecological variables such as climate and seasonal change. The usefulness of landscape data in epidemiological models depends on the use of the appropriate spatial scale, which can be determined empirically. Epidemiological models with a spatially structured host population can benefit from the explicit consideration of landscape structure.

Introduction

A major endeavor of landscape ecology is to understand the effect of landscape structure on the movement of individuals and the dynamics of populations. Landscape structure is generally defined in terms of 'composition' and 'configuration' (Dunning et al. 1992). These are, respectively, the kinds of patches present in the landscape and the amount of each, and the spatial relationships among them. The term 'fragmentation' is used here to mean the extent to which a landscape element is subdivided into separate units (Fahrig 1997), i.e., fragmentation is a component of landscape configuration.

A functional measure of landscape structure is 'connectivity', the degree to which the landscape facilitates or impedes the movement of individuals among patches (Taylor et al. 1993). When a landscape is composed of habitat patches embedded in a matrix used only for dispersal, the connectivity of the landscape is the combined result of landscape composition, landscape configuration, and the ease of movement of individuals through the matrix (Taylor et al. 1993). At present there is no commonly accepted measure of connectivity (Tischendorf and Fahrig 2000a). The measures that have been proposed vary widely, but they are all related in some way to the two main

components of landscape structure, composition and configuration (Tischendorf and Fahrig 2000b).

The potential effects of landscape structure on disease transmission are becoming increasingly recognized (Ostfeld et al. 1995, Taylor and Merriam 1996, Kitron 1998). We hypothesize that landscape connectivity can influence the rate at which infected individuals come into contact with susceptible individuals, and thereby influence the rate at which a disease spreads across a landscape. In a spatially divided population, the probability of an infectious host and a susceptible host coming into contact should be greater within subpopulations and smaller between subpopulations. The probability of such contacts also should be greater among subpopulations having more dispersal among them, than among subpopulations with less dispersal among them. This hypothesis leads to the prediction that landscape structure should affect disease incidence. In this study we tested this prediction for hantavirus incidence in deer mouse populations from across Canada.

The study system

The deer mouse

The deer mouse (*Peromyscus maniculatus*) is common throughout most of North America south of the tree line (Carleton 1989, p. 47) and has been described as 'probably the most widely distributed native small mammal on the continent' (Hooper 1968, p. 43). *P. maniculatus* is often described as a habitat generalist (Baker 1968), with some habitat specialization among subspecies (Wecker 1963). While there is some contradiction in habitat preferences reported in the literature (see Kaufman and Kaufman 1989), one generalization that can be made is that deer mice tend to choose habitats with greater vertical structural diversity (Wolff and Hurlbutt 1982; Kaufman and Kaufman 1989), for example, choosing mature trees over open areas or ground vegetation (Stah 1980; Barry et al. 1984; Parren and Capen 1985; Sekgororoane 1995), choosing coulees over open prairie (Morris 1992), and choosing among clearcuts those with higher diversity of slash and foliage height (Buckner and Shure 1985).

Dispersal occurs primarily in juveniles, and mostly during the breeding season (Wolff 1989). Juvenile dispersal distance averages less than 150 m (Baker 1968, p. 108), is usually less than 300 m (>89%, $n = 413$, from data in Stickel 1968, p. 395), and is rarely greater than 900 m (<2%, $n = 428$, from data in Blair 1950 and Stickel 1968). The longest reported dispersal distance by a deer mouse is 1220 m (Dice and Howard 1951 in Stickel 1968, p.395), while the longest reported homing distance by an experimentally relocated deer mouse is 1980 m (Teferi and Millar 1993).

During the summer, deer mice nest singly or occasionally in pairs or family groups, but they may nest communally during the winter (Wolff 1989, p. 282). In Canada, seasonal variation of deer mouse population levels is extensive, with fall population levels averaging more than twice as high as spring population levels (see review by Terman 1968).

Sin Nombre virus

Sin Nombre and Sin Nombre-like viruses are hantaviruses that are widely distributed across North America (Rowe et al. 1995). The deer mouse *P. maniculatus* is the main host (Childs et al. 1994). Sin Nombre virus (also originally referred to as Four Corners or Muerto Canyon virus) was identified after an outbreak of human disease in the southwestern U.S. in 1993 (Nichol et al. 1993). It has since been studied intensively because of the public health risk posed by the human disease, Hantavirus Pulmonary Syndrome (HPS). The epidemiology of Sin Nombre virus within the mouse population has been studied relatively little, however, and many important fundamental parameters remain unknown. These include the mode(s) of transmission between mice, and the time-course of infectivity.

The primary mode of Sin Nombre infection in humans is by inhalation of airborne virus particles released from the faeces and urine of infected mice, particularly as it dries. The mode(s) of transmission between mice is unknown. Inhalation of airborne virus particles may be the primary mode of transmission, but another possibility is blood contact during aggressive interactions. The rodent hosts of hantaviruses develop a persistent or chronic infection with no apparent disease symptoms (LeDuc 1987; Lee and van der Groen 1989).

Health Canada's Zoonotic Diseases laboratory at the Canadian Science Centre for Human and Animal Health in Winnipeg conducts hantavirus surveillance for all of Canada. This surveillance program relies on samples of rodent sera, or snap-trapped animals, sent to Health Canada for hantavirus testing by researchers and agencies from across the country. Samples are

tested for the presence of antibodies to Sin Nombre by an enzyme-linked immunosorbent assay (ELISA) procedure, modified from Centers for Disease Control (1994). This program has produced a bank of serological information from over 5000 rodents trapped from across the country. As the primary reservoir for Sin Nombre (Childs et al. 1994), deer mice make up the bulk of the rodents tested in the serological surveillance program.

Hypotheses

Our main objective was to use these data to test the prediction that landscape structure affects the distribution of Sin Nombre in deer mouse populations. Our underlying hypothesis was that landscape structure affects virus distribution through its affect on landscape connectivity. As explained above, direct measurement of landscape connectivity is problematic and there is no widely accepted measure (Tischendorf and Fahrig 2000a, b). We therefore tested effects of the 2 main components of connectivity, landscape composition and configuration, on virus distribution. In addition, since this was not a controlled experiment, we needed to include in the statistical analyses other variables that might affect the distribution of the virus. The variables and hypotheses are described below.

Landscape structure

Landscape composition

Deer mice occur at different densities in different habitat types (Wecker 1963; Stah 1980; Wolff and Hurlbutt 1982; Barry et al. 1984; Buckner and Shure 1985; Parren and Capen 1985; Kaufman and Kaufman 1989; Morris 1992, Sekgororoane 1995). We use 'preferred habitat' to mean a habitat type where published data show *P. maniculatus* occurring at higher densities compared to other habitat types available in that region.

The amount of habitat in a landscape (landscape composition), along with its configuration (see below), is an important factor determining landscape connectivity (Tischendorf and Fahrig 2000b) which, as argued above, should affect disease transmission. Therefore, we hypothesized that hantavirus incidence should be positively related to the amount of deer mouse habitat in the landscape.

Landscape Configuration

If landscape configuration affects landscape connectivity (Taylor et al. 1993), then landscape configuration should affect disease transmission and we would predict an effect of landscape configuration on hantavirus incidence in deer mouse populations. The exact effect will depend on the response of deer mouse movements to landscape fragmentation. If individuals are less likely to move across a more fragmented landscape, such fragmentation may form a partial barrier to the transmission of Sin Nombre, and *P. maniculatus* populations in fragmented landscapes would show a lower incidence of Sin Nombre infection. Alternatively, individuals may move farther in fragmented habitat, and Diffendorfer et al. (1995) found this to be the case for deer mice. In this case individuals may have a wider range of contacts, increasing the transmission probability. In this case we would expect *P. maniculatus* populations living in more fragmented landscapes to show a higher incidence of Sin Nombre infection.

Landscape Scale

Any discussion of the possible role of landscape structure in the epidemiology of Sin Nombre raises the question of appropriate spatial scale. The scale of a study is appropriate if the landscape is characterized at the scale at which the process(es) of interest operate. In this case the relevant scale is the maximum distance that individual deer mice move through the landscape. We characterized the landscapes at the presumed most appropriate scale (2 km) and also at a larger (double) and smaller (half) scales.

Other Variables

Seasonal Change

P. maniculatus populations typically undergo large temporal fluctuations in numbers. Population density is typically very low in the early spring, rises during the summer, and peaks in the fall. A high overwinter mortality reduces the population to very low density by the following spring. If transmission probability is a function of population density, we might expect the rate of transmission to accelerate during the summer and fall, and a peak in incidence of Sin Nombre infection to occur in the fall.

Climate

Risk of infection for humans is known to be greater when mouse excreta become dry, because virus particles become airborne much more readily in these

conditions. If we assume inhalation of airborne virus particles to also be an important mode of transmission between mice, we might expect a greater rate of transmission in drier climates. The rate at which excreta become dry is probably affected more strongly by individual microclimates but this information is not available, and broad patterns could also exist at the level of the overall climate, with some combination of temperature, precipitation, and/or drought.

Viral strain

Work by Morzunov et al. (1996) has identified phylogenetically distinct groups within the Sin Nombre complex, and suggests coevolution of various strains with phylogenetic groups of *P. maniculatus*. Two major strains of Sin Nombre have been identified in Canada, which are geographically allopatric, an eastern variant, occurring east of approximately the 95th meridian, and a western variant occurring approximately west of the 95th meridian (Morzunov, pers. comm.). There is a possibility that the occurrence of these different strains may be responsible for the fact that human mortality and morbidity from Sin Nombre is much higher in western North America than in eastern North America.

Buildings

While most of the mice tested as part of the national surveillance program were trapped in the wild on trap lines as part of ecological research or wildlife surveys, many were snap- trapped in human dwellings or other buildings. The demographic relationship between mice in buildings and the larger wild population is unknown. Mouse populations in human buildings may be more or less demographically distinct from wild populations. The within-patch dynamics of these populations (population density, contact behaviour, communal nesting, etc.) may also be quite different from those of wild populations. We might therefore expect a possible confounding effect from some mice being trapped inside buildings.

Hantavirus pulmonary syndrome cases

Some of the mice tested as part of the national surveillance program were trapped in and near the homes of people who had been diagnosed with Hantavirus Pulmonary Syndrome (HPS), the respiratory illness associated with Sin Nombre infection in humans. These sites were sampled specifically because a high probability of finding infected mice there was expected. Our statistical procedures needed to account for this potential bias.

Annual within-site variability

Variability in virus incidence among years within a site could potentially confound results when examining seasonal variability in virus incidence. Inter-annual within-site variability was examined to determine the validity of combining data from several years in the same analysis.

Methods

The objective of the analyses was to test for relationships between the probability that a deer mouse carries the Sin Nombre virus, and the predictor variables defining the landscape structure and other factors likely to affect virus incidence. The response variable in the analyses was the number of positive records (y: mice testing positive for Sin Nombre antibodies) out of the number of mice tested (n), for each trapping site across Canada.

We searched all records from Health Canada's Hantavirus surveillance program to determine which data could be used for the analyses, based on several criteria. The date of trapping, precise trapping location (hereafter referred to as the trapping 'site'), and size of trapping area had to be known. When date or location information was not recorded it could sometimes be found with the assistance of the individual or agency responsible for the trapping. Sites had to be at least 2 km apart in order to be considered spatially independent for this analysis. This minimum distance was chosen because it exceeds the greatest recorded individual dispersal distance for deer mice and greatly exceeds commonly observed individual dispersal distances. Thus more than one season would normally be required for infection to be carried between two sites separated by 2 km. The size of the trapping area had to be known because in some cases a batch of mice were taken from a large geographic area (>4 km^2). These were divided into separate sites if the precise trapping locations could be learned. For sites where no virus was detected, a minimum sample size (n) of 30 mice was necessary in order to have a 95% certainty that the virus was in fact absent from the sites. We therefore used the criterion $n \geq 30$, or $y \geq 1$. These criteria yielded records from 2837 mice, at 101 independent sites across Canada.

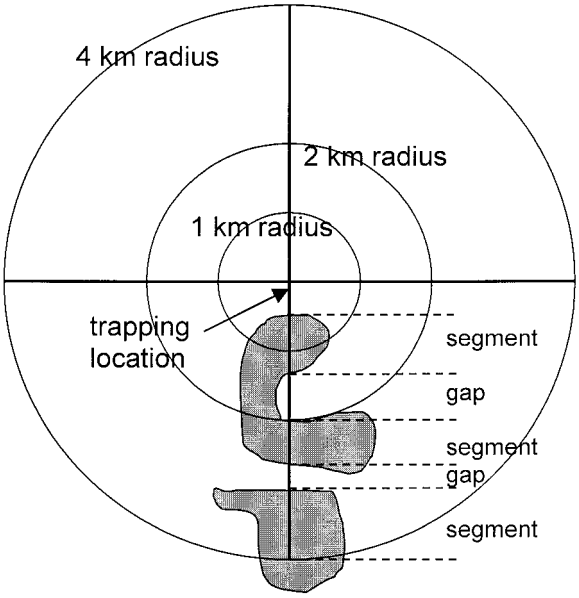

Figure 1. Transect sampling method used to characterize landscape structure at each site. Shaded area = preferred habitat. A pair of transects was used, each 8 km long, intersecting at the point of the trapping location. Using this method each landscape was characterized at the scale of a one, two, and four km radius.

Measuring landscape composition and configuration

For each site, a point corresponding to the trapping location was identified on an air photo. A pair of transects corresponding to 8 km each in length was established on the air photo(s), intersecting on the point of the trapping location (Figure 1), and placed parallel to the edges of the air photos, which were arbitrary in their relation to cardinal directions. Each landscape was characterized at three scales: 1, 2, and 4 km radius. Traditional transect sampling technique avoids intersecting transects since the resulting non-independence of transects hinders the combining and comparing of transects for analysis (Pielou 1977). In the present case, however, intersecting transects were deliberately used so that the indices of landscape composition and configuration (below) would characterize the landscape surrounding the trapping location, while being particularly sensitive to the area closest to the trapping location (Figure 1).

From the air photos, all habitat patches were classified as either preferred habitat or non-preferred habitat. Forest, crop fields, clearcuts, and coulees were all classified as preferred habitat in comparison to pasture, hay, open prairie, bog, and marsh (Barry et al. 1984; Buckner and Shure 1985; Morris 1992; Parren and Capen 1985; Sekgororoane 1995; Stah 1980; Wolff and Hurlbutt 1982).

Each point was recorded along the transect where it intersected a border between preferred habitat and another landscape element such that two phases were recorded along each transect: segments of preferred habitat and gaps of non-preferred habitat (Figure 1). Uninhabitable patches such as lakes, rivers, and asphalt were considered 'non-preferred' habitats for the analysis.

Landscape composition

Landscape composition (*Comp*) was represented by the proportion of the transect comprised of preferred habitat, that is:

$$Comp = (\Sigma Lh_i)/L,$$

where Lh_i = length of a transect segment comprised of preferred habitat, ΣLh_i = sum of all Lh_i for the transect, and L = length of the transect. *Comp* values range from 0 (no preferred habitat) to 1 (all preferred habitat). The mean value of *Comp* from the 2 intersecting transects was used for each site.

Landscape configuration

We developed an index of landscape configuration (*Conf*) that reflects the degree of fragmentation of the preferred habitat. The index needed to range from 0 to 1, and be independent of landscape composition (*Comp*). A review of the literature found no such index that was applicable to the transect method employed here (O'Neill et al. 1988, Gustafson and Parker 1994). The index was developed by (1) identifying the limit of resolution of the transect sampling procedure (see below) and expressing the length of the transect L as a number of limit-of-resolution units, (2) calculating the theoretical maximum possible number of habitat segments for each landscape, based on these quantities and the proportion of preferred habitat (*Comp*), and (3) expressing the extent of fragmentation as the ratio of the observed number of habitat segments to the maximum possible number of habitat segments.

The limit of resolution was defined as the shortest unit of length along the transect that could be resolved and classified as either preferred habitat or not, or in other words, the shortest possible segment or gap. This was 1 mm on the air photo transects, which was equivalent to 50 m on the ground. The maximum number of separate segments of habitat that could occur for a given total amount of habitat on a given transect

(*smax*) was then calculated as:

$$smax = \Sigma Lh_i, \text{ when } \Sigma Lh_i \leq 0.5L, \text{ and}$$
$$smax = L - \Sigma Lh_i + 1, \text{ when } \Sigma Lh_i > 0.5L,$$

where L = length of transect in 'limit-of-resolution units' and ΣLh_i = total amount of habitat on transect in 'limit-of-resolution units'. Note that L was an even number for our transects.

Landscape configuration was then expressed as the proportion:

$$Conf = (s - 1)/(smax - 1)$$

where s = observed number of habitat segments, and *smax* = maximum possible number of habitat segments. *Conf* values range from 0 (unfragmented) to 1 (maximally fragmented). We subtract 1 from both numerator and denominator so that $Conf = 0$ when the transect is all preferred habitat. The mean value of *Conf* from the 2 intersecting transects was used for each site.

Other variables

Strain
A dummy variable (*Strain*) coded for the two virus strains. Although the actual strain was not determined for each sample, as mentioned above there is a relationship between strain and longitude. All sites east of 95° longitude were classified as being in the range of the eastern variant ($Strain = 0$). All sites west of 95° longitude were classified as being in the range of the western variant ($Strain = 1$). Because of the correlation between *Strain* and longitude, it was impossible to make a confident inference about the real effect of virus strain. However, by including *Strain* we at least controlled for its potential effect when examining the other variables.

Climate
Ecoclimatic zone maps and climate data were used to estimate the mean annual temperature (*Temp*), mean total annual precipitation (*Precip*), and mean annual period of relative drought (*Drought*: the number of months during which mean temperature in °C exceeds mean precipitation in mm) for each site (Ecoregions Working Group 1989).

Hantavirus pulmonary syndrome
Rather than exclude these samples from the analysis because of bias, all sites were classified as to whether they were sampled in response to a human Hantavirus Pulmonary Syndrome case ($HPS = 1$) or not ($HPS = 0$), in order to detect and control for any effect of this bias.

Buildings
All sites were classified by a dummy variable (*Building*) to test for a possible difference between wild-trapped mice ($Building = 0$) and mice trapped in human dwellings ($Building = 1$).

Trapping date
The mean trapping date of all mice trapped at each site (*Date*) was used to control for differences among sites due to different trapping dates.

Analyses

To determine whether data from different years at a single site could be pooled, we conducted a Fisher's exact test to test for significant difference in virus incidence among years for the 5 sites where data existed for 2 years.

The data were then analyzed by stepwise multiple logistic regression analysis, where the response variable was the logit transformation (see Ashton 1972) of the ratio *y/n*, where *y* is the number of seropositive mice in each sample of *n* mice. We used the LOGISTIC procedure in the SAS statistical package (SAS 1996), with the SELECTION=STEPWISE option. This conducts both forward addition and backward elimination, using the significance criterion ($\alpha \leq 0.05$). Because temporal autocorrelation was assumed among samples taken throughout the season at each site, all serological data for each site were pooled, yielding one *y/n* proportion for each of the 101 sites.

Four stepwise logistic regression analyses were conducted. In the first model the landscape composition and configuration variables were excluded; only the other variables (*Date*, *Temp*, *Precip*, *Drought*, *Strain*, *HPS*, and *Building*) were included. The other 3 models included these variables, plus *Comp* and *Conf*, calculated at a 1, 2, and 4 km radius around the trapping location. Following examination of the residuals, the quadratic term, $Comp^2$ was also included.

Results

The Fisher's exact test detected no significant difference in virus incidence among years for the 5 sites for which data from 2 years were available. Data from all years were therefore pooled for subsequent analyses.

Table 1. Results and diagnostics for the four stepwise logistic regression analyses. The order-independent Wald X^2 statistic associated with each variable (d.f. = 1 for each term), and its significance level (in parentheses) are shown for all variables that were included in the models by the stepwise selection procedure (significant at $\alpha = 0.05$). n.a. = not applicable. The 1-km scale model had the best fit (highest % concordance, lowest % discordance and smallest residual X^2).

	Spatial scale of landscape data (radius)			
Variables	None	1 km	2 km	4 km
composition	n.a.	12.92 (0.0003)	5.6 (0.0180)	
composition^2	n.a.	15.48 (0.0001)	7.55 (0.0060)	
configuration	n.a.	10.86 (0.0010)	7.38 (0.0066)	29.66 (0.0001)
date		5.03 (0.0250)	5.20 (0.0225)	7.92 (0.0049)
strain				
precipitation				
temperature	9.26 (0.0023)	7.62 (0.0058)	5.86 (0.0155)	5.05 (0.0246)
drought				
HPS				
building	43.9 (0.0001)	32.4 (0.0001)	33.7 (0.0001)	37.3 (0.0001)
% concordant	59.1	70.1	67.2	67.5
% discordant	29.9	26.6	29.2	28.3
Residual X^2(df, p)	8.94 (5, 0.11)	2.41 (4, 0.66)	3.76 (4, 0.44)	10.35 (6, 0.11)

The 4 models resulting from the stepwise logistic regression analyses were generally similar (Table 1). At least one measure of landscape structure was significant at all 3 spatial scales. *Temp* and *Building* were significant in all four models. *Date* was significant in the three models that included landscape data. The shape of the relationship (sign of the parameter and curvilinearity) was consistent for each variable among all the models in which it was significant. *Strain*, *Precipitation*, *Drought* and *HPS* were not significant variables in any of the models.

The effect of landscape structure was highly significant and strongest in the model using data from a 1 km radius around the trapping location (Table 1). The regression equation for this model was:

$$\begin{aligned}\ln[P/(1-P)] \;=\; & -2.3 - 0.0045\,Date \\ & - 0.10\,Temp + 1.28\,Building \\ & + 2.29\,Conf - 4.68\,Comp \\ & + 4.96\,Comp^2\end{aligned}$$

where P = probability that a deer mouse tested positive for Sin Nombre.

Figures 2 to 5 show the shapes of the relationships between each variable in the model and the probability of a positive Sin Nombre test. In each case the other variables in the model were held constant at their mean values. All else being equal, virus incidence (i) was lowest at sites composed of 40% to 50% preferred habitat (intermediate *Comp*) and highest at sites with a small or large proportion of preferred habitat (Figure 2), (ii) was lowest in unfragmented landscapes and increased geometrically with fragmentation (Fig-

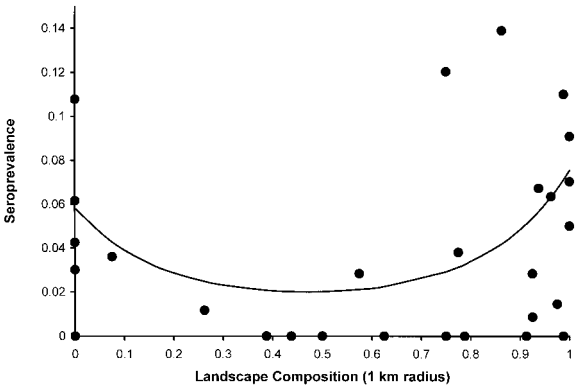

Figure 2. Seroprevalence in relation to landscape composition index at the 1 km radius scale. Solid line: seroprevalence predicted by landscape composition in the stepwise multiple logistic regression equation (sample size = 2,837 mice), with the other variables in the regression model held constant at the their mean values. Data points: for presentation, the data were sorted by landscape composition value and combined, so that each point on the graph represents the proportion of mice testing positive out of approximately 100 mice. Each point is then plotted against the median value of landscape composition for that group of mice.

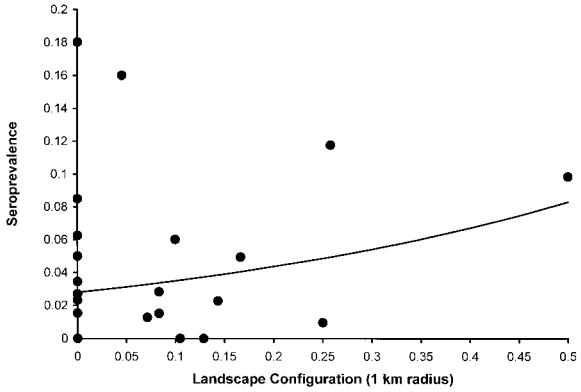

Figure 3. Seroprevalence in relation to landscape configuration index at the 1 km scale. Solid line: seroprevalence predicted by landscape configuration in the stepwise multiple logistic regression equation (sample size = 2,837 mice), with the other variables in the regression model held constant at the their mean values. Data points: for presentation, the data were sorted by landscape configuration value and combined, so that each point on the graph represents the proportion of mice testing positive out of approximately 100 mice. Each point is then plotted against the median value of landscape configuration for that group of mice.

ure 3), (iii) decreased during the breeding season (Figure 4), (iv) was lower at warmer sites (Figure 5), and (v) was higher among mice trapped in buildings than among mice trapped in the wild. The significant variables, ranked in decreasing order of size of their effect on predicted virus incidence (see Wald X^2 values in Table 1) were: *Building*, *Comp*, *Conf*, *Temp*, and *Date*.

Discussion

Our results indicate that landscape structure affects Sin Nombre virus in deer mice across Canada. In fact landscape composition and configuration had stronger effects on virus incidence than other more commonly measured variables such as climate variables and time of year. Below we speculate as to the possible causes for the forms of the relationships found.

Landscape composition

The results indicate a relatively strong effect of landscape composition on virus incidence. The hypothesis, that virus incidence should increase with increasing proportion of preferred habitat, was confirmed in landscapes with over 40% preferred habitat (Figure 2), suggesting that landscape connectivity increased over this range.

However, there was also a (smaller) increase in virus incidence at low proportions of preferred habitat. In a landscape with very little or no preferred habitat, deer mice are forced to live in less-preferred habitat, presumably in low overall numbers. Some animals have been shown to move more quickly through less-preferred habitat than through preferred habitat (Baars 1979; Rijnsdorp 1980; Wallin and Ekbom 1988; Hansson 1991; Diffendorfer et al. 1995; Andreassen et al. 1996; Matter 1996; Charrier et al. 1997; Collins and Barrett 1997; Pither and Taylor 1998). If this applies to deer mice, then their movement rates may be higher in landscapes with little preferred habitat than in landscapes with more (10–40%) preferred habitat. This would increase connectivity at very low habitat amounts, thus increasing virus transmission.

Landscape configuration

Landscape configuration also had a significant effect on virus incidence. Virus incidence increased with increasing fragmentation of preferred habitat. For a species that readily ventures into non-preferred habitats, and moves more quickly through non-preferred habitats than preferred habitats, habitat fragmentation can increase landscape connectivity. Increasing fragmentation increases the probability that mice in preferred habitat encounter habitat edges, which increases the likelihood of mice moving into less-preferred habitats. This likely explains the observation by Diffendorfer et al. (1995), that deer mice moved farther in more fragmented habitats. Fragmentation therefore results in higher landscape connectivity, which, we hypothesize, increases the rate of disease transmission.

Another possible explanation for the positive effect of landscape fragmentation on virus incidence is that population densities of small mammals generally increase with decreasing patch size, and are higher in habitat patches within fragmented landscapes than in unfragmented landscapes (Yahner 1992; Wolff et al. 1997; Dooley and Bowers 1998; Bowers and Dooley 1999). The mechanism for this is unknown (Diaz et al. 1999), but is most likely related to the positive edge effects shown by many small mammals (Godfryd and Hansell 1986; Rosenberg and Raphael 1986; Bowers et al. 1996; Kremsater and Bunnell 1999; Manson et al. 1999). If deer mouse densities are typically higher in smaller patches, then contact rates and virus transmission would also be higher in these patches, leading to higher virus incidence in more fragmented landscapes. The effect of patch size on population

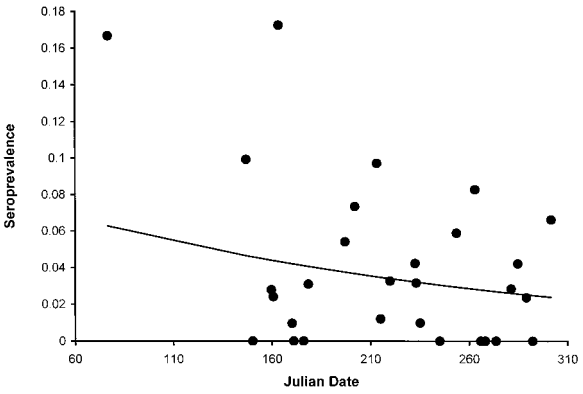

Figure 4. Seroprevalence in relation to date of trapping. Solid line: seroprevalence predicted by trapping date in the stepwise multiple logistic regression equation (sample size = 2,837 mice), with the other variables in the regression model held constant at the their mean values. Data points: for presentation, the data were sorted by trapping date and combined, so that each point on the graph represents the proportion of mice testing positive out of approximately 100 mice. Each point is then plotted against the median value of trapping date for that group of mice.

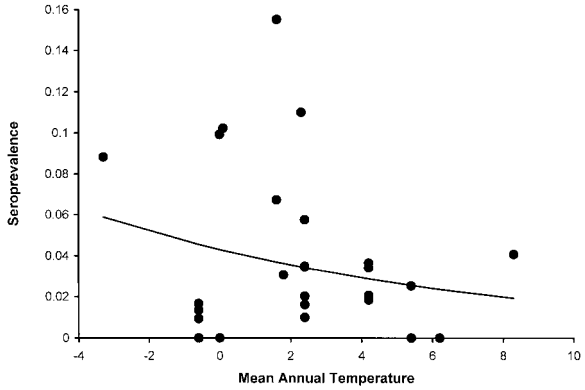

Figure 5. Seroprevalence in relation to mean annual temperature. Solid line: seroprevalence predicted by mean annual temperature in the stepwise multiple logistic regression equation (sample size = 2,837 mice), with the other variables in the regression model held constant at the their mean values. Data points: for presentation, the data were sorted by mean annual temperature and combined, so that each point on the graph represents the proportion of mice testing positive out of approximately 100 mice. Each point is then plotted against the median value of mean annual temperature for that group of mice.

density may also partly explain the increase in virus incidence at low habitat amounts (Figure 2), where patch sizes are smaller.

Spatial scale

The model using landscape structural variables at the 1 km radius scale had the best fit to the data (lowest residual X^2). Therefore, we suggest this as an appropriate scale for designing experiments and/or sampling schemes for future studies testing hypotheses about effects of landscape structure on the epidemiology of Sin Nombre in deer mouse populations. In many cases however, it would likely be more informative to repeat the method employed here, given the relative ease of simultaneous sampling and analysis at several scales.

One km may be a more accurate estimate of the minimum distance required for spatial independence than is the 2 km distance which we used in defining sites, but any dichotomous definition of spatial independence based on a minimum separation distance is a pragmatic simplification. The extent of spatial independence between sites varies with the characteristics of movement of individuals across the landscape, which will differ among landscapes.

Seasonal change

Although it is known that deer mouse populations increase during the breeding season from spring to autumn, we found virus incidence to decrease during this period (Figure 4). This indicates that the number of uninfected individuals increased more rapidly than did the number of infected individuals during this period. This could be due to higher mortality of infected individuals than of noninfected individuals and/or lower infection rate among offspring than among adults.

Differential mortality has not been directly investigated, but seems unlikely. The rodent hosts of hantaviruses develop a persistent or chronic infection with no apparent disease symptoms (LeDuc 1987; Lee and van der Groen 1989). Also, there is evidence that Sin Nombre has co-evolved with *P. maniculatus* (Chizhikov et al. 1996; Morzunov et al. 1996). A virus is rarely lethal to its primary host, since this characteristic would be selected against as a direct result of the differential mortality of the host.

The temporal pattern makes sense if adults are more likely to be infected by other adults than are offspring to be infected by their mother. This may well be the case if vertical transmission and perinatal transmission are low and if resistance of neonates to infection in the nest is high, for example due to maternal antibodies. In addition, if the reproductive rate of infected and uninfected females is assumed to be the same, then anything less than 100% infection of the offspring of infected females would result in a decreasing virus incidence during the reproductive season. During the overwintering period, communal nesting among adults would permit high transmission

rates and build up virus incidence to a higher level again the following spring. Field studies using nest boxes could resolve these questions, but antigen testing would likely be required in addition to antibody screening because very young offspring may test positive due to maternal antibodies, but not be infected with the virus.

Temperature

The negative relationship between virus incidence and mean annual temperature (*Temp*) was unexpected, because higher virus incidence rates have been reported from warmer regions in the United States. Our results suggest the opposite for Canada, where temperatures are generally colder. We have no explanation for this finding. Given the correlation between temperature and geography, it seems likely that these temperature relationships result from a spurious correlation(s) with some other variable.

Buildings

As discussed, the dynamics of subpopulations in buildings may be quite different from those of wild subpopulations. The very conditions that probably attract deer mice into buildings, a concentrated food supply and moderate microclimate, may increase transmission of Sin Nombre by increasing mouse population density and out-of-host survival of the virus, respectively.

Conclusions

We conclude that landscape structure can affect the pattern of distribution of a virus in its host population. In fact, landscape structure can have a more important effect on the distribution of the virus than other ecological variables such as climate and seasonal change.

Finally, Nee (1994) and Lawton et al. (1994) have observed similarities between epidemiological models and metapopulation models. The link between landscape structure and various metapopulation models is also a popular area of research (e.g., Henein and Merriam 1990; Fahrig and Merriam 1994). In this study we have attempted to integrate these three concepts and clarify the relationships among them. Our results suggest that epidemiological models should consider not only the demographic structure of the host population, but its spatial structure as well, and this can in some cases be effectively inferred from landscape structure.

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