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PHYSIOLOGICAL ECOLOGY - ORIGINAL PAPER

Differential regulation of pathogens: the role of habitat disturbance in predicting prevalence of Sin Nombre virus

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Abstract Deer mice (*Peromyscus maniculatus*) are the primary reservoir for Sin Nombre virus (SNV), a North American hantavirus that causes disease with high mortality in humans. Recent studies have proposed that habitat disturbance affects prevalence of SNV in deer mice; however, the outcomes proposed in these studies are in opposition to each other. Our objectives were to test these divergent hypotheses by: (1) measuring SNV infection in deer mice within a patchwork of disturbance; and (2) evaluating the relationships between SNV prevalence, population density and demography as possible mechanisms. In 2003 and 2004, we sampled 1,297 deer mice from 17 sites with varying levels of disturbance in the Great Basin Desert. Across sites and years, SNV prevalence varied from 0.0 to 38.9%. We found a negative relationship between SNV prevalence and disturbance. Although we found no direct relationship between SNV prevalence and deer mouse density, we found that density was highest on sites with the lowest levels of disturbance. The number of deer mice that survived across seasons (e.g., trans-seasonal survivors) differed across levels of disturbance and was greatest on our least disturbed study sites ($\bar{x} = 14.00\%$), moderate on sites with intermediate levels of disturbance ($\bar{x} = 5.61\%$) and zero on highly disturbed sites. On low-disturbance sites, a greater proportion of trans-seasonal survivors were SNV seropositive (28.80%) compared to the intermediate-disturbance sites (16.67). Collectively, our results indicate that habitat disturbance plays a predictive role in SNV prevalence, with highly disturbed sites having reduced long-term survival of deer mice, including survival of infected individuals.

Keywords Deer mice · Disease ecology · Disturbance · Hantavirus · Sin Nombre virus

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Introduction

In natural systems, the prevalence of pathogens is highly variable and can differ at both local and landscape levels. Recently, the role of anthropogenic disturbance in influencing prevalence of infectious agents has gained attention, although its effect is predicted to produce divergent outcomes. For example, Johnson and Chase (2004) suggested that habitat disturbance is implicated in parasitic infections responsible for amphibian malformations in North America. Disturbance in this system causes an increase in the sole intermediate host of the amphibian parasite. Likewise, agricultural development in Israeli deserts increases vegetation growth, which is linked to increased population growth of the primary vector for cutaneous leishmaniasis, *Phlebotomus papatasi* (Wasserberg et al. 2003). In contrast, parasitism in populations of northern redbelly dace

(*Phoxius eos*) declined in boreal lakes with significant deforestation (Marcogliese et al. 2001). In degraded wetland habitats, the concentration of trematodes increased with restoration efforts, likely due to the increase in avian species abundance and richness (Lafferty 1997; Lafferty and Kuris 2005). Collectively, these studies demonstrate that anthropogenic disturbance has considerable impacts on the life history dynamics of pathogens, vectors and hosts, and can significantly alter the prevalence of pathogens in the natural environment.

We evaluated the relationship between disturbance and pathogen prevalence using Sin Nombre virus (SNV) in rodents as a study system. The primary reservoir for SNV is the deer mouse (*Peromyscus maniculatus*), and infected deer mice shed virus in urine, saliva and possibly feces (Otteson et al. 1996). Transmission between hosts is believed to occur through aggressive interactions, such as biting, as evidenced by strong correlations between infection and external scarring (Boone et al. 1998; Calisher et al. 1999; Mills et al. 1997, 1999a). SNV infections have an initial acute phase followed by a persistent phase that is maintained for the life of the animal (Botten et al. 2000; Yamada et al. 1995). In infected deer mice, SNV is present in several endothelial tissues with the most critical for maintenance being the heart, lungs, and brown adipose tissue (Botten et al. 2003). The oscillations of viral RNA titers in the blood of both artificially infected, laboratory deer mice as well as naturally infected, wild deer mice suggest periods of recrudescence (Botten et al. 2003: Kuenzi et al. 2005). Although deer mice infected with SNV are thought to be largely asymptomatic (Botten et al. 2003; O'Connor et al. 1997), conflicting information exists. Marked septal edema in lung tissue and immune infiltrates in the liver have been documented (Netski et al. 1999). Furthermore, SNV infection causes a chronic response of the immune system in that high titers of neutralizing antibodies can be present at any time during the infection (Botten et al. 2003). SNV transmission to humans occurs via inhalation of aerosolized virus contained in deer mouse excrement (Doyle et al. 1998). SNV infection in humans can result in hantavirus pulmonary syndrome, a disease with high rates of mortality (Hjelle et al. 1994; Kilpatrick et al. 2004).

The SNV–deer mouse system is a good model with which to study the role of anthropogenic disturbance in patterns of pathogen prevalence, as SNV prevalence is spatially variable (Mills et al. 1998; Boone et al. 1998; Kuenzi et al. 1999; Otteson et al. 1996), and variability can occur independently of host densities (Biggs et al. 2000; Boone et al. 1998; Douglass et al. 2001). Prevalence of SNV in deer mice varies considerably among populations, i.e., 0–50% (Douglass et al. 2001; Kuenzi et al. 1999; Mackelprang et al. 2001; Otteson et al. 1996). Much of the variation in SNV prevalence among populations has been attributed to differences among habitat type, with the greatest prevalence found in piñon–juniper and Great Basin shrub habitats and the lowest prevalence in salt desert scrub habitats (Boone et al. 1998). However, prevalence of SNV can vary as much within a single type of deer mouse habitat, such as Great Basin shrub, as it does among habitat types (Mills et al. 1998).

Human alteration of habitat has been implicated as a critical factor mediating prevalence in the SNV–deer mouse system. Mackelprang et al. (2001) proposed that high SNV prevalence ($\sim$30% over 6 months) at a site in Utah was the result of anthropogenic disturbance created by off-road vehicles (ORV) on the study area. The authors suggested that the open spaces resulting from ORV disturbance significantly altered deer mice behavior in a manner that increased encounter rates, thereby increasing SNV transmission and prevalence. Similarly, in a meta-analysis of 101 sites across Canada, Langlois et al. (2001) reported that fragmented habitats had higher SNV prevalence than less fragmented habitats. The authors proposed that increased movement and local densities on fragmented sites led to increased encounter rates among potential hosts. In contrast, in a study comparing four sites in Colorado, Calisher et al. (2001) found the lowest incidence of SNV prevalence in deer mice on a site that had been disturbed by grazing and homesteading. They hypothesized that the low prevalence resulted either from disturbed areas having greater rates of population turnover resulting in reduced opportunities for SNV transmission, or because disturbed habitats were dispersal sinks for juvenile deer mice, an age class with a low likelihood of becoming infected with SNV.

Given our limited understanding of the role of anthropogenic disturbance in governing variation in SNV prevalence, we conducted an extensive study on disturbance and SNV dynamics. Our objectives were to test the existing hypotheses on habitat disturbance and SNV prevalence by: (1) measuring SNV infection in deer mice across varying levels of disturbance in Great Basin shrub habitat, and (2) evaluating potential mechanisms by which disturbance may affect SNV prevalence, including population density, demography, and survival of hosts.

Materials and methods

Study sites and sampling periods

Deer mice were non-destructively sampled from 17 different 3.14-ha sites near the West Tintic Mountains in the Great Basin Desert of central Utah (Juab and Utah counties) on lands administered by the U.S. Department of Agriculture and the Bureau of Land Management. Vegetative

communities of each site were dominated by big sagebrush (*Artemisia tridentata*) and Utah juniper (*Juniperus osteosperma*). Sections of this area such as Little Sahara Recreation Area and surrounds are extremely popular for ORV driving, receiving over 215,000 ORV users per year (Long and Blahna 2001). Pronounced ORV trails are widespread throughout the area. ORVs create a substantial and persistent disturbance in desert habitats. For example, 100 passes by a single-track ORV (motorbike) results in a trail 3 times as wide as tire width, removes all annual vegetation, damages larger shrubs, and compacts soil 5 times more than that of an undisturbed site (Webb 1983). We selected sites to represent the spectrum of disturbance in the area based on shrub cover and bare ground, ranging from roughly 1.2% shrub cover and 62.2% bare ground at sites with the greatest levels of disturbance to 48.1% shrub cover and 6.1% bare ground on the sites with the lowest levels of disturbance. We limited our investigation to disturbance caused by ORV use, as it is the predominant type of disturbance in this area. The lowest disturbance sites are part of a long-term research area and thus, have restricted ORV access (Fig. 1a), whereas the mid- and high-disturbance sites have received varying levels of ORV use over the past several decades (Fig. 1b, c). To minimize various sources of large-scale geographic variation among sites, the sites we studied were separated by <100 km.

Sites were sampled for deer mice in "spring" (May and June) and "fall" (August, September and October) of 2003 and 2004 during 15-day periods that coincided with the new moon. Although we monitored a total of 17 sites during 2003 and 2004, only 12 sites could be sampled during any single season due to time limitations. Of the 17 sites, five were sampled in every season and the remaining 12 were sampled in some, but not all seasons (Table 1). We removed sites UTL-12-Mid, UTL-13-Mid and UTL-14-High from the study in fall 2004 because during the fall 2003 and spring 2004 trapping, we discovered that their popularity for target practice was incompatible with small rodent trapping and crew safety. These sites were replaced with sites TJ-15-Mid, TJ-16-Mid and TJ-17-High, which were selected for their similar habitat structure. Sites LS-6-High and LS-11-High were also removed in fall 2004 because of their extremely low densities of deer mice (<1 mouse/ha) and were replaced by sites TJ-18-High and TJ-19-High.

Deer mouse sampling

At each site, animals were live-trapped (Sherman traps) on a "web" of 148 traps over 3.1 ha (Mills et al. 1999b). To ensure that the small mammal population at each site was exhaustively sampled, trapping continued during a single season until recapture rates exceeded 90%, which was never >5 nights. High recapture rates provide statistical confidence in estimating SNV prevalence on sites with few deer mice. After capture, animals were identified to species, weighed and sexed. All individuals were uniquely marked with numbered ear-tags. We collected ~0.2 ml of blood from the retro-orbital sinus of all deer mice upon initial capture of each trapping season. Blood was immediately stored on dry ice until transfer to a −80°C freezer. Following processing, animals were released at location of capture. All personnel involved in trapping and handling

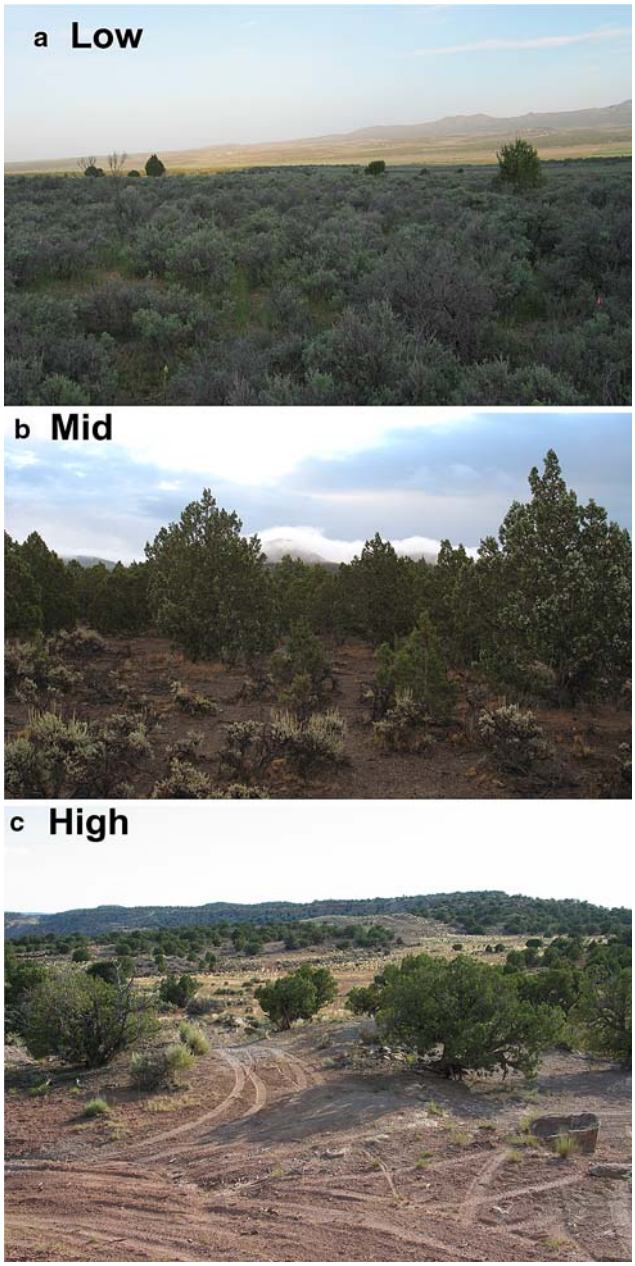

Fig. 1 Photographs of representative levels of disturbance (i.e., mean indices of vegetative cover and bare ground) for **a** low-, **b** mid- and **c** high-disturbance study sites near the West Tintic Mountains in the Great Basin Desert of central Utah

Table 1 Summary of deer mice (*Peromyscus maniculatus*) sampled and prevalence[a] of Sin Nombre virus (SNV) at 17 sites in central Utah from 2003 to 2004. *Spring* May, June; *Fall* August, September, October

Site identifier[b]	Spring 2003		Fall 2003		Spring 2004		Fall 2004	
	Prevalence	Number of deer mice captured	Prevalence	Number of deer mice captured	Prevalence	Number of deer mice captured	Prevalence	Number of deer mice captured
TJ-3-Low	13.6	44	9.1	55	34.3	35	38.9	36
TJ-4-Low	8.2	85	5.9	51	16.3	49	24.2	66
LS-5-Mid	18.5	27	6.9	29	11.5	26	4.2	24
LS-6-High	0.0	10	0.0	6	12.5	8	–	–
LS-7-Low	18.2	33	5.6	36	25.9	27	19.5	41
LS-8-Low	16.7	24	10.0	20	21.7	23	29.2	24
LS-9-Low	–[c]	–	9.1	22	10.0	10	4.8	21
LS-10-Low	–	–	7.7	26	8.7	23	15.0	20
LS-11-High	–	–	11.1	9	0.0	3	–	–
UL-12-Mid	–	–	0.0	38	18.2	22	–	–
UL-13-Mid	–	–	0.0	26	15.4	26	–	–
UL-14-High	–	–	0.0	17	30.4	23	–	–
TJ-15-Mid	–	–	–	–	–	–	15.0	20
TJ-16-Mid	–	–	–	–	–	–	33.3	15
TJ-17-High	–	–	–	–	–	–	10.0	20
TJ-18-High	–	–	–	–	–	–	13.3	15
TJ-19-High	–	–	–	–	–	–	0.0	15
Mean (±SE)	12.5 (5.6)		5.5 (3.6)		17.1 (7.5)		17.3 (9.8)	
Total deer mice sampled		223		335		275		317

[a] Prevalence of SNV was measured as the total number of deer mice positive for Sin Nombre viral antibodies divided by the total number of deer mice sampled at each site in each season

[b] Site identifiers include the geographic location [Tintic Junction (*TJ*), Little Sahara I*LS*), Utah Lake (*UL*)], the numerical site code (*3–19*) and the disturbance level (*Low*, *Mid*, *High*)

[c] Dashes indicate sites that were not sampled in a particular season

rodents took precautions for working with animals potentially infected with hantavirus (Centers for Disease Control and Prevention 1995) and general techniques for capturing and processing animals were approved by the Institutional Animal Care and Use Committee at the University of Utah.

Antibody detection

In a Bio Safety Level-3 laboratory at the University of Nevada, Reno, we performed enzyme linked immunosorbent assays (ELISA) for antibodies (IgG) against SNV in deer mouse blood, as described previously by Otteson et al. (1996) and Feldman et al. (1993). ELISA results for SNV antibodies have a concordance of about 70% with the presence of viral RNA in blood as determined by reverse-transcriptase polymerase chain reaction (PCR) (Otteson et al. 1996, Rowe et al. 1995). The remaining 30% include adults with low viral titers that may escape detection by PCR, animals that are recently infected (~3 weeks) but have not yet mounted a detectable IgG response and uninfected juveniles who have received maternal antibodies while nursing from SNV seropositive dams.

Disturbance estimation

To estimate the level of ORV disturbance on each study site, we inventoried vegetation in 2004 using the line-intercept method (Canfield 1941). In this process, vegetation was point sampled at 0.5-m intervals along each of the twelve 100-m transects of the trapping web. Thus, on each 3.14-ha site, we sampled 2,400 points. Vegetative cover was classified as either shrub, tree or herbaceous, whereas non-vegetative cover categories were divided into cryptogamic crust, moss, litter, rock and bare ground. Cryptogamic crusts and mosses are abundant in the Great Basin Desert where soils are undisturbed and can almost completely cover the ground of an undisturbed area. Cryptogamic crusts and mosses are not affected by most natural disturbances other than fire (Johansen et al. 1984). However, both are quickly removed by anthropogenic

disturbance (e.g., walking, motorized travel) and grazing (Cole 1990; Anderson et al. 1982). Thus, the categories of cryptogamic crust and moss represent areas that have had little disturbance. In contrast, the category of bare ground, is defined as an area with no cryptogamic crust or moss and best represents disturbed areas. On the study sites, bare ground is most likely due to anthropogenic disturbance in that the there is no evidence of recent fires that would remove the cryptogamic crust. We did not include a category of bare ground for obvious ORV trails (i.e., tire tracks) because it underestimates ORV use for two reasons. Trails with heavy use are compacted and leave little trace of tire tracks. Additionally, tire treads on slopes with heavy snow pack or water drainage can be removed by melting snow and drainage.

At each site for each coverage category, the total number of intercepts was summed and divided by the total number of points to generate a percentage for each category. We limited the disturbance estimate used in the analysis to percentage of shrub cover and percentage of bare ground for two reasons. First, both tree and herbaceous cover are not predictive of ORV use as this type of disturbance rarely results in removal or destruction of large trees and is often followed by colonization by invasive grasses (Hirst et al. 2003). Thus, ORV disturbance may not change tree cover and may increase herbaceous plant cover. Secondly, shrubs are affected by ORV use and shrubs provide the primary nesting sites for deer mice (Smith and Urness 1984). Bare ground is an indicator of areas where cryptogamic soils have been disturbed, mostly likely by anthropogenic forces. To determine whether shrub cover and bare ground changed from 2003 to 2004, we compared vegetation inventories that were collected on six of the sites in 2002 to that of the 2004 data.

Population demographics

To determine the age structure of the study populations, deer mice with body mass below 14 g were considered to be juveniles and deer mice with body masses greater than 14 g were considered to be adults (Calisher et al. 2001, Borucki et al. 2000). To ensure that these estimations of animal age were reasonable for the populations, we verified that mean body masses of individuals assigned as adults and juveniles based on body mass (>14 or <14 g, respectively), corresponded to mean body masses of adults and juveniles, as determined by visual estimation of external reproductive condition (i.e., females = lactating or perforate; males = scrotal). We estimated the percentage of the population represented by trans-seasonal survivors as the number of recaptures divided by the total number deer mice captured in the current season on each site (Abbott et al. 1999; Calisher et al. 2001; Kuenzi et al. 1999). A trans-seasonal survivor is defined as an individual recaptured in any subsequent trapping session, not just the trapping session immediately following the initial capture.

Deer mouse density and Sin Nombre prevalence

Deer mouse density (deer mice/ha) in each season was calculated using the program DISTANCE (version 4.1; Thomas et al. 2004). We calculated SNV antibody prevalence (total number of SNV seropositive deer mice divided by the total number of deer mice) independently for each site in each season. We determined trans-seasonal infection level as the number of SNV seropositive recaptured deer mice divided by the total number of recaptured individuals for each consecutively sampled site in each season.

Statistical analysis

Because we were interested in breaking down the causal chain between SNV prevalence and disturbance, we conducted our analyses in a step-wise manner to ask: (1) what is the relationship between disturbance and SNV prevalence, (2) what is the relationship between SNV prevalence and deer mouse density, and (3) what is the relationship between disturbance and deer mouse density? We investigated the relationship between ORV disturbance and SNV prevalence using general linear mixed modeling (GLIM). GLIM does not require balanced sampling of longitudinal repeated data (SYSTAT version 10 2000; Cnaan et al. 1997), and thus was appropriate considering our unbalanced study design (e.g., sites repeatedly sampled, but not equally). In preliminary assessments of SNV prevalence, residual variation was non-normal and heteroscedastic, so we used a square-root transformation of SNV prevalence as the dependent variable. The percentage of shrub cover, season, year and season × year were considered fixed effects and site was entered as a random effect to account for repeated sampling. To evaluate the relationship between SNV prevalence and deer mouse density, we also used GLIM. The square root of SNV prevalence was the dependent variable. As deer mouse density estimates were positively skewed and leptokurtic, they were also transformed for normality. The square root of density was considered to be a fixed effect and site was a random effect. Using GLIM, we also evaluated the relationship between disturbance and deer mouse density; in this model, the square root of deer mouse density was the dependent variable, which shrub cover was a fixed effect and site was a random effect.

ANOVA was used to compare overall differences in SNV prevalence between spring and fall and between 2003 and

2004. We were unable to use a repeated measures design because not all sites were sampled during each sampling period. Pairwise differences were investigated using least squares means comparisons with Tukey–Kramer adjustments for multiple comparisons. To determine whether the inconsistency in the sampling of study sites affected observed patterns of prevalence across seasons and years of our study, we analyzed the five sites that were sampled during each sampling period with a repeated measures ANOVA. In this model, seasonal prevalence was the dependent variable and site was the independent categorical factor.

ANOVA was also used to compare overall population densities of deer mice on our study sites between spring and fall and between 2003 and 2004. Paired *t*-tests were used to determine whether disturbance, including both the percentage of shrub cover and the percentage of bare ground, changed on our study sites between 2003 and 2004.

We used general linear models (GLM) to evaluate differences in the age structure of the study populations across levels of disturbance, as well as across years and seasons of the study. In these models, the percentage of adults was the dependent factor and independent factors included the percentage of shrub cover, the percentage of bare ground, season, year, and season × year. Backward stepwise elimination ($\alpha = 0.15$) was used to reduce general models to the most parsimonious version. Upon finding differences in both SNV prevalence and the percentage of adults in our study populations across years of the study, we opted to repeat our initial three-part GLIM analysis to determine if the relationship between disturbance and SNV prevalence remained constant when only adult deer mice were included in the analysis. This analysis also provided information about how disturbance influenced the age structure of our study populations. In our first analysis we included the square root of SNV prevalence in adults as the dependent variable, shrub cover, season, year and season × year as fixed effects and site as a random effect to account for repeated sampling. In our second GLIM analysis, the square root of SNV prevalence in adults was the dependent variable, whereas the square root of adult population density was a fixed effect and site was a random effect. In our third GLIM analysis, the square root of adult deer mouse density was the dependent variable, shrub cover was a fixed effect and site was a random effect.

We used ANOVA to determine whether trans-seasonal survival differed across levels of disturbance. Low overall numbers of trans-seasonal survivors required aggregation of study sites into disturbance categories rather than using continuous data for disturbance estimations. Each site was assigned a general disturbance score (1 − proportion of shrub cover + proportion of bare ground) and sites were aggregated into disturbance categories (low, mid or high) based on natural intervals between disturbance scores. Of the 17 sites, seven were classified as low disturbance (score = 0.62–0.85), six were classified as mid disturbance (score = 0.98–1.15) and four were classified as high disturbance (score = 1.28–1.56). In each season, we sampled a minimum of two sites in each disturbance category.

Results

Habitat disturbance

Disturbance varied considerably across sites (Fig. 1). Bare ground ranged from 6.1 to 62.6% and shrub cover ranged from 1.2 to 48.1% among sites. On sites classified as low disturbance, bare ground ranged from 6.1 to 27.6% and shrub cover ranged from 30.9 to 48.1%. On sites with intermediate levels of disturbance, bare ground ranged from 20.8 to 48.6% and shrub cover ranged from 11.0 to 33.8%. On high-disturbance sites, bare ground ranged from 29.7 to 62.2% and shrub cover ranged from 1.2 to 21.3%. Shrub cover and bare ground did not differ significantly between 2003 and 2004 (shrub cover $t_5 = 1.40$, $P = 0.22$; bare ground $t_5 = -1.61$, $P = 0.17$). These results also indicate that our activity on the study sites did not significantly increase disturbance.

SNV prevalence

Over the course of our 2-year study, we sampled 1,150 individual deer mice (Table 1). Across sites, SNV prevalence varied from 0.0 to 38.9% (Table 1). The annual average SNV prevalence more than doubled from 2003 to 2004 (9.0 vs. 17.2%; $F_{1,\ 38} = 7.79$, $P < 0.01$; Fig. 2a), there was no difference in prevalence between seasons (14.8 vs. 11.4%; $F_{1,\ 38} = 1.39$, $P = 0.25$). We found similar fluctuations in SNV prevalence when the five sites that were sampled in each season of the study were considered independently. Prevalence on these five sites doubled from 2003 to 2004 (2003 = 11.3% vs. 2004 = 22.6%; $F_{1,\ 16} = 9.67$, $P < 0.01$) and across years, but did not vary between seasons (spring = 18.5% vs. fall = 15.3%; $F_{1,\ 16} = 0.74$, $P = 0.40$).

Relationship between SNV prevalence, habitat disturbance and deer mouse density

There was a positive relationship between the percentage of shrub cover and SNV prevalence (estimate = 0.38, $Z = 2.42$, $P = 0.02$); however, there was no relationship between SNV prevalence and fixed effects including

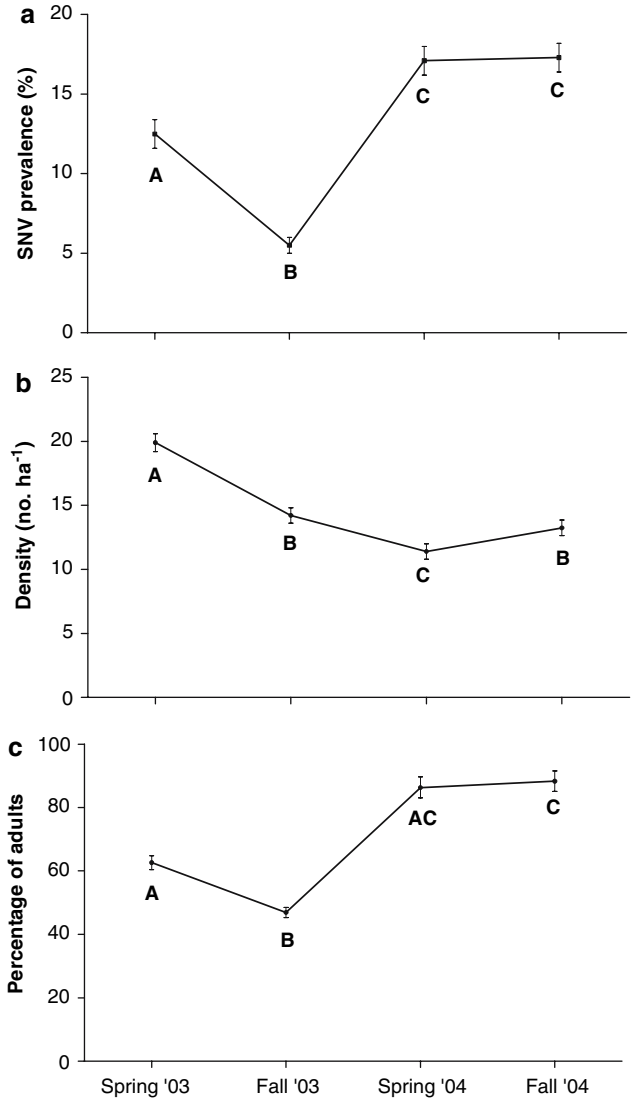

Fig. 2 **a** Prevalence of Sin Nombre virus (*SNV*) measured in deer mouse (*Peromyscus maniculatus*), **b** deer mouse density, and **c** percentage of adults in deer mouse populations in Central Utah sampled from spring 2003 (May, June) to fall 2004 (August, September, October) (mean ± SE). In spring 2003, six populations (sites) were sampled whereas in all the other sampling periods, 12 populations were sampled (see Table 1 for sampling details). SNV prevalence was calculated by the number of deer mice testing positive for Sin Nombre viral antibodies divided by the total number of deer mice captured in a population in a particular season. Deer mouse density (deer mice/ha) in each season was calculated by the program DISTANCE. Adults were individuals with body masses greater than 14 g. *Different letters* indicate significant ($P < 0.05$) differences among seasons as determined with post hoc comparisons of least squares means

season ($Z = 0.19$, $P = 0.85$), year ($Z = 1.04$, $P = 0.31$), or season × year ($Z = 1.27$, $P = 0.20$). Site was not a significant random effect in this model ($Z = 0.16$, $P = 0.44$).

Deer mouse densities (estimated by DISTANCE) on the study sites ranged from 1.50 to 42.50 deer mice/ha (Fig. 2b). Deer mouse densities did not differ across seasons ($F_{1,\ 39} = 0.37$, $P = 0.55$) of the study; however, deer mouse density was higher in 2003 than in 2004 (least squares mean = 17.06 vs. 12.31; $F_{1,\ 39} = 3.64$, $P = 0.05$). We found no relationship between SNV prevalence and deer mouse density (fixed effect, $Z = 1.50$, $P = 0.13$), and site was not a significant random effect ($Z = 0.16$, $P = 0.44$). However, there was a positive relationship between deer mouse density and shrub cover (fixed effect, estimate = 31.37, $Z = 3.34$, $P = 0.01$), but site was not a significant random effect ($Z = 2.15$, $P = 0.20$).

Population demographics and SNV prevalence

The percentage of adults in the study populations increased from 2003 to 2004 ($F_{1,\ 39} = 21.67$, $P < 0.01$; Fig. 2c). Upon examination of patterns of SNV prevalence in adult deer mice only, we found a positive relationship between the percentage of shrub cover and prevalence (estimate = 0.47, $Z = 2.19$, $P = 0.03$); however, there was no relationship between SNV prevalence in adults and fixed effects including season ($Z = 0.69$, $P = 0.49$), year ($Z = -2.83$, $P = 0.78$), or season × year ($Z = -0.88$, $P = 0.38$). Site was not a significant random effect in this model ($Z = 0.36$, $P = 0.36$). We found no relationship between adult SNV prevalence and adult population density ($Z = 1.89$, $P = 0.06$), and site was not a significant random effect ($Z = 0.44$, $P = 0.33$). There was also no significant relationship between adult population density and shrub cover ($Z = 1.42$, $P = 1.16$), and site was not a significant random effect in the model ($Z = 0.01$, $P = 0.50$).

Trans-seasonal survival

Across seasons, trans-seasonal survival differed across levels of disturbance ($F_{2,\ 6} = 21.38$, $P < 0.01$; Fig. 3); survival was greatest on the least disturbed study sites ($\bar{x} = 14.00\%$, SE = 1.77) and moderate on sites with intermediate levels of disturbance ($\bar{x} = 5.61\%$, SE = 1.96). We did not recapture any animals on sites with the highest levels of disturbance, suggesting that trans-seasonal survival in these habitats was rare. We did not recapture any deer mice from non-consecutive sampling seasons. There was a negative relationship between trans-seasonal survival of deer mice and the percentage of bare ground on the study sites (coefficient = −0.36; $F = 8.75$, $P < 0.01$; $r^2 = 0.27$), but trans-seasonal survival was not related to shrub cover. Within sites, trans-seasonal survival did not differ across pairs of consecutive seasons (e.g., spring 2003 to fall 2003, fall 2003 to spring 2004, etc.).

The low-disturbance sites had the greatest number and proportion of infected trans-seasonal survivors. Across all

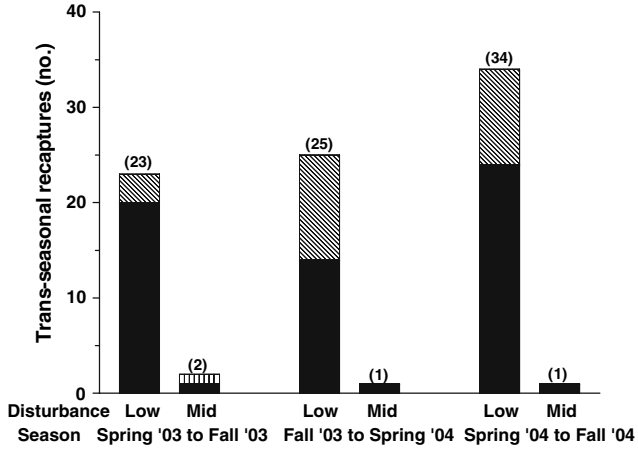

Fig. 3 Trans-seasonal survival of uninfected (*solid fill*) and SNV infected (*patterned fill*) deer mice at study sites with low and mid levels of disturbance in spring 2003 to fall 2004. Trans-seasonal survival is calculated as the number of deer mice recaptured over consecutively sampled seasons. There were no trans-seasonal recaptures on sites with high levels of disturbance

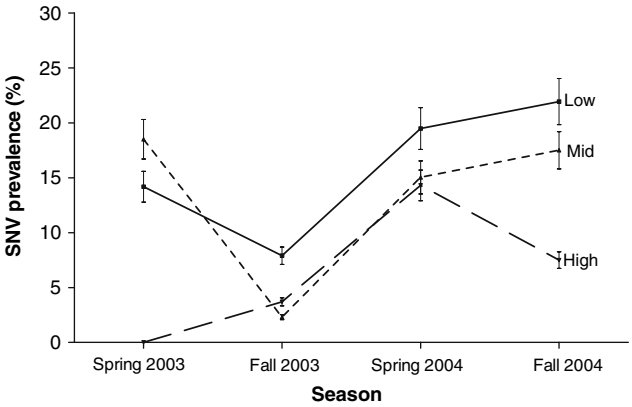

Fig. 4 General relationship between disturbance level (*High*, *Mid* and *Low*) and SNV prevalence (number infected out of total captured) in deer mice monitored at sites from spring 2003 to fall 2004. Prevalence is reported as the mean (±SE) for each site within a particular disturbance level

seasons, 29% of trans-seasonal survivors on the low-disturbance sites were SNV seropositive (Fig. 3). On sites of intermediate disturbance, there were far fewer trans-seasonal survivors who were SNV seropositive (16.67%; Fig. 3). There were no infected trans-seasonal survivors on the high-disturbance sites because there were no trans-seasonal survivors on those sites.

Discussion

The central objective of our study was to test two mutually exclusive hypotheses about the role habitat disturbance plays in the prevalence of SNV in deer mouse populations. Our results indicate that habitat disturbance plays a predictive role in SNV prevalence, as we found that prevalence was generally highest on sites with the lowest levels of disturbance (Fig. 4). Although we found no direct relationship between SNV prevalence and deer mouse density, we found that density was highest on sites with the lowest levels of disturbance. Given that the only demographic factor that differed among the disturbance categories was trans-seasonal survival, it is likely that reduced population turnover on the low-disturbance sites contributed to elevated SNV prevalence. Thus, the results support in part, the Calisher et al. (2001) model that disturbance decreases prevalence. We found no support for the hypotheses presented by both Mackelprang et al. (2001) and Langlois et al. (2001), that disturbance increases prevalence. Our results underscore the complexity of the SNV–deer mouse system. Below we discuss in more detail the interplay of disturbance, deer mouse density and population demography in governing the dynamics of SNV.

Disturbance, SNV prevalence and deer mouse density

As stated above, our results indicate that habitat disturbance is negatively correlated with SNV prevalence. Our results also suggest that disturbance has a large impact on both population densities and population turnover of deer mice, as the percentage of bare ground on the study sites was negatively correlated with trans-seasonal survivorship in both 2003 and 2004. Of the individuals recaptured across seasons, those on sites with lower levels of disturbance were much more likely to be SNV seropositive than individuals captured on sites with higher levels of disturbance. One interpretation of these results is that habitat structure imposes constraints on animal behavior (e.g., movement, aggression) or susceptibility to infections. As animal densities increase and food becomes limiting, deer mice on highly disturbed sites may be forced to emigrate to more favorable habitats, thereby increasing population turnover. Alternatively, as densities increase on highly disturbed sites, deer mice may be required to travel across open spaces more frequently compared to undisturbed sites. The greater use of open space by deer mice in disturbed habitats could result in higher predation rates of deer mice, leading to increased rates of population turnover. Over time, higher rates of population turnover in disturbed habitats could reduce the prevalence of SNV. Although transient animals could increase prevalence via increased movement of infected individuals into naïve communities, we did not find evidence for this. Our findings are consistent with those of Douglass et al. (2001), who did not find elevated SNV prevalence in transient animals compared to residents on a site. It is possible that while transients may move greater distances, they may be less likely than residents to engage in aggressive territorial encounters, therefore reducing opportunities for SNV transmission.

The strong effect of population turnover on SNV prevalence could easily extend to situations in which anthropogenic disturbance is not a contributing factor. For example, severe environmental conditions (e.g., drought, early frost) could result in large fluctuations in trans-seasonal survival. Such an alteration in trans-seasonal survival could result in chaotic SNV dynamics within a site. The role of trans-seasonal survival on SNV dynamics warrants further research and could be particularly important in predictive models.

SNV prevalence increased on the study sites between 2003 and 2004, whereas deer mouse density declined during this period. Other studies have found or suggested similar patterns. Biggs et al. (2000) predicted that SNV prevalence should be higher at low population densities. They suggested that a reduction in food resources reduces density but increases contact rates and transmission opportunities among deer mice searching more intensively for limited food resources. Abbott et al. (1999) found a similar relationship between density and hantavirus prevalence in populations of pinyon mice and brush mice (*Peromyscus boylii*), which they suggested was the result of infected individuals having a greater likelihood of survival across seasons at low population densities. Others have proposed that seemingly inverse relationships between deer mouse density and SNV prevalence may result from "delayed density-dependence", in which prevalence of hantavirus in a particular season is positively correlated to the density of hosts in a previous season (Calsiher et al. 1999; Mills et al. 1999a; Niklasson et al. 1995). Although speculative at this stage of our investigation, these hypotheses present plausible mechanisms for how SNV prevalence could be high despite low host densities. Our findings contrast the "el Niño" model of SNV dynamics, which suggests that prevalence is directly related to density of the host community. In this model, increased nest sites and food resources that are associated with increased precipitation during el Niño events are often accompanied by a subsequent "boom" in rodent populations (Parmenter et al. 1999). Increases in SNV prevalence have been reported following increases in host density (Calisher et al. 2002) and accordingly, the el Niño model predicts that SNV prevalence will be highest during seasons or years when deer mouse density is greatest (Glass et al. 2000; Parmenter et al. 1999).

SNV prevalence and deer mouse demography

Changes in population age structure across all study sites from 2003 to 2004 mirrored changes in SNV prevalence during that period. From spring to fall 2003, the percentage of adults in the study populations declined slightly, as would be expected if the fall sampling period, following summer births, contained a larger portion of young-of-the-year deer mice than the spring sampling period. However, from spring to fall 2004, the percentage of adults in the study populations increased while overall population density decreased. The decline in population density and the increased proportion of adults in the study populations indicate that reproductive success was lower in 2004 than in 2003. The changes in population age structure that occurred from 2003 to 2004 offer a mechanistic explanation for how SNV prevalence could be high in 2004 when deer mouse density was low. SNV prevalence can be high despite low population densities if the population is adult-biased. The "juvenile dilution effect" has been described in previous studies of SNV (Calisher et al. 2001; Mills et al. 1999a), and may partially account for "delayed density-dependence" in SNV prevalence in that seasonal patterns of births and SNV transmission are non-synchronous. Adults in general are more likely than younger age classes to acquire an SNV infection, in part because they are more likely to engage in behaviors such as fighting that promote transmission (Childs et al. 1987; Glass et al. 1998; Hinson et al. 2004; Klein et al. 2004). In addition, the juvenile deer mice of dams infected with SNV are not competent hosts because they possess SNV antibodies acquired while nursing (Douglass et al. 2001). These antibodies remain active for several weeks after weaning (Botten et al. 2000). If juvenile deer mice with maternal antibodies are exposed to SNV during this period, they are unlikely to become infected. Thus, the percentage of adults in the population often reflects patterns of "actual" SNV prevalence. That we found similar relationships among SNV prevalence, disturbance and deer mouse population density when both the entire population was considered and when only adult deer mice were considered demonstrates that these relationships remain constant despite variations in population age structure.

Synthesis

Collectively, our results indicate that there is a negative relationship between SNV prevalence and habitat disturbance. Although habitat disturbance appears to influence both SNV prevalence and deer mouse population density, we found no direct relationship between prevalence and density. Rather, our results indicate that maintenance of SNV over time requires long-term persistence of deer mice and, in particular, of infected individuals. Our results suggest that disturbed habitats may not support this long-term persistence. In a broader sense, our findings underscore the intricacies of population demography and habitat quality in pathogen ecology, demonstrate the complexity of predicting prevalence in the natural environment and underscore the need for more large-scale multiple-year studies.

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