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Original Contribution

Testing Mechanisms of the Dilution Effect: Deer Mice Encounter Rates, Sin Nombre Virus Prevalence and Species Diversity

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Abstract: Species diversity has been shown to decrease prevalence of disease in a variety of host–pathogen systems, in a phenomenon termed the Dilution Effect. Several mechanisms have been proposed by which diversity may decrease prevalence, though few have been tested in natural host-pathogen systems. We investigated the mechanisms by which diversity influenced the prevalence of Sin Nombre virus (SNV), a directly transmitted virus in deer mice (*Peromyscus maniculatus*). We monitored both intra and interspecific encounters of deer mice using foraging arenas at five sites in the Great Basin Desert with disparate levels of species diversity to examine two potential mechanisms which may contribute to the dilution of SNV prevalence: (1) reduced frequency of encounters between deer mice, or (2) reduced duration of contacts between deer mice. We also investigated the relationship between deer mouse density and these mechanisms, as density is often predicted to influence both inter and intraspecific encounters. Results of our study indicate that frequency of intraspecific interactions between deer mice was reduced with increased diversity. Species diversity did not impact average duration of encounters. Density was correlated with absolute, but not relative rates of encounters between deer mice, suggesting that encounters may be influenced by factors other than density. Our study indicates that species diversity influences the dynamics of SNV by reducing encounters between deer mice in a trade-off between intra and interspecific interactions.

Keywords: deer mice, Dilution Effect, encounter rate, hantavirus, Sin Nombre virus, species diversity

Introduction

Species diversity has been shown to decrease the prevalence of infectious disease in a variety of host–pathogen systems. This phenomenon, termed the Dilution Effect, has been documented for animal pathogens such as *Borrelia burgdorferi* (Ostfeld and Keesing, 2000) and Laguna Negra virus (Yahnke et al., 2001), as well as several plant pathogens (Knops et al., 1999; Mitchell et al., 2002, 2003). Several, non-mutually exclusive mechanisms have been proposed by which species diversity may decrease pathogen prevalence (Keesing et al., 2006). For example, the presence of other species in a community may reduce intraspecific encounters between hosts, resulting in fewer opportunities

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for pathogen transmission. Alternatively, species diversity may reduce transmission probability if the presence of other species reduces the duration of intraspecific encounters between hosts. Species diversity may also reduce prevalence by reducing host survival (Keesing et al., 2006). Although several mechanisms have been proposed, few of these potential mechanisms have been tested empirically in natural host–pathogen systems (Keesing et al., 2006). Identifying the significance of mechanisms underlying the Dilution Effect is critical for understanding and predicting the dynamics of many infectious agents in natural host populations.

We investigated the mechanisms through which species diversity influenced the prevalence of Sin Nombre virus (SNV), a directly transmitted disease in deer mice (*Peromyscus maniculatus*). Other *Peromyscus* species (Otteson et al., 1996) and the desert woodrat (*Neotoma lepida;* Dearing et al., 1998) may serve as secondary SNV reservoirs. SNV transmission between rodents is primarily by direct contact, presumably during aggressive interactions that involve biting and scratching, as evidenced by the strong correlation between SNV infection and scarring (Mills et al., 1997, 1999; Boone et al., 1998; Calisher et al., 1999). SNV infection in rodents is chronic, and survival of certain age classes appears to be reduced (Douglass et al., 2001).

Previous studies have suggested that SNV dynamics follow the Dilution Effect, as increased species diversity was associated with reduced SNV prevalence (Mills, 2005; Clay, 2007) [Clay, in review]. Likewise, our previous work found that increased species diversity reduced deer mouse density and survival, which may directly or indirectly reduce SNV prevalence (Clay, 2007) [Clay, in review]. Studies of deer mouse behavior suggest that SNV prevalence may also be altered by reduced encounters between deer mice. For example, deer mice shift microhabitat use to avoid encounters with other rodent species, including kangaroo rats (*Dipodomys* spp.) and pocket mice (*Perognathus parvus*) that do not host SNV, and they avoid pinyon mice (*P. truei;* Kritzman, 1974; Ambrose and Meehan, 1977; Llewellyn, 1980; Larsen, 1986; Llewellyn and Jenkins, 1987; Falkenberg and Clarke, 1998) that are putative secondary reservoirs for SNV. When encounters between deer mice and other species occur, they often result in fighting and other aggressive interactions (Kritzman, 1974; Ambrose and Meehan, 1977; Llewellyn, 1980; Falkenberg and Clarke, 1998). Shifts in deer mouse behavior or microhabitat use may reduce SNV prevalence if deer mice have more interspecific encounters and fewer intraspecific encounters. Species diversity may also influence SNV transmission probability, if hosts spend less time in contact with one another when encounters occur (Keesing et al., 2006). Laboratory studies suggest that SNV is transmitted less efficiently than other hantaviruses (Botten et al., 2002); this inefficiency may be exacerbated and prevalence may be negatively affected if the duration of intraspecific contact between deer mice is reduced by the presence of other species.

To investigate the mechanisms underlying the relationship between species diversity and SNV prevalence, we monitored both intra and interspecific encounters of deer mice in the Great Basin Desert using foraging arenas at five study sites with varying levels of species diversity. The primary goal of our study was to examine whether species diversity reduced SNV prevalence by either of two specific mechanisms: (1) reduced frequency of encounters between deer mice, or (2) reduced duration of contacts between deer mice (Keesing et al., 2006). We also investigated the relationship between deer mouse density and these two mechanisms, as density is often predicted to influence both inter and intraspecific encounters (Anderson and May, 1979; Arneberg et al., 1998). We also sought to identify the species that deer mice most frequently encountered, apart from conspecifics. We predicted that with increased species diversity, deer mice would have more encounters with non-SNV hosts, such as Ord's kangaroo rat (*Dipodomys ordii*) or Great Basin pocket mouse (*Perognathus parvus*). Understanding deer mouse interactions with other rodent species may provide further insight about the role of species diversity in the prevalence of SNV.

Methods

Study Sites

Deer mice were surveyed in a mark-recapture study conducted at five sites near the West Tintic Mountains in the Great Basin Desert of central Utah (Juab County), on lands administered by the US Department of Agriculture and the Bureau of Land Management. To maintain independence and avoid inter-site migration, all sites were located >700 m apart. Study sites were dominated by big sagebrush (*Artemisia tridentata*) and, to a lesser extent, Utah juniper (*Juniperus osteosperma*).

Rodent Sampling

Rodent sampling occurred in "Spring" (May, June) and "Fall" (September, October) of 2005 during 15-day periods that coincided with the new moon. For three nights at each site, rodents were live-trapped using 148 traps (H.B. Sherman Traps, Inc., Tallahassee, FL) distributed in a "web" configuration over 3.1 hectares, following the methods of Mills et al. (1999). This trapping regime is standardly used in studies estimating SNV prevalence (Mills et al., 1999) and community surveys of rodents (Brown, 1998).

Upon capture, rodents were identified to species and we recorded the sex, weight, and reproductive condition of each individual. All captured rodents were marked with uniquely numbered ear tags (National Band and Tag, Co., Newport, KY), and uniquely coded Passive Integrated Transponder (PIT) tags (BioMark, Inc., Boise, ID) were injected subcutaneously between the scapulas. Approximately 0.2 ml of blood was drawn via the retro-orbital sinus from all deer mice at the time of initial capture for each sampling period. Blood was stored immediately on dry ice until transfer to an −80°C freezer. After processing, all rodents were released at the point of capture. All workers implemented precautions for handling animals potentially infected with hantavirus (Mills et al., 1995), and all techniques for capturing and handling animals were approved by the Institutional Animal Care and Use Committee of the University of Utah (IACUC #0203011, #0503011).

SNV Antibody Detection

Enzyme linked immunosorbent assays (ELISA) were used to screen for IgG antibodies to SNV. Because deer mice produce virus-specific IgG antibodies continuously after infection with SNV, presence of antibodies is a reliable indicator of SNV infection (Borucki et al., 2000; Botten et al., 2003; Safronetz et al., 2005).

In this process, wells of polyvinyl chloride microtiter plates (Dynatech Laboratories, Chantilly, VA) were coated overnight at 4°C with recombinant nucleocapsid antigen diluted 1:2000 in phosphate buffered saline (PBS). A non-hantavirus recombinant antigen was used as a negative control. After incubation, unbound antigen was removed from wells by washing three times with wash buffer. Deer mouse sera were heat inactivated by placing in a 55°C water bath for 30 min. Heat inactivated sera was diluted 1:100 in serum-dilution buffer, containing powdered nonfat milk, Tween 20, and 10× PBS in a 1:1:20 ratio. The diluted sera solution was and added to the antigen-coated wells and plates were then incubated at 37°C for 60 min. Plates were then washed three times with wash buffer (1:20 Tween and 10× PBS) and incubated at 37°C for 30 min with 100 µl of ABTS Microwell Peroxidase Substrate Solution (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD; Borucki et al., 2000). Absorbance (405 nm) was recorded with a Versa Max Tunable Microplate Reader (VWR International, West Chester, PA) and values >3 SD of the negative control wells contained on each plate were considered positive for anti-SNV antibodies (Borucki et al., 2000).

Species Diversity and Density Estimates

Rodent capture data were used to calculate species diversity and density on each study site. Species diversity was estimated using Gini-Simpson Index ($D = 1 - \sum p_i^2$; Gini, 1912). For all statistical comparisons, deer mouse densities were estimated at each site using the program DISTANCE, a regularly used program for estimating density, particularly when employing trapping webs (version 4.1; Thomas et al., 2004). We also estimated the density of all rodents on a per area basis (no./hectare) for each species at all sites, since some of the non-deer mouse species were few in number, and DISTANCE estimates are not reliable with small sample sizes (Buckland, 2001; Lehmer et al., 2007). These data were not used for statistical analysis, but rather were included strictly for informational purposes.

Approximating Encounters at Foraging Arenas

Encounters between deer mice and with other marked rodents were approximated at the study sites by using foraging arenas equipped with PIT antennae and data loggers (FS2001FT-ISO, Biomark, Inc., Boise, ID), powered by 14.1-volt batteries. Time constraints combined with limited PIT equipment meant that sites were sampled consecutively, not simultaneously, over the course of 1 month. Within 1 week of mark-recapture sampling, 12 foraging arenas were placed at each site for three nights. Arenas were placed throughout the site in locations where deer mice had been captured during the mark-recapture study. At the start of each night, foraging arenas were filled with of 2 L of fine grain sand (Jurassic Playsand, Salt Lake City, UT) mixed with 6 g of certified weed-free millet in a round plastic tray (30 cm diameter). As millet comprised $<1\%$ of volume, rodents had to actively forage for seed in the sand. Millet seeds are comparable in size to seeds that naturally occur in the Great

Basin (Crist and Friese, 1993) and the stocking density in the arenas was consistent with the density of seeds in the seed bank (Allen and Nowak, 2008). Furthermore, seeds were often present in the morning indicating that there were other food sources available. Arenas were closed at sunrise and millet was sifted out each morning.

PIT antennae were placed below the foraging arenas with data loggers to continuously record the identity and time of each visiting individual. After recording rodent arena visits for three consecutive nights, data from the loggers were downloaded onto a laptop computer. From these data, we determined which individuals visited arenas, documented potential encounters between individuals (defined as the presence of two individuals at a foraging arena within <15 s of one another), as well as the duration of each event at arenas.

We quantified encounters between rodents in two ways. We estimated unique encounters between rodents at foraging arenas, which we estimated as the number of distinct individuals each mouse encountered regardless of the number of times they encountered one another. We used these data to determine if the probability of intraspecific encounter between deer mice was altered with species diversity. We also estimated the relative rate of intraspecific deer mouse encounters at each site by looking at the total number of encounters that occurred between rodents, which did not account for unique pairs or the duration of the encounter.

Statistical Analysis

We investigated the relationship between species diversity and the absolute rate of intraspecific encounter between deer mice using logistic regression. In this model, the dependent variable was unique deer mice encounters, and each was binomially coded as intra or interspecific. Species diversity and deer mouse density (based on density estimates calculated using program DISTANCE) were continuous independent factors.

We also evaluated the effect of species diversity on relative rates of intraspecific encounters by comparing the proportion of intraspecific deer mouse encounters at each site to species diversity, using generalized linear modeling (GLMM). In this analysis, we used the proportion of intraspecific deer mouse encounters (number of intraspecific deer mouse encounters/total intra and interspecific encounters) because preliminary examination of the data revealed large variation in the absolute number of intra and interspecific contacts across sites. Although there were more intraspecific encounters at low diversity sites compared to high diversity sites, there was a sevenfold difference in absolute contacts among the sites with low diversity; thus, we used proportions to standardize encounters particularly among low diversity sites for the GLMM. Species diversity and deer mouse density were continuous fixed effects, whereas site was treated as a random effect. In this model, we specified a normal distribution for the dependent variable.

To determine the influence of species diversity on the duration of encounters, we examined the average duration of intraspecific encounters of deer mice at study each site. Using GLMM, deer mouse density and species diversity were fixed effects, and site was treated as a random effect. As the average duration of encounters was not normally distributed, we specified a gamma distribution in this model.

Using general linear mixed modeling (LMM), we examined the relationship between SNV prevalence and reduced host encounters, as well as reduced duration of encounters. In separate analyses, we compared proportion of intraspecific encounters between deer mice and the average duration of deer mouse encounters to SNV prevalence. Site was entered as a random effect in each model. Variables in all statistical models were considered statistically significant if $P \leq 0.05$, and of biological interest if $P > 0.05$ but <0.10.

Results

Species Diversity, Density, and SNV Prevalence

Over the course of our study, we sampled 585 rodents, 300 of which were deer mice (*P. maniculatus*). We captured 285 individuals of seven other rodent species including: Ord's kangaroo rat (*Dipodomys ordii*), pinyon mouse (*P. truei*), Great Basin pocket mouse (*Perognathus parvus*), desert woodrat (*Neotoma lepida*), western harvest mouse (*Reithrodontomys megalotis*), sagebrush vole (*Lemiscus curtatus*), and northern grasshopper mouse (*Onycomys leucogaster*).

Species diversity varied considerably across sites and sampling periods, with Gini-Simpson Index D values ranging from 0.20 to 0.75 (Table 1). Low diversity communities ($D = 0.20$–0.28) consisted of three species, with deer mice comprising the majority of the population ($>80\%$). The highest diversity community ($D = 0.75$) had seven species, including deer mice ($<36\%$ of total). Deer mice were

Table 1. Species Diversity (D), Deer Mouse Density, and SNV Prevalence for All Sites Sampled in "Spring" (May, June) and "Fall" (September, October) 2005, in Juab County, Utah[a]

Site ID	Sampling period	Species diversity (D)	Deer mouse density	SNV prevalence
3	Spring	0.63	11.5	0.39
	Fall	0.67	12.5	0.12
4	Spring	0.37	19.0	0.51
	Fall	0.58	21.0	0.20
5	Spring	0.70	14.5	0.10
	Fall	0.74	18.0	0.20
16	Spring	0.28	8.0	0.19
	Fall	0.20	28.0	0.07
19	Spring	0.75	8.5	0.05
	Fall	0.74	13.5	0.07

[a]Species diversity was calculated using the Gini-Simpson Index (D). Deer mouse densities were estimated using the program DISTANCE, and do not match the untransformed data in Table 2. Deer mouse densities estimated by DISTANCE were entered in all models with density in statistical comparisons. SNV prevalence was estimated for deer mice at each site, in each season (number of antibody-positive deer mice/number deer mice captured).

Table 2. Density of All Rodent Species at All Sites Sampled in "Spring" (May, June) and "Fall" (September, October) 2005, in Juab County, Utah[a]

Site ID	Sampling period	Species density (no./ha)						
		Deer mice (*Peromyscus maniculatus*)	Ord's kangaroo rat (*Dipodomys ordii*)	Sagebrush vole (*Lemiscus curtatus*)	Desert woodrat (*Neotoma lepida*)	Great Basin pocket mouse (*Perognathus parvus*)	Pinyon mouse (*Peromyscus truei*)	Western harvest mouse (*Reithrodontomys megalotis*)
3	Spring	7.42	0	2.26	0	3.55	0.97	0
	Fall	8.39	0	2.26	0	10.00	0.32	2.58
4	Spring	12.58	0	1.61	0	1.61	0.65	0
	Fall	13.23	0	2.26	0	7.42	0	0.97
5	Spring	9.35	1.94	0.32	4.84	1.61	2.26	0
	Fall	11.29	2.90	0	6.77	2.9	3.23	0.65
16	Spring	5.16	0	0.32	0	0	0.65	0
	Fall	18.39	0	0.97	0	1.29	0	0
19	Spring	5.48	2.26	0	4.19	1.94	1.29	0
	Fall	8.71	1.61	0.32	6.77	5.16	0.32	0.65

[a]For the purpose of comparison, densities were estimated as total number of animals per species captured at each site divided by the area (3.1 ha). DISTANCE was not used due to small sample sizes.

numerically dominant at all sites across both Spring and Fall sampling periods with one exception; at one site (Site 3), deer mice were the second most abundant species after Great Basin pocket mice during the Fall sampling period. Deer mouse density ranged from 8.0 to 28.0 deer mice per hectare as estimated by DISTANCE and from 5.2 to 18.4 when not transformed by DISTANCE (Table 1). Density of non-deer mice species ranged from 0 to 10 rodents/ha (not DISTANCE transformed; Table 2). SNV prevalence in deer mice ranged from 5.9 to 51.3% across sites and sampling periods (Table 1).

Encounters at Foraging Arenas

The number of unique intraspecific deer mouse encounters at foraging arenas ranged from 0 to 75 across all sites and

sampling periods. We found that species diversity did influence that absolute rate of encounter between deer mice, as the probability of intraspecific encounter between deer mice decreased significantly with increased species diversity (Figure 1a, Logistic regression, coefficient = −0.278, SE = 0.083, df = 1, $P = 0.008$). In this analysis, we also found that the probability of intraspecific encounter between deer mice increased with deer mouse density (Figure 1b, Logistic regression, coefficient = 1.260, SE = 0.429, df = 1, $P = 0.003$).

Of all the encounters observed at the arenas, species diversity also affected the relative rates of encounters between deer mice. The proportion of intraspecific deer mouse encounters, which ranged from 0 to 100%, was negatively correlated with increased species diversity (Figure 2a; GLMM, $t = -4.23$, $P = 0.02$). In this case, there was no significant effect of deer mouse density (Figure 2b, GLMM, $t = -1.07$, $P = 0.36$). For the random term, site, variance component = 0.000052 (SE ± 0.000049).

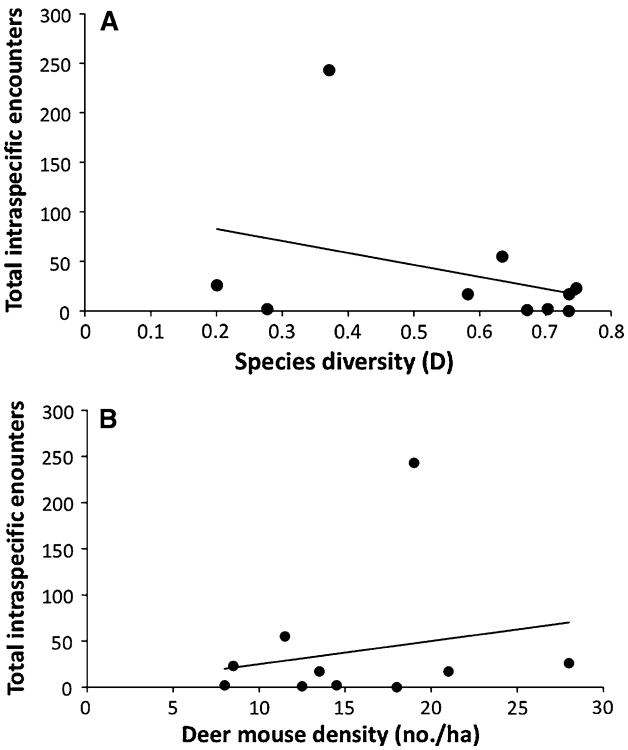

Figure 1. The absolute rate of intraspecific encounter between deer mice was negatively correlated with species diversity (**a:** Logistic regression, coefficient = −0.278, SE = 0.083, df = 1, $P = 0.008$). In this analysis, we also found that the probability of intraspecific encounter between deer mice increased with deer mouse density (**b:** Logistic regression, coefficient = 1.260, SE = 0.429, df = 1, $P = 0.003$). Sites were sampled in "Spring" (May, June) and "Fall" (September, October) 2005, in Juab County, Utah.

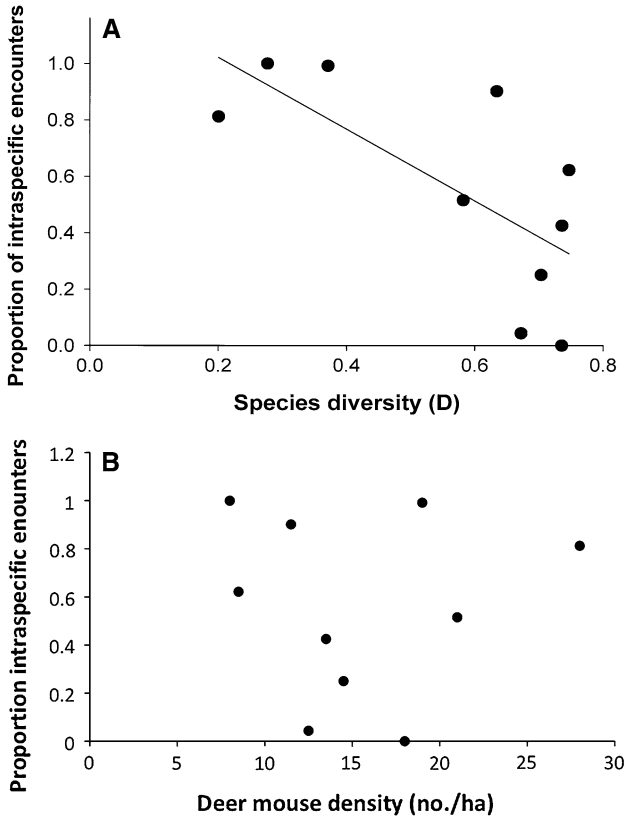

Figure 2. The relative rate of intraspecific encounter between deer mice (number of intraspecific deer mouse encounters/total intra and interspecific encounters) was negatively correlated with species diversity (**a:** GLMM, $t = -4.23$, $P = 0.02$). In this case, there was no significant effect of deer mouse density (**b:** GLMM, $t = -1.07$, $P = 0.36$). For the random term, site, variance component = 0.000052 (SE ± 0.000049). Sites were sampled in "Spring" (May, June) and "Fall" (September, October) 2005, in Juab County, Utah.

Duration of Encounters

The average duration of encounters between deer mice ranged from 0 to 47.9 s across sites and sampling periods. There was no significant effect of species diversity (GLMM, $t = 1.03$, $P = 0.41$) or deer mouse density (GLMM, $t = 2.18$, $P = 0.16$) on the average duration of intraspecific encounters between deer mice. For the random term, site, variance component = 0.48 (SE ± 0.27).

Intraspecific Encounters and SNV Prevalence

Sites with a greater proportion of intraspecific encounters had higher SNV prevalence (GLIM, estimate = 0.27, $Z = 2.99$, $P < 0.01$). For the random term, site, variance component = 0.005 (SE ± 0.003). The average duration of intraspecific deer mouse encounters (GLIM, estimate = 0.002, $Z = 0.57$, $P = 0.57$) was not correlated with SNV

Table 3. Identity and Frequency of Deer Mouse Intra and Interspecific Encounters across All Sites Sampled in Spring and Fall, 2005, Juab County, Utah[a]

Species	Deer mouse (*Peromyscus maniculatus*)	Great Basin pocket mouse (*Perognathus parvus*)	Ord's kangaroo rat (*Dipodomys ordii*)	Sagebrush vole (*Lemiscus curtatus*)	Pinyon mouse (*Peromyscus truei*)
No. unique contacts with deer mice	116	18	5	5	1

[a]Great Basin pocket mouse, Ord's kangaroo rat, and sagebrush vole do not host SNV.

prevalence. For the random term, site, variance component = 0.02 (SE $\pm$ 0.01).

Interspecific Encounters

The majority of deer mouse encounters with non-conspecifics at foraging arenas occurred with Great Basin pocket mice ($N = 18$), followed by Ord's kangaroo rats ($N = 5$) and sagebrush voles ($N = 5$; Table 3). We observed one encounter between a deer mouse and a pinyon mouse.

Discussion

The principal objective of our study was to examine two mechanisms by which species diversity reduced SNV prevalence: either by reducing the frequency of encounters between deer mice or by reducing duration of contacts between deer mice. The results of our study indicate that intraspecific interactions between deer mice were inversely related to changes in species diversity, as the absolute and the relative rates of intraspecific deer mouse encounters were lower at sites with greater diversity. However, our findings also suggest that the presence of other species did not alter the average duration of intraspecific deer mouse encounters. While deer mouse density was positively correlated with the absolute rate of encounter between deer mice, it was not correlated with the relative rate or the average duration of deer mouse encounters. Our study highlights the complex nature of intra and interspecific interactions in the rodent community and the importance of understanding the mechanisms by which diversity influences pathogen prevalence in natural host populations.

Lower SNV prevalence was correlated with fewer intraspecific encounters between deer mice, as prevalence was lower at sites with a lower proportion of intraspecific deer mouse encounters. This pattern is consistent with the predictions of the Dilution Effect hypothesis, as species diversity has the potential to reduce pathogen prevalence in natural populations by reducing encounters between hosts (Keesing et al., 2006). In communities of low species diversity, deer mice encounter one another frequently, increasing the opportunity for SNV transmission. In contrast, our study suggests that deer mice have approximately half as many encounters with one another in high diversity communities; our results show that these conditions also result in deer mice having a larger number of interspecific encounters, primarily with individuals that do not host SNV.

Previous work suggested that SNV is transmitted between deer mice less efficiently than other hantaviruses (Botten et al., 2002). As such, we predicted that the presence of other species would reduce the transmission probability of SNV by reducing the duration of deer mouse encounters. Contrary to our predictions, species diversity did not influence the duration of intraspecific interactions, as measured by the average duration of deer mouse contacts. We interpret these findings to indicate that diversity does not alter the manner in which deer mice interact with other deer mice, i.e., the presence of other species does not decrease the length of the interactions between deer mice.

It was noteworthy that neither the relative rates nor the duration of encounters were altered by changes in deer mouse density, suggesting that the patterns we observed were not due to simply fewer deer mice at high diversity sites. This result implies that in our study system, encounters between deer mice were not strongly density-dependent. The lack of density-dependence may imply that deer mice encounters, and thereby SNV dynamics, were more frequency-dependent. However, the data do not support this interpretation fully. In a frequency-dependent system, hosts are thought to have a relatively fixed number of encounters per unit time. In this situation, greater species diversity would increase interspecific encounters between hosts and non-hosts, thereby decreasing intraspecific encounters between hosts (Keesing et al., 2006). In

our system, although the probability of intraspecific encounters decreased with high diversity, and deer mice had more encounters with species that do not host SNV, the number of interspecific encounters was low over all and did not compensate for the reduction in intraspecific encounters. Thus, the presence of other species appears to markedly alter the behavior of deer mice in the foraging arenas. In these diverse communities, deer mice are at the lower end of the size distribution and thus may be forced to forage in less desirable microhabitats. Scent marking by other species at the trays could, in part, be responsible for this change in behavior. For example, Great Basin pocket mice emit a pungent odor (noticeable to humans) that deer mice may detect and use to avoid interactions with pocket mice.

While our study indicates that deer mouse behavior is influenced by the presence of other species, we did not find a particular demographic group of deer mice to alter its behavior more than any other. Specifically, male and female deer mice both had encounters at foraging areas with other species, and neither group altered its behavior more than the other at higher levels of species diversity. We also did not find patterns suggesting that deer mice of particular age classes were more influenced by other species than the rest of the population. In the future, a manipulative study could examine whether sex, body mass, or other demographic factors influence deer mouse responses to the presence of other species.

An increase in interspecific encounters does not necessarily result in a reduction in pathogen transmission. Prevalence can increase or remain consistent with increased species diversity, when interspecific encounters are with species that can also host the pathogen (Amplification Effect hypothesis; Keesing et al., 2006). If the majority of interspecific encounters for deer mice were with another putative SNV host, such as pinyon mice or desert woodrats, diversity could have increased SNV prevalence. However, our observations at the foraging arenas indicate that the majority of interspecific encounters that deer mice had were with Great Basin pocket mice and Ord's kangaroo rats, species that are not reservoirs for SNV. Thus, observations of encounters at the foraging arenas are consistent with predictions of the Dilution hypothesis in that, as diversity increases, the number of encounters with hosts decreases. The encounter results are also consistent with the observed decrease in SNV prevalence in higher diversity communities (Mills, 2005; Clay, 2007).

Because we documented encounters between rodents at foraging arenas, our study may reflect phenomena unique to foraging behavior, rather than being reflective of overall rodent interactions. However, confrontations involving food resources are likely opportunities for SNV transmission, as deer mice have been shown to fight aggressively over food resources (King, 1957; Healey, 1967). Encounters during foraging are also a more persistent opportunity for SNV transmission since foraging is a daily behavior, whereas disputes over mates and nest resources vary temporally. Several studies have documented overlap in food resources between deer mice and sympatric rodent species (Douglas, 1969; Brown et al., 1979), suggesting that interspecific confrontations are likely to occur over food resources as well. While our study may not capture all of the possible interactions between rodents, it likely captures a substantial and ongoing aspect of intra and interspecific encounters.

We acknowledge the correlative nature of this study and its shortcomings. Since our study was conducted in nature with free-ranging populations of rodents, we were unable to control certain for factors. For example, although we marked rodents for three nights prior to making our observations, we could not control for unmarked animals entering the study site or potential differences in trappability between species. Furthermore, we were unable to manipulate the system through the addition and removal of species. Thus, despite finding that at highly diverse sites deer mice had fewer interactions with conspecifics and more interspecific interactions, we cannot rule out that the reduction in conspecifics encounters was due to other factors. It is possible that the differences in intraspecific encounter rates at high and low diversity sites resulted from factors that drive the differences in the species communities, such as habitat structure. Future studies with access to outdoor enclosures (deer mice infected with SNV cannot be held indoors except in BSL 4 level containment) could experimentally manipulate communities, such that density and diversity could be controlled, and all individuals in the community could be tracked. Despite the fact that our study lacked such control, one of its strengths is that it represents natural ecological conditions for free-ranging deer mice and other rodent species, their interactions, and the possible transmission of SNV.

Results of our study support the Dilution Effect hypothesis, as they suggest that species diversity may have widespread influence on pathogen dynamics. Our work specifically demonstrates that species diversity can play a

critical role in the dynamics of Sin Nombre virus by reducing the frequency of contacts between disease hosts. Species diversity does not appear to reduce prevalence by reducing the duration of contact when encounters between deer mice do occur. This study also indicates that encounters between deer mice, and therefore SNV transmission, are more likely to be frequency-dependent. As predicted by the Dilution Effect, this suggests that SNV prevalence may be reduced when deer mice trade-off intraspecific encounters that transmit disease for interspecific encounters that do not. This study highlights the complexity of intra and interspecific interactions of wild rodents, and emphasizes the importance of such interactions on the dynamics of pathogen transmission in the natural environment.

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