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Behavioural differences: a link between biodiversity and pathogen transmission

Laurie Dizney*, M. Denise Dearing

Department of Biology, University of Utah, Salt Lake City, UT, U.S.A.

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Biodiversity often serves to reduce zoonotic pathogens, such that prevalence is lower in communities of greater diversity. This phenomenon is termed the dilution effect, and although it has been reported for several pathogens (e.g. Sin Nombre virus, SNV), the mechanism is largely unknown. We investigated a putative mechanism, by testing the hypothesis that higher biodiversity alters behaviours important in pathogen transmission. Using deer mice (*Peromyscus maniculatus*) and SNV as our host–pathogen system, and a novel surveillance system, we compared host behaviours between high- and low-diversity communities. Behaviours were observed on foraging trays equipped with infrared cameras and passive integrated transponder (PIT) tag readers. Deer mice inhabiting the more diverse site spent less time in behaviours related to SNV transmission compared to deer mice from the less diverse site. The differences were attributed to the composition of behavioural phenotypes ('bold' versus 'shy') on the sites. Bold deer mice were 4.6 times more numerous on the less diverse site and three times more likely to be infected with SNV than shy deer mice. Our findings suggest that biodiversity affects pathogen transmission by altering the presence of different behavioural phenotypes. These findings have implications for human health and conservation.

Biodiversity is being lost at an unprecedented rate (Pimm & Raven, 2000). Along with this loss comes the loss of ecosystem services that intact ecosystems provide (Cardinale et al., 2012). One such ecosystem service is pathogen regulation. The negative correlation between biodiversity and pathogen prevalence has been termed the dilution effect (Ostfeld & Keesing, 2000a, 2000b), and has been best studied with respect to Lyme disease in white-footed mice, *Peromyscus leucopus* (Keesing et al., 2010; LoGiudice et al., 2008; Ostfeld & LoGiudice, 2003; Schauber, Ostfeld, & Evans, 2005). Recent research suggests that the dilution effect applies to many host–pathogen systems (Carlson, Dyer, Omlin, & Beier, 2009; Ezenwa, Godsey, King, & Guptill, 2006; Johnson, Lund, Hartson, & Yoshino, 2009; Thieltges, Bordalo, Caballero-Hernandez, Prinz, & Jensen, 2008), including several rodent-borne hantaviruses (reviewed in Khalil et al., 2014). A consistent picture has emerged that loss of biodiversity leads to increased pathogen prevalence and incidence of human disease (Civitello et al., 2015; Keesing et al., 2010), although discussion remains at what scale it applies (Salkeld, Padgett, & Jones, 2013; Wood & Lafferty, 2013). Although the mechanism underlying the dilution effect has been elucidated for Lyme disease (Keesing et al., 2009, 2006), for most other pathogens it is unknown. Moreover, the mechanism is likely different for vectored pathogens (e.g. Lyme disease) than for directly transmitted pathogens, such as hantaviruses, where behaviour potentially plays a key role.

We investigated transmission dynamics of Sin Nombre hantavirus (SNV) by studying the effect of community diversity on the behaviour of deer mice (*Peromyscus maniculatus*), the natural host of SNV (Childs et al., 1994; Nichol et al., 1993). Transmission of SNV between hosts is hypothesized to be through aggressive behaviour, based on the strong correlation between scarring and infection found in several studies (Boone et al., 1998; Calisher, Sweeney, Mills, & Beaty, 1999; Douglass et al., 2001; Mills, Ksiazek, Peters, & Childs, 1999). In order for SNV to be transmitted then, two events must occur: (1) an infected deer mouse must encounter an uninfected deer mouse and (2) an aggressive act must take place. If community diversity were to affect either of these events, then transmission would be altered.

Documenting the behaviour of hosts with respect to disease transmission is challenging on many fronts. First, behaviour is inherently difficult to study in natural settings; however, this approach is necessary because behaviours change when animals

* Correspondence and present address: L. Dizney, 5000 N Willamette Blvd, Portland, OR 97203, U.S.A.
E-mail address: dizney@up.edu (L. Dizney).

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are brought into a laboratory setting (Calisi & Bentley, 2009). Second, the majority of zoonotic pathogens are hosted by rodents (Woolhouse & Gowtage-Sequeria, 2005), which are small and often nocturnal, making direct observations challenging. Third, behaviours involved in transmission are likely rare events, further complicating the problem. To overcome these issues, we developed and deployed a novel field surveillance system to observe rodent behaviour unaffected by human presence. Our system integrates passive integrated transponder (PIT) technology, which uniquely identifies each individual, with infrared video surveillance. In this way, the identity of each individual captured on video, as well as any demographic data collected during PIT tag insertion, is known.

The primary goal of our research was to investigate a putative mechanism of the dilution effect (i.e. whether host behaviour differs with community complexity). Previous research revealed that SNV prevalence is lower in communities with greater diversity (Clay, Lehmer, St Jeor, & Dearing, 2009; Dizney & Ruedas, 2009; Mills et al., 1997; Root et al., 2005). We predicted that reduced diversity would increase intraspecific encounter probability ('encounter behaviour') and aggressive interactions in deer mice. Clay, Lehmer, Previtali, St Jeor, and Dearing (2009) found that the largest individuals within a population engage in the most contacts. Other investigators discovered that large males (Calisher et al., 1999; Douglass, Calisher, Wagoner, & Mills, 2007; Douglass et al., 2001; Mills et al., 1999) and deer mice in reproductive condition (Clay, Lehmer, Previtali, et al., 2009; Douglass et al., 2001; Mills et al., 1997) have higher infection prevalence than the population at large, suggesting increased encounter and aggressive behaviour. We therefore examined demographic differences (average mass and proportion of male and reproductive deer mice) between sites to see whether they explained behavioural differences. We predicted that large, reproductive and SNV-positive male deer mice would show the highest levels of encounter behaviour.

METHODS

Small Mammal Sampling

Our study sites were located in Juab County, Utah, on Bureau of Land Management property within the Great Basin Desert. Vegetation was largely big sagebrush, *Artemisia tridentata*, and Utah juniper, *Juniperus osteosperma*. This research was part of a 10-year study on SNV in deer mouse populations conducted across 12 sites (Clay, Lehmer, Previtali, et al., 2009; Clay, Lehmer, St Jeor, et al., 2009). For the present study, we chose two of the 12 sites that varied the most with respect to a diversity index (Shannon H': 0.93 versus 1.37, $t = 10.69$, $P = 7.73 \times 10^{-26}$). We visited each site in May, July and September of 2010 and 2011. We trapped and marked rodents on both sites simultaneously over 3 nights, but we monitored behaviour separately for each site, spending 4 nights on each site. Trapping and monitoring were done around the new moon because desert rodents are known to decrease foraging and total activity as illumination (moonlight) increases (Falkenberg & Clark, 1998; Kotler, 1984).

Small mammals were trapped for 3 consecutive nights per sampling period using a web sampling design consisting of 148 Sherman live-traps over a 3.14 ha area (Mills, Childs, Ksiazek, Peters, & Velleca, 1995). Traps were left open from dusk to dawn, and checked each morning for captures. Data collected included species, mass, sex and reproductive status. For SNV analysis, a blood sample (0.1–0.2 ml) was taken upon initial capture of each visit from the retro-orbital sinus of all rodents except Great Basin pocket mouse, *Perognathus parvus*, and Ord's kangaroo rat, *Dipodymys ordii*, which are not known to be SNV reservoirs (Childs et al., 1994). In fact, no rodent species other than deer mice were found to be SNV-positive in this study. Before bleeding, the eye was anaesthetized with one drop of Proparacaine HCl 0.5% ophthalmic solution to minimize possible pain associated with bleeding. The amount of blood taken was equal to or less than 1% of the body mass of any captured individual, following established guidelines (http://oacu.od.nih.gov/ARAC/documents/Rodent_Bleeding.pdf). Only experienced researchers performed the bleeding, which generally took about 30 s. The eye was then gently squeezed shut to stop bleeding (usually less than 5 s) and monitored again when the rodent was released. Retro-orbital bleeding is the standard method of blood collection in hantavirus studies because (1) it leaves no external wound that could lead to later infection, (2) it is fast, which minimizes handling time and stress to the rodent and (3) it gives the high-quality sample required for SNV testing (http://oacu.od.nih.gov/ARAC/documents/Rodent_Bleeding.pdf). Occasionally (in 4/155 captures), we found a deer mouse with a nonfunctioning eye, which we attributed to our bleeding method. Three of the four deer mice were subsequently recaptured, which suggested to us that they could still find food and defend their territories; the fourth deer mouse was captured during our last trapping event, and thus we never had an opportunity to recapture it. Once blood was collected, blood samples were put on dry ice until transfer to an −80 °C freezer. Enzyme-linked immunosorbent assays (ELISA) were used to detect SNV-specific IgG antibodies in the blood samples (Feldmann et al., 1993). Although SNV infection is a chronic infection, viremia is sporadic and brief (Botten et al., 2000, 2003) and therefore difficult to measure. In contrast, IgG antibodies are produced for life after infection with SNV (Botten et al., 2000), and thus, ELISA is the standard method of assessing infection status. Before release, all rodents were marked with a uniquely numbered PIT tag (TX1400ST, BioMark, Inc., Boise, ID, U.S.A.) injected just below the skin between the scapulae with a sterile, 12-gauge needle. The tags were encased in glass to prevent tissue irritation, were 12 mm long and weighed 0.06 g (approximately 0.2–0.6% of the weight of any captured individual). Because of its small size and the fact that PIT-tagged rodents were recaptured at the same rate as non-PIT-tagged rodents (approximately 30%), we think it unlikely that the PIT tags caused changes in behaviour. The only issue we encountered with PIT-tagged rodents was that, in approximately 2.5% of them, the tag came out. When the study was over, the tags were left in the rodents because of the low survivorship of rodents across seasons (~14%; Lehmer, Clay, Pearce-Duvet, St Jeor, & Dearing, 2008) and the invasive techniques necessary for removal. This research complied with the Institutional Animal Care and Use Committee of the University of Utah (IACUC no. 0802012) and the ASAB/ABS Guidelines for the Use of Animals in Research. Additionally, all workers followed guidelines for working with animals potentially infected with SNV (Mills et al., 1995).

Deer Mouse Surveillance

After 3 nights of trapping and marking animals, nine surveillance stations, each 50 m apart in a 3×3 grid, were distributed throughout the same area. Each surveillance station consisted of a foraging tray (30 cm diameter) over a PIT tag antenna. A foam ring encircled the tray and acted as a ramp. The antenna was connected to a data reader (FS2001FT-ISO, Biomark, Inc.), which was powered by a 12 V battery. The reader stored data from PIT-tagged rodents on or within a 0.5 m radius of the trays so that identification, arrival and departure times, and the presence of multiple individuals were known. In addition, an infrared video camera (MESSOA, Model SCR351-HN1), mounted 1 m above ground on a metal pole, was directed at the foraging tray and connected with an above-ground cable to a centrally located computer. The computer was powered by a generator (EU 1000, Honda) and stored the video imagery (four

frames/s). Software from TimeScience™ integrated the video and reader data such that the identity and behaviour of each rodent was coordinated with its demographic data and infection status. Cameras and readers were operated from dusk to shortly after dawn. We intended to conduct surveillance for 4 nights at each site during each visit. However, due to hazardous weather and road conditions, on two occasions surveillance was conducted for 3 nights per site.

The foraging trays were filled with 3 g of millet seed mixed into 2 litres of sand. Presence of this food source was unlikely to change the behaviour of any rodent visitors for several reasons. First, the amount and size of the seed was comparable to that naturally present (Allen & Nowak, 2008; Christ & Friese, 1993). Second, animals had to actively forage to obtain seed, as they would under natural conditions. Finally, on most trays there was seed left in the morning, indicating the presence of equally, or more, productive food sources.

Each night half of the trays were placed under sagebrush, and termed 'protected'. The other half of the trays were at least 1 m away from any sagebrush, and termed 'exposed'. The trays were switched each night, such that any one tray spent half the nights in a covered position and half the nights in an exposed position. For each tray, protected and exposed positions were no more than 2 m apart.

Community and Population Analysis

We determined density (per 3.14 ha) by averaging the number of initial deer mouse or other rodent captures per season per site. The number of rodents on trays was the total number of all unique rodents, including deer mice, which visited each tray each night. The number of deer mice on trays was a measure of unique deer mice that visited each tray each night. Trays that were not visited by any rodents were not included in either measure. We also evaluated demographic characteristics (average mass and proportion of males and reproductive individuals) for deer mice. Since we could not control for pregnancy, mass was compared between males only. A deer mouse was considered to be reproductive if the testes were scrotal (males) or the vagina was perforate and/or nipples were enlarged (females). Proportions were calculated as the number of male or reproductive deer mice captured on each site divided by the total number of deer mice captured on the site. Small mammal diversity was measured using the Shannon index ($H' = \sum p_i \log p_i$) and compared between sites as shown in Brower, Zar, and von Ende (1997). SNV prevalence was determined for the 2-year study period by dividing the number of infected deer mice by the total number of deer mice captured per site. Continuous data (deer mouse and other rodent density, number of rodents or deer mice on trays, male deer mouse mass) were compared between sites with a Student's *t* test. Prevalence and proportions were analysed with a chi-square binomial proportion test.

Behavioural Analyses by Site

Most deer mice were observed only during the 4-night surveillance period directly following their capture; for these deer mice, behaviours were summed over the 4 nights, giving one value per behaviour per 4-night surveillance period. To account for pseudoreplication, behaviours of deer mice observed in more than one surveillance period (recaptures) were averaged across the two surveillance periods, giving a single value per deer mouse per behaviour. Behaviours were then compared between sites. We could not analyse behaviours on a finer scale (i.e. seasonally) because of the small number of deer mice observed on the trays in some seasons.

The following behaviours were deemed to increase the probability of encountering other deer mice: more time on trays, a higher tray-by-night index, greater distance travelled and a higher exposed tray index. Time on trays was the average amount of time a deer mouse spent on a tray during a visit. We created a tray $\times$ night index (number of unique trays visited $\times$ number of nights the deer mouse was observed during a 4-night surveillance period) to account for the small number of each in a surveillance period (nine trays and 4 nights). We assumed that the greater the distance a deer mouse travelled, the more likely it was to encounter another deer mouse, and we approximated this value by adding the linear distance between consecutively visited trays. The first tray visited each night received a value of 1 m. If the same tray was visited several times successively, the distance for each visit was considered 1 m, which is the minimum distance that a deer mouse would have to travel to be out of range of the cameras and readers. Therefore, these values represent the minimum distance travelled. Our previous work documented more intraspecific encounters on exposed trays compared to protected trays (chi-square proportion test: 0.0015 versus 0.0009, $P = 0.023$). We believe this is due to exposed trays being more visible and offering fewer covered escape options than trays under sagebrush. We were interested in both the total amount of time deer mice spent on exposed trays as well as the proportion of total tray time this represented. To account for both, we created an exposed tray index: (exposed time/total time) $\times$ exposed time.

The encounter behaviours (more time on trays, higher tray $\times$ night index, greater distance travelled per surveillance period and a higher exposed tray index) were based on data from the PIT tag antennae and readers. Aggression was assessed from video data. An aggressive interaction was defined by either a chase or a fight between two deer mice. Chasing included any pursuit of a deer mouse by another with no observed contact, whereas fighting was characterized by aggressive contact. Due to generator failure, video data was approximately one-third that of reader data and therefore, aggression was determined from 24 free-ranging individuals (compared to 70 unique deer mice detected on the trays with the PIT tag readers). The video data was collected from portions of 16 nights from both sites over the 2 years.

We also analysed indirect measures of predator avoidance behaviour. One indirect measure of predation risk is 'giving-up density' (GUD), which represents the amount of food at which an animal stops foraging because its harvest rate no longer exceeds the costs of foraging, the missed opportunities costs (not participating in alternative activities) and the risks of predation (Brown, 1988). In other words, an animal should forage until the benefits of foraging no longer exceed the costs. To calculate GUD, seed remaining in a tray was sieved out each morning and weighed, and then a new 3 g was added to the tray. Only trays where a deer mouse was the last forager (31% of trays) were included in the GUD analysis (Brown, 1988), and were averaged per site. Vigilance is another indirect measure of predation risk (Lima & Bednekoff, 1999). We defined vigilance as a deer mouse circling a tray or being on a tray but not feeding and with the head up, presumably looking for other animals. Time in vigilance was assessed from video data and averaged per site. All behaviours were compared between sites with a Student's *t* test.

Behavioural Analysis by Individual

We analysed the four encounter behaviours for which we had data on all animals (time on trays, tray $\times$ night index, distance travelled and exposed tray index) with a principal components analysis (PCA). Based on the PCA analysis, we categorized deer mice on a bold–shy axis (Wilson, Clark, Coleman, & Dearstyne,

1994). All four variables were first normalized using a log transformation.

We examined the relationship of boldness to SNV infection status and demographic data using logistic regression with binomial errors and the logit link function. Mass, sex and reproductive condition were the demographic variables included in the full model. The model was simplified by stepwise (backward) elimination using analysis of deviance and chi-square statistics.

All statistics were performed in R (R Development Core Team, 2006). Differences were considered statistically significant if $P \leq 0.05$.

RESULTS

A total of 155 uniquely tagged individuals visited the trays, with deer mice making up 45% ($N = 70$) of the visitors. Of the 70 deer mice, 38 were captured on the more diverse site and 32 on the less diverse site. Eight deer mice were recaptures, or observed during two surveillance periods, versus 62 seen in a single surveillance period. All eight recaptures had the same SNV and reproductive status for both surveillance periods.

Over 44 nights, we collected more than 3000 h of data from the PIT tag readers and approximately 1000 h of video data. Tagged deer mice were on the trays approximately 55 h, or 1.7% of the total time that trays were available. Other species visiting the trays were *P. parvus* ($N = 76$), pinyon mouse, *Peromyscus truei* ($N = 5$), and western harvest mouse, *Reithrodontomys megalotis* ($N = 4$). Together, the three other species spent an additional 81 h on the trays, or 2.6% of the total time that trays were available. Based on our observations, deer mice appear to be generally solitary rodents. Most of the tray time involved deer mice foraging alone. Of the encounters between deer mice captured on video (24 encounters, or 0.8% of total video time), 13 were aggressive interactions (chasing or fighting), 10 involved one animal avoiding the other, and one involved two animals sharing the tray.

Community and Population Analysis

The two sites were chosen based on the difference in diversity of small mammals for the 8 years previous to this study. During the 2 years of our study, the less diverse site continued to have significantly lower H' (Table 1). On the less diverse site, we captured deer mice ($N = 89$), *P. parvus* ($N = 53$) and *R. megalotis* ($N = 13$). On the diverse site, we captured deer mice ($N = 66$), *P. parvus* ($N = 97$), *R. megalotis* ($N = 15$), *P. truei* ($N = 34$) and *Dipodomys ordii* ($N = 3$). Despite differences in diversity, the two sites shared many similar characteristics. There was no statistical difference in density of deer mice or other rodents between the two sites, or in the total number of animals or deer mice that visited the trays (Table 1). The demographics of the two populations of deer mice were also similar. There was also no difference in the average size of deer mice or in the proportions of males or reproductive individuals (Table 1). Despite these similarities, SNV prevalence was four times higher on the less diverse site (Table 1).

Behavioural Analysis by Site

Deer mice on the less diverse site exhibited increased encounter behaviour compared to deer mice on the more diverse site (Table 2). They occupied the trays about 2.4 times longer per visit, had a tray × night index that was 1.8 times higher, travelled at least three times farther per surveillance period and spent almost 5.8 times more time on exposed trays than deer mice on the more diverse site (Table 2). In addition to increased encounter behaviour, deer mice on the less diverse site also engaged in five times the number of aggressive interactions compared to deer mice on the diverse site. However, this finding was not significant (Table 2), probably due to the small number of aggressive interactions captured on camera ($N = 13$).

There were also differences between sites in predator avoidance behaviours. Deer mice on the less diverse site had lower GUD (Table 2), meaning they stayed on the trays and continued harvesting until significantly less seed was left compared to the diverse site. Deer mice on the less diverse site also spent less time in vigilance behaviour when compared to deer mice on the more diverse site (Table 2).

Behavioural Analysis by Individual

Based on PCA, deer mice were categorized as 'shy' or 'bold'. PCA is especially useful when the variables, in our case behaviours, are likely to be correlated. This redundancy allows PCA to reduce the original variables into a smaller number of artificial variables called principal components (PC). The PCs are ordered such that PC1 is the combination of original variables that explains the largest amount of variation in the original data. In our data set, PC1 included all four behaviours and accounted for 72.3% of the variation, so we subsequently considered only PC1 in the categorization of animals (Table 3). Within PC1, each animal was assigned a single score, with a score of 0 being average. Fifty-seven deer mice were categorized as shy (<1 SD above average) and comprised the majority of the population on both the less diverse and more diverse sites (71.1% and 93.8%, respectively). Deer mice exhibiting the highest levels of encounter behaviour (>1 SD above average) were considered bold. The bold group consisted of 11 deer mice (28.9%) from the less diverse site and two deer mice (6.2%) from the more diverse site,

Table 1
Comparison of site characteristics using Student's *t* test (density, number of animals and deer mice on trays and Shannon H') or chi-square binomial proportion test (all proportions and Sin Nombre hantavirus, SNV, prevalence)

	Less diverse	More diverse	t or χ^2	P
Site similarities				
Deer mouse density (per 3.14 ha)[a]	14.1±2.5	11.0±2.7	0.85	0.41
Other rodent density (per 3.14 ha)[a]	11.0±4.0	19.1±13.7	1.40	0.19
Number of rodents on trays[a]	3.03±0.22	2.50±0.23	1.44	0.15
Number of deer mice on trays[a]	2.04±0.12	1.74±0.15	1.45	0.15
Male deer mouse mass (g)[a]	18.9±0.55	18.6±0.75	0.35	0.73
Proportion of males	0.60	0.53	0.14	0.70
Proportion of reproductive deer mice	0.79	0.75	0.01	0.92
Site differences				
Shannon H'	0.87	1.26	8.87	<0.001
SNV prevalence in deer mice	0.36	0.09	5.88	0.01
Proportion of bold deer mice	0.29	0.06	4.51	0.03

[a] Mean ± SE.

Table 2
Comparison of deer mouse behaviours between sites using Student's t test (all encounter behaviours and GUD) or chi-square binomial proportion test (proportion of time vigilant)

	Less diverse	More diverse	t or χ^2	P
Encounter behaviours and aggression				
Time on trays (s)[a]	102.9±17.3	43.6±8.9	2.83	0.006
Tray×night index[a]	8.8±1.6	4.9±0.9	2.02	0.048
Distance travelled (m)[a]	381.3±99.6	124.4±48.5	2.15	0.035
Exposed tray index (s)[a]	650.4±238.7	112.8±78.7	1.94	0.054
Aggressive interactions[a]	1.08±0.38	0.20±0.20	1.45	0.163
Predator avoidance behaviours				
GUD (g)[a]	0.81±0.07	1.29±0.17	2.99	0.003
Proportion of time vigilant	0.04	0.07	57.8	<0.001

GUD: giving-up density.
[a] Mean ± SE.

Table 3
Loadings and total proportion of variance for the first principal component (PC1) from a principal components analysis based on four behaviours considered important in pathogen transmission in deer mice

Behavioural variables	PC1
Total tray time	0.570
Tray×night index	0.612
Distance travelled	0.198
Exposed tray index	0.511
Total proportion of variance	0.723

leading to a 4.6-fold disparity in the proportion of bold deer mice between sites ($\chi^2_1 = 4.51$, $P = 0.034$; Table 1).

In the logistic regression characterizing bold deer mice, only positive SNV status remained in the final model (odds ratio = 6.00; 95% confidence interval = 0.51–3.12; $P = 0.006$). Bold deer mice were three times more likely to be infected than shy deer mice (46.1% versus 15.7%). Mass, sex and reproductive status did not improve the fit of the model and were therefore excluded.

DISCUSSION

Greater levels of biodiversity are often associated with lower incidences of pathogen infection. A putative cause for this pattern is that host behaviour is impacted by community complexity such that hosts engage less often in behaviours that transmit pathogens in more diverse communities compared to less diverse communities. We found heterogeneities in the behaviours of deer mice with respect to community diversity. Our findings suggest that community complexity affects behaviours that impact transmission dynamics of SNV. On average, there were higher levels of encounter (bold) behaviour on the less diverse site than the more diverse site. Boldness and positive SNV status were significantly associated, supporting a behavioural basis to transmission.

An alternate interpretation to our findings is that bold behaviour is a result of SNV infection rather than the cause. Many pathogens have been shown to directly alter behaviour through adaptive manipulation (Brown, 2005; Thomas et al., 2005). A good example is the killifish (*Fundulus parvipinnus*), which exhibits conspicuous behaviours when infected with larval trematodes, and which, in turn, makes the killifish more susceptible to predation by birds, the final host (Lafferty & Morris, 1996). Behaviour can also be manipulated indirectly in a variety of ways, including foraging efficiency, altered time budgets and predator avoidance (Barber, Hoare, & Krause, 2000). Hantaviral manipulation of the host has not received much attention. One study on male Norway rats, *Rattus norvegicus*, infected with Seoul hantavirus suggests that infection increases aggression in males (Klein, Zink, & Glass, 2004). However, two studies on SNV suggest that infection is the consequence of increased encounter behaviours and not the cause (Clay, Lehmer, Previtali, et al., 2009; Dizney & Dearing, 2013).

We recognize that these inferences are based on one high- and one low-diversity site and as such the interpretations are limited. The cost of this single surveillance system (>$60 000 USD) and the effort involved in surveillance (team of four people for 11 nights per site per season) prohibited adding additional sites to our study. Thus, the replication in this study is at the level of the surveillance station and not at the level of the site. Instead, we feel the limitations to our study are in part mitigated by both the vast quantity of data and the large degree of behavioural differences found between the two sites.

There could be factors other than diversity leading to behavioural differences between sites, including density. However, deer mouse density did not differ significantly between sites, nor did the number of deer mice on the foraging trays. The presence or number of competitors on the trays might also promote behavioural differences, as deer mice are known to avoid certain species of rodents (Ambrose & Meehan, 1977; Falkenberg & Clarke, 1998; Larsen, 1986; Llewellyn & Jenkins, 1987). However, there was no difference between the sites with respect to the number of individuals of other species on the trays. We analysed mass as an indicator of overall health (Fairbairn, 1977), which could potentially alter behaviour, and we found no difference between sites. Males have been shown to be more aggressive (Wolff, 1989), and a higher proportion of males in a population would likely be reflected in changes in average behaviour. Reproductive condition has also been shown to modify behaviour by increasing aggressiveness in both male and female deer mice (Wolff, 1989). Yet there was no difference in the proportion of males or reproductive individuals between the sites. Abiotic differences probably do not play a role either; both sites were part of the Great Basin ecosystem, less than 25 km apart and at similar elevations (1707 and 1768 m), and as such, had similar vegetation and weather regimes. Based on previous research, the two sites have similarly low levels of anthropogenic disturbance and openness (Clay, Lehmer, Previtali, et al., 2009; Clay, Lehmer, St Jeor, et al., 2009). There may be other factors affecting deer mouse behaviour that we did not measure. However, we consider the two sites to be similar in most aspects, suggesting that the source of behavioural heterogeneity is community diversity.

On average, deer mice on the less diverse site engaged in higher levels of all encounter behaviours measured. However, this difference was not the result of increased boldness across all individuals in the population. Rather, the difference was driven by the greater number, both in actual and relative terms, of bold deer mice on the less diverse site. In fact, when all 13 bold deer mice were removed from the analysis, none of the four behaviours differed between sites (data not shown; $P > 0.15$ for all). In humans, different personality types are recognized, where bold people act boldly in most, if not all, situations. A large number of studies have found 'personalities' in a wide array of species, from monkeys to ants (Gosling, 2001). In nonhuman populations of animals, suites of correlated behaviours have been termed 'behavioural syndromes', with individuals showing a behavioural phenotype, such as bold or shy (Sih, Bell, & Johnson, 2004). Bold individuals in our study were more likely to be infected with SNV than shy deer mice, suggesting that increased encounter behaviour increases pathogen transmission. Counter to our predictions, bold deer mice were not identifiable based on size, sex or reproductive status.

Behavioural phenotypes can be a product of genes and/or the environment (Sih & Bell, 2008). Considerable research suggests that behavioural types are heritable and linked to fitness (reviewed

in Réale, Reader, Sol, McDougall, & Dingemanse, 2007), which implies limited plasticity (Sih et al., 2004). If behavioural phenotypes are genetically based, then biodiversity could affect SNV transmission by selecting for different behavioural phenotypes. For example, in a less diverse ecosystem, boldness appears to increase access to high-quality resources (more time on the foraging trays), which in turn could increase fitness. Therefore, a bold phenotype would be selected for, and since bold individuals have increased encounter behaviour, SNV transmission would increase. Conversely, in a more diverse ecosystem, boldness would be selected against; if more predators and more types of predators are found as diversity increases, then increased encounter behaviours would also increase the risk of predation. The resulting community of mostly shy individuals, whose behaviour decreases the probability of encountering other deer mice, would dilute SNV prevalence. Alternatively, if behavioural phenotypes are a product of environmental experience, then communities of varying complexity should have different make-ups of behavioural types. A gene*environment interaction is also possible (Bell & Sih, 2007; Carere, Welink, Drent, Koolhaas, & Groothuis, 2001). Regardless of whether behavioural phenotypes are genetically or environmentally based, decreased diversity appears to allow a bold type to succeed, whereas increased diversity appears to suppress it.

While we did not directly assess predation risk, our findings suggest that it could be a strong selective or environmental force on behavioural phenotypes. Giving-up density (GUD) is an indicator of costs of foraging, missed opportunities and predation risk (Brown, 1988). Given that our foraging trays had the same amount and type of seed and substrate, and that we were comparing the same species at the same time of year in similar populations, foraging and missed opportunity costs should have been the same between sites. An interpretation of the higher GUD on the diverse site is that it reflects an increased risk of predation (Brown, 1988). Furthermore, as predation risk increases, vigilance should increase concomitantly with GUD (Brown, 1999). We found increases in both GUD and vigilance on the diverse site, further supporting predation as a possible force acting on behavioural phenotypes.

Our study suggests that behavioural heterogeneity is a potential mechanism underlying the dilution effect. Specifically, low diversity appears to promote bolder behavioural phenotypes, which have increased encounters with conspecifics. These increased encounters lead to increased pathogen transmission, not only among bold individuals but also among the shy deer mice with which they interact. Our results could have implications for both pathogen transmission and conservation. Maintaining biodiversity could limit the behavioural phenotypes responsible for the majority of SNV transmission, thus potentially decreasing SNV prevalence and the risk to humans.

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