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DISEASE ECOLOGY

Heterospecific competitors and seasonality can affect host physiology and behavior: key factors in disease transmission

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Abstract. Ecological and environmental factors can influence the transmission of infectious diseases. They can accomplish this via effects on host susceptibility and exposure to infection, which are governed by host physiology and behavior, respectively. To better inform disease control, more information is needed about how extrinsic factors affect physiological and behavioral processes that determine transmission. We investigated how heterospecific competitors and seasonality may influence host susceptibility and intraspecific contact rates using a directly transmitted disease system, the North American deer mouse (*Peromyscus maniculatus*)—Sin Nombre hantavirus (SNV) system. In grasslands of western Montana, USA, deer mice compete with dominant voles (*Microtus* spp.) and shrews (*Sorex* spp.) and experience a seasonal temperate climate. Higher SNV transmission occurs primarily during spring/summer, when changes in physiology and behavior may serve as influential contributors. We hypothesized that (1) voles, and to a lesser extent shrews, will induce chronic stress, suppress immunity, and may change contact rates of deer mice; and (2) during spring/summer, deer mice may experience chronic stress, suppressed immunity, and higher contact rates, which may help explain the reported seasonality in SNV transmission. Over two years, we trapped small mammals at four grids in western Montana. Deer mice were sampled for feces and blood and evaluated for scar numbers, demography, and body condition scores (BCSs). We evaluated stress physiology with fecal corticosterone metabolites (FCMs), neutrophil/lymphocyte (N/L) ratios and BCSs, immunity with white blood cell (WBC) counts, and contact rates with scar numbers. We found that shrew density was negatively associated with stress response FCMs, suggestive of chronic stress. Additionally, although complex interactions existed, shrew and vole densities were negatively associated with BCSs, but differentially with scar numbers. N/L ratios were higher in spring/summer, whereas WBC counts were lower in summer, suggestive of chronic stress and suppressed immunity, respectively. Our results suggest that (1) heterospecific competitors may differentially influence disease transmission via stress physiology and contact rates, and that (2) chronic stress, suppressed immunity, and higher contact rates may help explain why higher SNV transmission has been previously reported during spring/summer in Montana. Our findings may extend to other directly transmitted disease systems.

Key words: animal behavior; fecal glucocorticoid metabolites; generalized linear regression trees; rodent-borne zoonosis; wildlife infectious disease.

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Introduction

Globally, emerging infectious diseases are causing wildlife populations to decline and species to go extinct (Daszak et al. 1999, Williams et al. 2002, Smith et al. 2006). Extrinsic (i.e., ecological and environmental) factors may drive a rise in disease emergence, because they can influence two key determinants of disease transmission. These include host susceptibility and exposure to infection, which are governed by host physiology and behavior, respectively (Hawley et al. 2011). Therefore, to better inform wildlife conservation and disease management, it is imperative that we identify physiological and behavioral mechanisms that link extrinsic factors to disease transmission so we can better understand and predict their impacts on wildlife disease dynamics.

Extrinsic factors may impact host physiology via glucocorticoid (GC) hormones, crucial regulators of the stress response. Although an acute rise in GCs from a stressor is considered adaptive, persistently elevated GCs (i.e., chronic stress) are maladaptive because they can lower immunity and increase susceptibility to infection (Dickens and Romero 2013, Dantzer et al. 2014). Stress physiology is typically evaluated not only with blood GCs but also with metabolized GCs in feces called fecal cortisol/corticosterone metabolites (FCMs) (Palme 2019). When vertebrates experience chronic stress, their baseline FCMs can rise and their stress response to an acute challenge (i.e., stress response FCMs) can decline (Busch and Hayward 2009). However, GC secretion patterns can be highly variable, which can make interpretation of hormone data a cumbersome task. Therefore, it is best practice to employ several measures that span GC metabolism (i.e., downstream effects of GCs) to attain a more comprehensive evaluation of stress physiology (Breuner et al. 2013, Dantzer et al. 2014).

There are various downstream measures of GCs (reviewed in Breuner et al. 2013). For example, because GCs increase neutrophils and decrease lymphocytes in the peripheral circulation, individuals under chronic stress are expected to have higher neutrophil/lymphocyte (N/L) ratios (Davis et al. 2008), while they are expected to have lower body condition scores (BCSs) from GC-induced breakdown of fat and muscle reserves (Sapolsky et al. 2000). However, the effect of GCs on immunity is much less straightforward because the components of the immune system (e.g., humoral vs. cellular) may respond differently to chronic stress (Sapolsky et al. 2000). Despite this inherent complexity, total white blood cell (WBC) counts are largely expected to decline with chronic stress (Martin 2009).

Heterospecific competitors may influence host susceptibility and exposure to infection. In particular, competitors may increase host susceptibility to infection via GCs. For example, Santicchia et al. (2018) found that invasive gray squirrels (*Sciurus carolinensis*) increased baseline FCMs in Eurasian red squirrels (*Sciurus vulgaris*), and Narayan et al. (2015) showed that invasive cane toads (*Rhinella marina*) decreased body condition in Fijian ground frogs (*Platymantis vitiana*). Therefore, interspecific competition can influence stress physiology and, presumably, host immunity. Additionally, heterospecific competitors may alter host behavior, thereby affecting exposure to infection. For example, Glass and Slade (1980) found that prairie voles (*Microtus ochrogaster*) avoided space that was used by reproductive cotton rats (*Sigmodon hispidus*), and Gutman and Dayan (2005) found that common spiny mice (*Acomys cahirinus*) inhibited the nocturnal activity of golden spiny mice (*Acomys russatus*). Taken together, heterospecific competitors may influence disease transmission via both host physiology and behavior.

Environmental factors that fluctuate seasonally, such as food availability and ambient temperature, may influence stress physiology, immunity, and behavior, which may explain why disease prevalence can vary seasonally in wildlife hosts (Altizer et al. 2006). Particularly in temperate habitats, seasonal environmental factors can affect stress physiology and immunity of wildlife, which may have ramifications for disease transmission (Nelson and Demas 1996). In fact, most vertebrates undergo seasonal changes in baseline and stress-induced GCs, with higher baseline GCs expected during the breeding period, although this can be species-specific (Reeder and Kramer 2005, Romero et al. 2008). Similarly, vertebrates undergo seasonal changes in immunity. For example, birds and small mammals enhance their immunity in winter (Martin et al. 2008). Wildlife can also exhibit seasonal changes

in behavior that may affect disease transmission. For example, by aggregating in flocks during winter, house finches may experience seasonal exposure to *Mycoplasma gallisepticum* (Altizer et al. 2004), and by engaging in aggressive interactions during their breeding season, bank voles (*Myodes glareolus*) may experience seasonal exposure to Puumala hantavirus (Escutenaire et al. 2002). In summary, seasonality in environmental factors may impact disease transmission via host physiology and behavior.

To examine the physiological and behavioral links between extrinsic factors and mechanisms affecting disease transmission, we conducted an observational study in grasslands of western Montana, USA, with the North American deer mouse (*Peromyscus maniculatus*; hereafter deer mouse), the reservoir host for the directly transmitted Sin Nombre hantavirus (SNV). This is an appropriate system for examining how physiology and behavior may link extrinsic factors to transmission for two main reasons. Firstly, there has been considerable research regarding the "diluting" effect of species diversity on disease risk (i.e., dilution effect; sensu Keesing et al. 2006) across rodent–hantavirus systems (e.g., Suzán et al. 2009, Dearing et al. 2015, Khalil et al. 2016). However, the dilution effect observed may not be due to diversity per se but due to ecological processes, such as interspecific competition between host and nonhost species, which may happen to correlate with diversity (Johnson et al. 2015). Regardless, we still require more information about the physiological and behavioral mechanisms behind the dilution effect that may be applicable to other directly transmitted disease systems (Rubio et al. 2017, Luis et al. 2018). Secondly, although seasonality has been extensively studied as a crucial driver for disease dynamics across rodent–hantavirus systems (Luis et al. 2015, Voutilainen et al. 2016), potential physiological and behavioral mechanisms have received comparatively less attention. Therefore, we used the deer mouse–SNV system as a model to investigate how ecological and environmental factors affect physiological and behavioral mechanisms of disease transmission.

At our study site, deer mice experience a temperate climate with strong seasonality and coexist with voles (*Microtus* spp.) and shrews (*Sorex* spp.). This study system allowed us to examine how heterospecific competitors (i.e., voles and shrews) and seasonality were associated with measures of deer mouse physiology (i.e., stress physiology and immunity) and behavior (i.e., contact rates), which has implications for SNV transmission. Given that demography, parasites, and habitat may influence physiology and behavior, they were also accounted for in our analyses (Dantzer et al. 2014).

Voles are considered dominant competitors of deer mice. For example, Grant (1971) found that meadow voles excluded deer mice from grasslands through aggressive interactions. However, we found no evidence to suggest that shrews are also dominant over deer mice, and because of their much smaller size, we reasoned that they are most likely less dominant. Consequently, we hypothesized that dominant voles will induce chronic stress, depress immunity, and/or alter contact rates of deer mice, with shrews having a lesser effect (Table 1). We did not have a priori hypotheses as to how contact rates may be altered because previous field studies generated inconsistent findings (Clay et al. 2009, Rubio et al. 2017, Luis et al. 2018).

In Montana, SNV transmission is typically highest during spring/summer, which coincides with the breeding season of deer mice during which they will engage in aggressive intraspecific encounters, leading to the accumulation of scars (Douglass et al. 2001, Bagamian et al. 2012). Field studies from nearby Idaho suggest that stress physiology of deer mice varies seasonally given that deer mice were found to have lower FCMs during fall than summer (Harper and Austad 2001). Deer mouse immunity may also vary seasonally as indicated through laboratory experiments where deer mice under short-day conditions (i.e., mimicking winter conditions in North America) had higher WBC counts compared with long-day conditions (Blom et al. 1994). Given all the evidence above, we also hypothesized that deer mice will experience chronic stress, suppressed immunity, and higher contact rates during spring/summer (Table 1).

Methods

Study system

Deer mice are the primary reservoir for SNV in North America (Childs et al. 1994). At grasslands

Table 1. List of hypotheses for how heterospecific competitors and seasonality will associate with stress physiology, immunity, and contact rates of deer mice, with corresponding predictions and empirical support for each measured variable.

Hypothesis	Metric	Prediction	Support?
Heterospecific competitors	↑ Stress	↑ Baseline FCMs	No
		↓ Stress response FCMs	Yes (shrews)
		↑ N/L ratio	No
		↓ BCS	Yes (both)
	↓ Immunity	↓ WBC counts	No
	- or Δ contacts	- or Δ scar numbers	Yes Δ (both)
Spring/ summer seasons	↑ Stress	↑ Baseline FCMs	No
		↓ Stress response FCMs	No
		↑ N/L ratio	Yes (both)
		↓ BCS	No
	↓ Immunity	↓ WBC counts	Yes (summer)
	↑ Contacts	↑ Scar numbers	Yes (summer)

Notes: Seasonality hypotheses received support if there was consistency across both years. Heterospecific competitors include voles and, to a lesser extent, shrews. BCS, body condition score; FCMs, fecal corticosterone metabolites; N/L ratio, neutrophil/lymphocyte ratio; WBC, white blood cell.

in western Montana, deer mice coexist with few other small mammals, which include meadow (*Microtus pennsylvanicus*) and montane voles (*Microtus montanus*), and vagrant (*Sorex vagrans*) and montane shrews (*Sorex monticolus*; Carson et al. 2006). None of these small mammals are considered to be competent SNV hosts (Mills et al. 2010).

Variables measured

We evaluated stress physiology with four measures that span GC metabolism: (1) baseline FCM levels, (2) stress response FCM levels (FCMs after overnight trap confinement minus baseline), (3) N/L ratio, and (4) BCS. Immunity was evaluated with total WBC counts. We used number of scars to evaluate contact rates because positive correlations between the presence of scars and SNV infection in deer mice have been found (e.g., Douglass et al. 2001). Despite its limitations, we thought number of scars was suitable for two reasons: (1) SNV is primarily directly transmitted via bites that can result in scars (Mills et al. 1999, Warner et al. 2019), and (2) it allowed us to incorporate a measure of contact rates, which are notoriously difficult to quantify in the field.

Site description and livetrapping

Four 1-ha grids were established, where each grid had 100 trap stations 10 m apart in a 10 × 10 array (Kuenzi et al. 2001). These were located at the Ninepipe Wildlife Management Area, Montana, USA, and were at least 1600 m away from each other. The most common grasses were intermediate wheatgrass (*Agropyron intermedium*), smooth brome (*Bromus* sp.), and timothy grass (*Phleum pratense*) (see Supporting Information for more grid descriptions).

Small mammals were livetrapped from November 2016 to August 2018. Grids A and B were trapped from October to November 2016, February to December 2017 (excluding June and July for A, and July for B), and March to July 2018. Grids C and D were trapped from October to November 2017 and March to August 2018. We trapped two grids concurrently at a time (i.e., grids A and B together, grids C and D together) once a month for three nights. We initially removed voles from grid B by euthanasia in August 2017–April 2018, so we could assess removal effects on deer mice (Vole *N* removed = 25). However, we considered this approach to be unsuccessful because voles were trapped consistently despite removal. Although removal may have influenced vole densities, we still typically trapped voles most often at this grid (Fig. 1). Therefore, we still had enough variation in vole densities to address our competitor hypotheses. We speciated euthanized or dead voles by examining their dentition; meadow voles have an extra cusp on their upper-middle molar (Hall and Kelson 1959). We did not speciate shrews but, most likely, they were vagrant and montane shrews (Carson et al. 2006).

We baited non-folding Sherman live traps (H. B. Sherman, Tallahassee, Florida, USA) with peanut butter and oats and supplied them with polyester bedding. Traps were opened around dusk and checked approximately four hours later when trap-induced stress is less likely to influence baseline FCMs (Harper and Austad 2001, Eleftheriou et al. 2020). Voles and shrews were released after processing, whereas deer mice were returned to their traps so they could

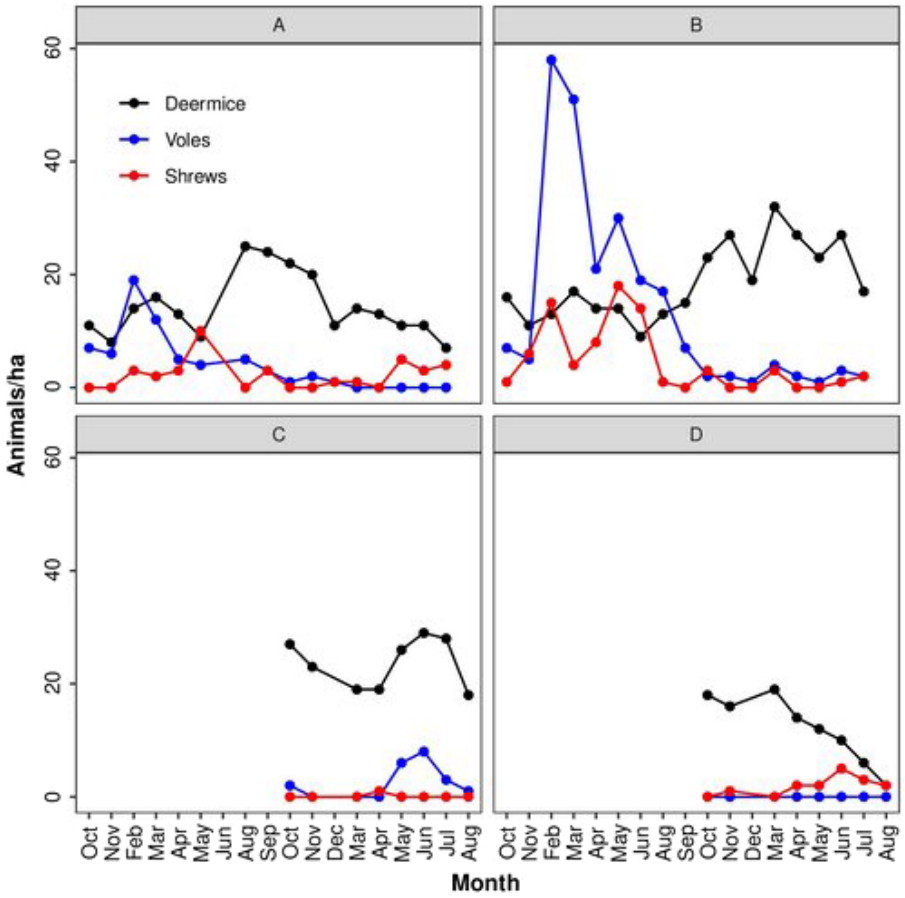

Fig. 1. Small mammal densities for each grid (A–D) across study period (October 2016 to August 2018). Deer mouse and vole densities were estimated using Minimum Number Alive index. Shrew densities were estimated using unique captures per trapping session.

be processed again around dawn before releasing on site. This allowed evaluation of the FCM response to an acute stressor (i.e., trap confinement; Eleftheriou et al. 2020).

Animal sampling

At the initial check, traps that contained animals were taken to a central station for processing. We tagged deer mice with metal ear tags (National Band and Tag, Newport, Kentucky, USA), collected feces, and returned them to their traps until around dawn, which is when we collected more feces and blood, and also weighed them. Sex, presence of fleas, and reproductive status were also noted. A BCS was estimated between one minus and five plus by palpation of tissue at the base of the tail, with five plus being extremely obese (Ullman-Culleré and Foltz 1999). BCS estimation was performed by the same experienced investigator throughout the entire course of the study. We also examined deer mice for scars, which we counted, if present. We assigned deer mice to four scar categories starting in April 2017: (0) no scars, (1) ≤4, (2) 5–8, and (3) ≥9 scars. Age was estimated from weight (juveniles < 14 g, subadults 14–17 g, and adults >17 g; Fairbairn 1977). Voles were ear-tagged, weighed, sexed, and evaluated for BCS and reproductive status. Given that shrews cannot be ear-tagged, we began marking them in April 2017 with a permanent marker. Because recaptures of marked shrews were rare (two recaptured April 2017–August 2018), we are confident we did not count the same animals twice within

the same trapping session. Active reproductive status in female rodents was determined via the presence of a perforate vagina, pregnancy, and/or lactation, and in male rodents via the presence of scrotal testes. Blood was collected with heparinized capillary tubes (Fisher Scientific, Pittsburgh, Pennsylvania, USA) from the retro-orbital capillary sinus, after topical anesthesia with proparacaine (Akorn, Lake Forest, Illinois, USA). Blood and feces were immediately frozen. We followed safety guidelines for working with animals potentially infected with hantavirus (Mills et al. 1995). All animal procedures were approved by the Institutional Animal Care and Use Committee (027-16ALDECS-051016) at the University of Montana, Missoula, MT. Land access was granted by Montana Fish, Wildlife and Parks.

FCM analyses

We heated feces in a laboratory oven within a biosafety cabinet at ~63°C for two hours to inactivate any SNV (J. N. Mills, *personal communication*) and reach constant weight. Dried feces were ground into powder, and 0.040 (±0.005) g was weighed out for extraction. One ml of 80% methanol was added to powdered samples, vortexed for 30 min at 1500 rpm, and centrifuged for 20 min at ~2500 g (Eleftheriou et al. 2020). Supernatants were frozen until analyses. Because corticosterone is the main glucocorticoid in *P. maniculatus* (e.g., Bradley and Terman 1981), we quantified FCMs using a corticosterone enzyme immunoassay (EIA) after supernatants were diluted (typically 1:80). We followed manufacturer's instructions (Assay Designs, Ann Arbor, Michigan, USA) but also used a reference wavelength of 650 nm. This EIA has been validated with deer mouse feces (Eleftheriou et al. 2020). Intra-assay and inter-assay coefficients of variation were <15% and 20%, respectively.

WBC count analyses

In healthy deer mice, the most common WBCs in circulation are lymphocytes and neutrophils (Schountz et al. 2014). To evaluate WBCs, we followed the methodology by Eleftheriou and Luis (2020). We stained blood smears with modified Wright stain (Sigma-Aldrich, St. Louis, Missouri, USA) and counted WBCs using light microscopy. Total WBC counts were estimated by counting cells from the feathered edge toward the smear's center for 20 fields at 400×. The mean count of 20 fields was multiplied by 2000 to get an estimate of cells/μL. At 1000×, we counted lymphocytes, neutrophils, monocytes, eosinophils, and basophils out of 100 WBCs.

SNV antibody detection

Because deer mice never resolve SNV infections, antibodies are a reliable marker of infection (Mills et al. 1999). Therefore, we detected infected deer mice by the presence of SNV antibodies in blood samples. We coated 96-well plates with SNV recombinant nucleocapsid antigen and followed an enzyme-linked immunosorbent assay (ELISA) protocol (Schountz et al. 2007) to determine antibody presence.

Statistical analyses

To examine seasonal effects, months were grouped to create a season variable where we assigned October–February to fall/winter, March–May to spring, and June–August to summer. We also grouped juveniles with subadults into one group we called non-adults to increase sample size. Minimum number alive (MNA) was used as an index of deer mouse and vole densities (sensu Krebs 1966) because it works well for small mammals (e.g., Luis et al. 2010). The number of unique captures was used as an index for shrew densities. We used linear and generalized mixed-effects regression trees, which use model-based recursive data partitioning, where each tree node is a regression coefficient (Fokkema et al. 2018). When individuals were sampled more than once, tag number was a random effect. We considered regression trees as appropriate because they can easily handle and identify complex interactions among many potential predictor variables that could be overlooked with traditional statistical approaches that use stepwise variable selection (Strobl et al. 2009). We used linear mixed-effects trees for each of these response variables: baseline FCMs, BCSs, N/L ratios, and WBC counts. For scar numbers, we used a generalized linear mixed-effects tree with a Poisson error structure (Fokkema et al. 2018). However, for stress response FCMs, we used a generalized linear tree with a Gaussian error structure and no random effects because deer mice were sampled only once (Hothorn and

Zeileis 2015). The trees included the following predictor variables: deer mouse, vole, and shrew densities, trapping grid, season, reproductive status, age, sex, flea presence, and SNV infection status. Statistical significance was set to $\alpha = 0.05$. Back-transformed means with standard errors are presented. We performed analyses in R (R Core Development Team 2018) within R Studio (RStudio Team 2015), using R package "glmertree" (Fokkema et al. 2018) to build mixed-effects regression trees and R package "partykit" (Hothorn and Zeileis 2015) to build generalized linear trees. All response variables except stress response FCMs, BCSs, and scar numbers were natural log-transformed to meet normality and homoscedasticity assumptions. We excluded any extreme outliers that were identified during this process and noted below whenever this was done.

Results

Livetrapping

We captured 289 individual deer mice (1028 captures) and 152 individual voles (211 captures). Additionally, we had 131 shrew captures. There was variability in animal densities across space and time (Fig. 1). Nearly all voles identified to species were meadow voles (~93%, $n = 27$).

Stress physiology measures

We only found a temporal effect on baseline FCMs. Deer mice had higher FCMs in fall/winter 2017 (12030.13 ± 1503.06 ng/g, $n = 149$, $P < 0.001$) compared with other times (7547.49 ± 1112.90 ng/g, $n = 307$). Initially, only presence of shrews was associated with lower stress response FCMs (19526 ± 2614 ng/g, $n = 61$, $P < 0.001$). However, after removing one extreme outlier (174703.9 ng/g) the more nuanced effect of shrew density was apparent (Fig. 2). Shrew density >4/ha was then associated with lower stress response FCMs (10565 ± 3201 ng/g, $n = 14$, P = 0.01). When shrew density was 1- 4/ha, deer mice had higher stress response FCMs (21537 ± 3071 ng/g, $n = 49$). However, deer mice had even higher stress response FCMs when shrews were absent (31612 ± 3471 ng/g, $n = 67$, P = 0.04). In summary, as shrew density increased, stress response FCMs decreased, indicative of chronic stress with a higher shrew density.

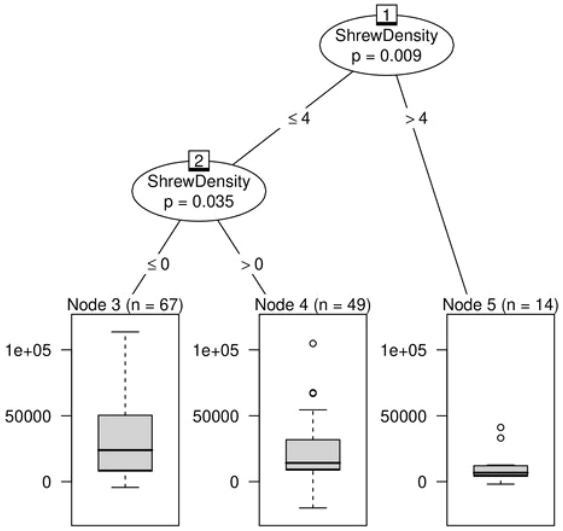

Fig. 2. Regression tree for stress response fecal corticosterone metabolites of deer mice. Each terminal node provides a sample size with a box plot for that subgroup. Density is per hectare. Statistical significance was set to 0.05.

Significant predictors for N/L ratio were season, grid, and age (Fig. 3). N/L ratios were lowest in deer mice at grid A (0.42 ± 0.05, $n = 61$) compared with other grids (0.70 ± 0.10, $n = 115$, $P = 0.002$), only in fall/winter. In spring/summer, adults had higher N/L ratios (1.43 ± 0.18, $n = 279$, $P < 0.001$) compared with non-adults (0.69 ± 0.15, $n = 22$).

Significant predictors for BCS were reproductive status, shrew and vole densities, season, and age (Fig. 4). Nonreproductive deer mice had higher BCSs (3.07 ± 0.05, $n = 138$, $P < 0.001$) when shrew density was ≤1/ha. When shrew density was >1/ha, BCSs were lower (2.79 ± 0.09, $n = 76$). Reproductive non-adults had higher BCSs (3.18 ± 0.12, $n = 27$, $P = 0.001$) than reproductive adults. For adults, the presence of voles had a significant effect on BCSs (P < 0.001), and was associated with a temporal effect, where deer mice had lower BCSs in spring and summer 2018 (2.34 ± 0.08, $n = 116$), compared with other times (2.64 ± 0.08, $n = 109$, $P = 0.002$). When voles were absent, higher shrew density (>1/ha) was associated with lower BCSs (2.28 ± 0.12, $n = 37$), compared with lower

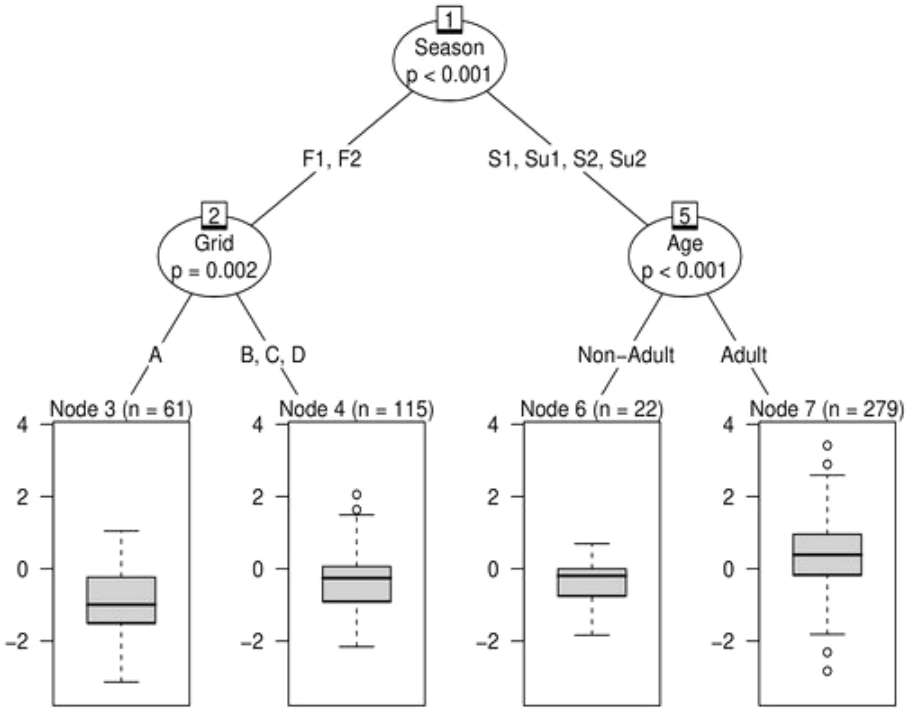

Fig. 3. Regression tree for ln neutrophil-to-lymphocyte (N/L) ratio of deer mice. Each terminal node provides a sample size with a box plot for that subgroup. Grids A and B were trapped from October'16 to July'18. Grids C and D were trapped from October'17 to August'18. Juveniles and subadults were grouped into "Non-Adult." F1, S1, Su1, F2, S2, and Su2 represent fall/winter'16, spring'17, summer'17, fall/winter'17, spring'18, and summer'18, respectively. Statistical significance was set to 0.05.

density (≤1/ha; 2.92 ± 0.11, n = 46, P < 0.001). In summary, deer mice had lower BCSs when heterospecific competitors were present, and reproductive adults had lower BCSs compared with other age/reproductive classes.

Immunity

Significant predictors for WBC counts were grid and season. Deer mice at grids A and B had lower WBC counts compared with grids C and D (P < 0.001; Fig. 5), which is most likely a temporal artifact of which grids were trapped together every month. For all grids, WBC counts were higher (A and B: 2239.37 ± 139.00/μL, n = 230; C and D: 3273.81 ± 340.23/μL, n = 118) in fall/winter and spring, and lower in summer (A and B: 1511.72 ± 146.38/μL, n = 81; C and D: 2271.31 ± 295.96/μL, n = 52, P = 0.002). To meet the normality assumption, we had to exclude two outliers that had counts of 100 WBCs each from grids A and B.

Scar numbers

Significant predictors for scar numbers were season, vole and shrew densities, and reproductive status. Deer mice had more scars in spring 2018 and across both summers, compared with spring 2017 and fall/winter 2017 (P < 0.001; Fig. 6). During spring and fall/winter 2017, reproductive deer mice had more scars (1.20 ± 0.17, n = 94) than nonreproductive individuals (0.85 ± 0.09, n = 115, P = 0.04). In spring 2018 and both summers, intermediate vole density (1-3/ ha) was associated with more scars (2.33 ± 0.28, n = 96, P < 0.001), compared with when voles were absent or at a higher density (>3/ha; 1.59 ± 0.20, n = 101, P = 0.001). Shrews only mattered when voles were absent; then, with a shrew density >1/ ha, deer mice had more scars (2.13 ± 0.30, n = 45) than when shrew density was ≤1/ha (1.53 ± 0.22, n = 58, P = 0.013). Taken together, the identity of heterospecific competitors was important in how a number of scars in deer mice were affected.

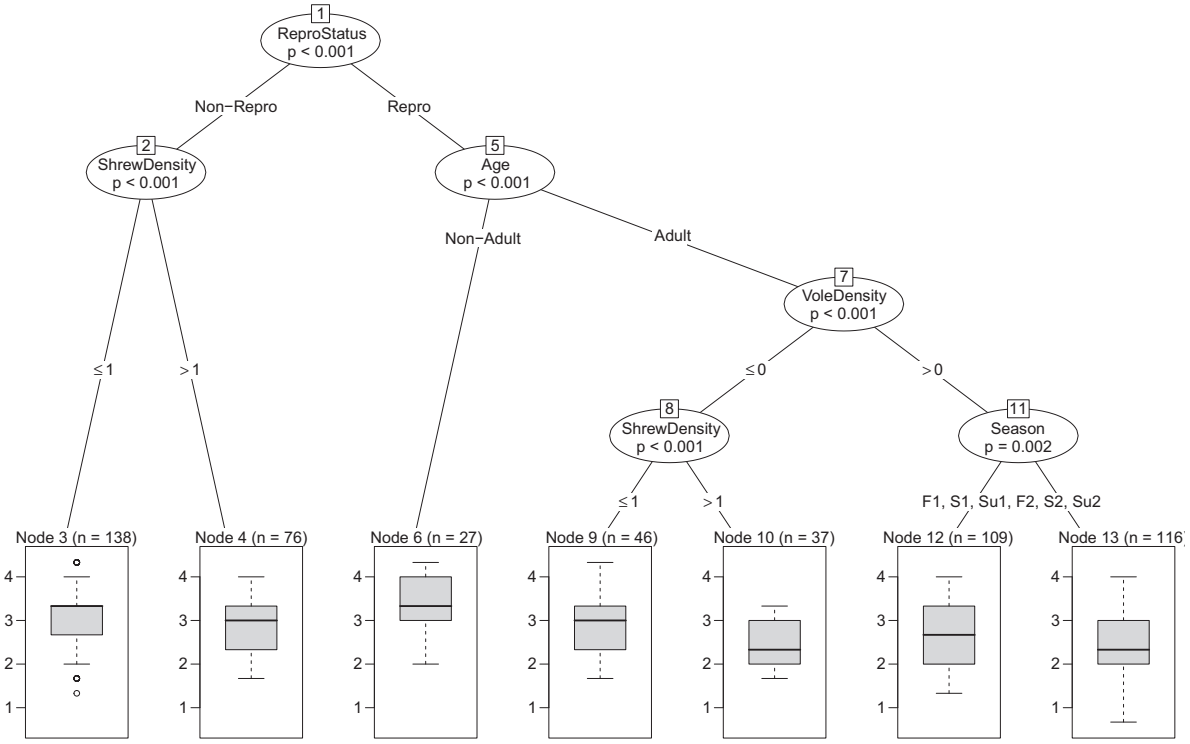

Fig. 4. Regression tree for body condition score of deer mice. Each terminal node provides a sample size with a box plot for that subgroup. "ReproStatus" stands for reproductive status. Juveniles and subadults were grouped together into "Non-Adult." F1, S1, Su1, F2, S2, and Su2 represent fall/winter'16, spring'17, summer'17, fall/winter'17, spring'18, and summer'18, respectively. Density is per hectare. Statistical significance was set to 0.05.

SNV infection

We detected 23 infected deer mice with most of them (n = 21) from grids where voles were also trapped. All, except for one, were trapped from August 2017 to August 2018 (Fig. 1). Because of a low number of infected deer mice, there was not enough power to perform formal statistical tests for evaluating competition or seasonality effects on SNV prevalence.

Discussion

We showed that two key determinants of SNV transmission, host susceptibility and exposure to infection, were associated with ecological and environmental factors. In particular, we examined how heterospecific competitors and seasonality were associated with (1) stress physiology and immunity (i.e., host susceptibility) and (2) scar numbers (i.e., host exposure to infection) in deer mice. Broadly, we found significant associations between both factors and select measures of stress physiology, immunity, and contact rates (summarized in Table 1).

Unfortunately, a low number of SNV-infected deer mice made it difficult to evaluate direct relationships between SNV prevalence and transmission with any of our physiological and behavioral measures. This was not surprising given the low deer mouse densities at our site during the study. Deer mouse density varies widely over space and time and has been shown to be the main driver of SNV prevalence (Luis et al. 2015, 2018). The densities at our field site ranged between 2 and 32 deer mice/ha (Fig. 1), with most hovering around the critical host density needed for SNV to invade (estimated at ~17 deer mice/ha at another site in Montana; Luis et al. 2015). Given the deer mouse densities observed, we would expect to see, at most, one infected individual when using the epidemiological model of Luis et al. (2015), which is similar to what we observed (maximum of three infected individuals per any given month). Although deer

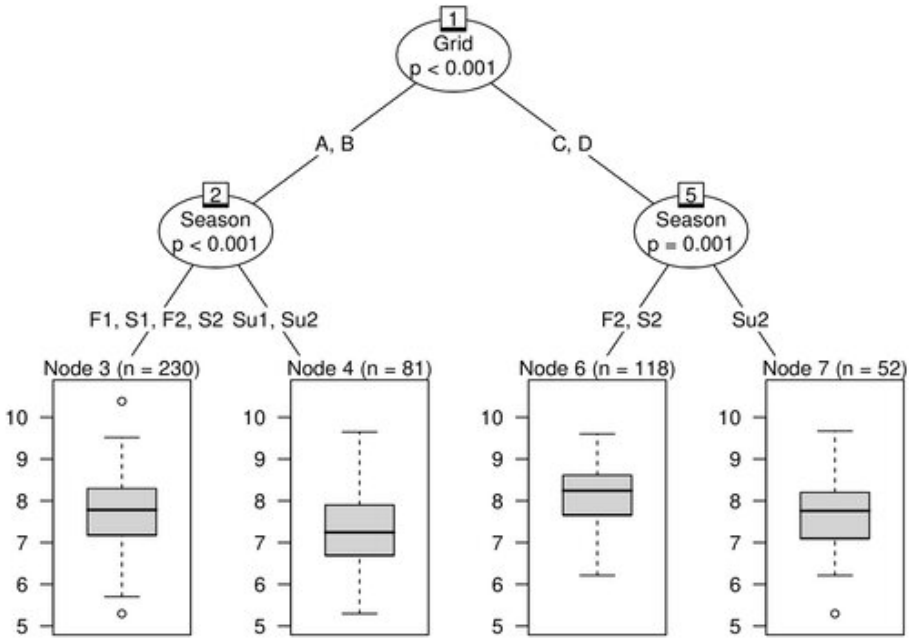

Fig. 5. Regression tree of white blood cell counts of deer mice. Each terminal node provides a sample size with a box plot for that subgroup. Grids A and B were trapped from October'16 to July'18. Grids C and D were trapped from October'17 to August'18. F1, S1, Su1, F2, S2, and Su2 represent fall/winter'16, spring'17, summer'17, fall/winter'17, spring'18, and summer'18, respectively. Statistical significance was set to 0.05.

mouse density appears to be the most important driver of SNV prevalence, Luis et al. (2018) showed that species diversity can also affect the SNV transmission rate (for a given deer mouse density), with SNV transmission rate higher in communities with more diverse heterospecific competitors. However, Luis et al. (2018) could not discern if this increase in transmission rate was due to changes in host susceptibility or contact rates. Here, we present evidence that heterospecific competitor densities may induce chronic stress, which could potentially result in suppressed immunity and increased susceptibility to infection. We also present evidence that competitors may potentially alter contact rates, which could impact exposure to infection, as evidenced by changes in scar numbers.

Interspecific competition

We found evidence that heterospecific competitor densities were associated with chronic stress in deer mice. Shrews were associated with two stress measures (stress response FCMs and BCSs), and voles were associated with one (BCSs). Although we hypothesized, based on support from the literature, that voles are dominant over deer mice, we reasoned that shrews are most likely less dominant given the lack of evidence to suggest otherwise. Thus, we expected shrews to minimally influence deer mouse stress physiology, but we found that shrew density, and not vole density, was negatively associated with stress response FCMs, indicative of chronic stress at a higher shrew density. Because shrew and deer mouse diets can overlap (i.e., insects; Rychlik and Jancewicz 2002, Witmer and Moulton 2012), exploitative competition may be intense enough to induce chronic stress in deer mice. In contrast to our findings, we expected to see an effect of voles on deer mouse baseline FCMs because (1) they are dominant over deer mice, and (2) a positive correlation between vole densities and deer mouse baseline FCMs has been found previously (Fredebaugh et al. 2013). However, we may have missed an effect because we did not sample frequently enough (e.g., weekly) or the effect was too subtle to detect. The absence of changes in N/

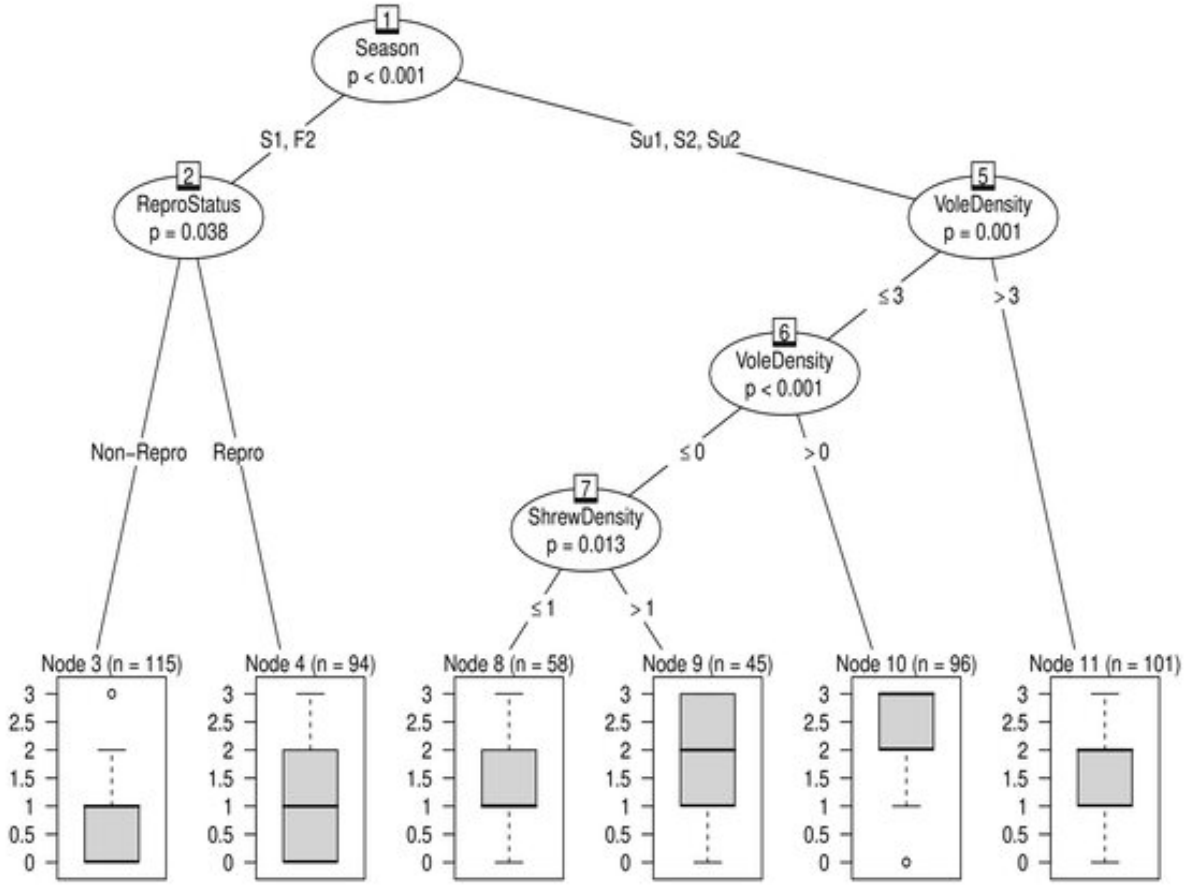

Fig. 6. Regression tree for scar numbers of deer mice. Each terminal node provides a sample size with a box plot for that subgroup. Density is per hectare. "ReproStatus" stands for reproductive status. S1, F2, Su1, S2, and Su2 represent spring'17, fall/winter'17, summer'17, spring'18, and summer'18, respectively. Statistical significance was set to 0.05.

L ratios was also unexpected but may have been due to the aforementioned reasons.

Both vole and shrew densities were negatively associated with body condition scores (BCSs) in deer mice. BCSs are one measure of chronic stress. However, they could indicate lower food availability, another potential result of interspecific competition. Regardless of the cause, individuals with lower BCSs may be more susceptible to infection (Beldomenico et al. 2009). We found that the presence of voles, and higher shrew density, was associated with lower BCSs in reproductive adults only. However, shrew density was associated with larger changes (Fig. 4). This pattern may ensue if reproductive adults compete with voles and shrews relatively more, perhaps due to their higher energetic demands.

Despite associations between heterospecific competitors and stress physiology, we found no association with WBC counts, the immunity measure. The complex and multifaceted relationship between stress physiology and immunity makes it difficult to choose the best suited immune measure that can capture the desired response (Martin 2009). Although relatively cheap and simple to attain, WBC counts may increase in response to infection or inflammation, potentially masking stress-induced reductions (Davis et al. 2008). Nevertheless, our findings suggest that we may need to explore other immunity measures that are more functional in nature, such as lymphocyte proliferation assays, which allow for a real-time immune challenge (Demas et al. 2011).

We also found that vole and shrew densities were differentially associated with deer mouse scar numbers, primarily during the breeding period. Interestingly, the relationship between vole density and scar numbers was non-monotonic. Deer mice had the most scars at intermediate vole densities (between 1 and 3/ha), with fewer scars either when voles were absent or present at densities >3/ha (Fig. 6). However, at higher shrew densities (>1/ha), deer mice had more scars than when shrews were absent. Although we did not have clear a priori predictions on how contact rates might change in response to interspecific competition, our findings suggest that deer mice may change their behavior based on what competitor is present. Such behavioral changes were reported by Clay et al. (2009) in deer mice from Utah, USA, at sites with high small mammal diversity, although Rubio et al. (2017) found no change in deer mice kept in outdoor enclosures with dominant Merriam's kangaroo rats (*Dipodomys merriami*). We acknowledge that using scar numbers as a proxy for contact rates has limitations (e.g., wounds healed with no scars), so our findings related to this measure need to be evaluated with caution. Thus, controlled experiments where technology is used to better estimate contact rates are needed (e.g., Dearing et al. 2015).

In summary, our findings suggest that the identity of competitor species may matter in how transmission is affected because deer mouse physiology and behavior responded differently to vole and shrew densities. Studies of other disease systems have demonstrated the importance of species identity in affecting disease transmission in the focal host, such as with Lyme disease (vector-borne transmission, LioGuidice et al. 2003) and chytridiomycosis (environmental transmission, Venesky et al. 2014). However, our study is one of the few to examine consequences of competitor species on both physiological and behavioral traits of the host that have implications for transmission of directly transmitted diseases. Given our results, it is crucial that we continue to test how heterospecific competitors can differentially affect host physiology and behavior so we can improve how we understand and predict disease dynamics across diverse communities.

Seasonality

There was no clear seasonality (consistent between years) in baseline or stress response FCMs. Although seasonal variation in photoperiod necessarily affected how long deer mice were in overnight confinement, this did not appear to be influential for stress response FCMs because season was not selected as a predictor (Fig. 2). Our findings are not entirely surprising because a review by Romero (2002) found no consistent seasonal patterns in baseline or stress-induced FCMs (i.e., after trap confinement) in mammals.

We found seasonality in N/L ratios, where deer mice had higher N/L ratios in spring/summer compared with fall/winter. Seasonal environmental factors that influence GCs may also affect N/L ratios, although these two measures may evaluate the stress response differently across time (Davis et al. 2008, Goessling et al. 2015). However, inflammation and infection may also affect N/L ratios (Davis et al. 2008). Given that deer mice were not tested for infection other than with SNV, we cannot discern if the seasonality observed was explicitly due to GCs. However, given the large sample size and consistent pattern across years, seasonality in stress physiology is a more parsimonious explanation. It is noteworthy that seasonal variation in photoperiod necessitated that time in confinement also varied by season. Although this may have influenced the findings, seasonality in N/L ratios (or heterophil-to-lymphocyte ratio in birds and reptiles) has been observed in other vertebrates (e.g., Norte et al. 2009, Goessling et al. 2016). In contrast to N/L ratios, we did not find clear seasonality in BCSs, despite seasonal changes in body condition occurring in other vertebrates (e.g., Milenkaya et al. 2013, Pokharel et al. 2017).

There was clear seasonality in the immunity measure, WBC counts, which were higher in fall/winter and spring, but lower in summer. Similar to N/L ratios, inflammation or infection may also influence WBC counts and possibly time in confinement as well (Davis et al. 2008). However, evidence consistent with our field data from previous studies of small mammals, including deer mice, proposes that seasonality is the most likely parsimonious explanation for our findings (Blom et al. 1994, Martin et al. 2008).

Aggressive encounters between deer mice that lead to scars are associated with breeding and typically occur in spring/summer (Bagamian et al. 2012). Similar changes in scarring from intraspecific fights occur seasonally in other vertebrates (Christian 1970, Woodroffe and Macdonald 1995). Such seasonal changes in aggressive encounters can result from changes in home range dictated by reproduction and food availability (e.g., Perelberg et al. 2003). For the most part, our study confirms this pattern. We also observed that deer mice had more scars across summers and spring 2018 compared with fall/winter. However, lower scar numbers in spring 2017 did not fit this pattern. Perhaps food availability, which we did not measure, was greater in spring 2017, which may have led to fewer aggressive encounters.

Taken together, higher N/L ratios in spring/summer, lower WBC counts in summer, and more scar numbers in summer may provide valuable insight as to why previous studies in Montana found that SNV transmission is higher in spring/summer. However, experimental studies would be needed to causally link these findings to SNV transmission in deer mice.

Conclusions

Overall, we found evidence that heterospecific competitors and seasonality were associated with select physiological and behavioral measures in deer mice. However, we found no effects of deer mouse density, which was surprising given that density has been associated with stress physiology and intraspecific contact rates in rodents (Harper and Austad 2004, Ostfeld and Keesing 2019). Similarly, there were no effects from flea infestation or SNV infection (although we very likely lacked power to detect an effect). The absence of these associations suggests that neither deer mouse density nor parasitism was as important as heterospecific competitors and seasonality in affecting physiological and behavioral measures. However, if deer mouse density were high enough for a long enough duration to sustain SNV transmission and increase infection, perhaps those associations could manifest. We should note that because we did not measure temporal or spatial heterogeneity in vegetation cover across grids, we cannot ascertain its consequences on physiological and behavioral measures. However, because grid was not a significant predictor in most models, it seems likely that vegetation cover itself did not play a widely influential role.

The mechanistic approach of our study can inform other disease ecology studies that seek to identify potential physiological and behavioral links between extrinsic factors and disease transmission. Going forward, conducting controlled experiments in outdoor enclosures where host densities are held constant will allow for more robust evaluations of the effects of interspecific competition and seasonality on stress physiology, immunity, and intraspecific contact rates in our system and other directly transmitted disease systems.

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Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3494/full