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Long-Term Patterns of Immune Investment by Wild Deer Mice Infected with Sin Nombre Virus

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ABSTRACT

Immunocompetence of animals fluctuates seasonally. However, there is little consensus on the cause of these fluctuations. Some studies have suggested that these patterns are influenced by changes in reproductive condition, whereas others have suggested that differences result from seasonal variations in energy expenditures. The objective of our study was to examine these contrasting views of immunity by evaluating seasonal patterns of immune response and reproduction in wild populations of deer mice *Peromyscus maniculatus* exposed to Sin Nombre virus (SNV). Over three consecutive fall (September, October, November) and three consecutive spring (March, April, May) sampling periods, we used titration enzyme-linked immunosorbent assay (ELISA) to quantify virus-specific antibody production in 48 deer mice infected with SNV. Levels of reproductive hormones were quantified using ELISA. SNV antibody titers reached their lowest level during November (geometric mean titer [GMT] = 420) and their highest levels during September (GMT = 5,545) and May (GMT = 3,582), suggesting that the immune response of deer mice to SNV has seasonal patterns. The seeming decrease in antibody titer over winter coupled with the consistency in body masses suggests that during winter,

immunocompetence may be compromised to offset the energetic costs of maintenance functions, including those associated with maintaining body mass. Deer mice showed distinct sex-based differences in SNV antibody production, with males producing higher antibody titers (GMT = 3,333) than females (GMT = 1,477). Levels of reproductive hormones do not appear to influence antibody production in either males or females, as there was no correlation between estradiol concentrations and SNV antibody titer in female deer mice ($r^2 = 0.26$), nor was there a significant relationship between levels of testosterone and SNV antibody titers in males ($r^2 = 0.28$). Collectively, this study demonstrates that immunocompetence of wild deer mice is seasonally variable; however, reproduction is not the primary stressor responsible for this variation. Rather, the data suggest that deer mice may compromise immunocompetence during winter to offset other maintenance costs during this period.

Introduction

Mammalian species regularly experience seasonal fluctuations in a number of physiological processes, including those associated with metabolism, thermoregulation, and reproductive activity (Ruby and Zucker 1992; Moffat et al. 1993). Recently, a handful of studies demonstrated that mammals may also experience seasonal fluctuations in immune system function. However, results of these studies do not reveal a consistent pattern: some studies indicate that immunocompetence reaches an annual low during the winter months, whereas other studies have shown that mammals upregulate immune function during winter. For example, immunocompetence of wild European rabbits (*Oryctolagus cuniculus*) is inhibited during winter, which corresponds to differences in their seasonal patterns of infection with the gastrointestinal nematode *Trichostrongylus retortaeformis* (Cornell et al. 2008). In contrast, wild cotton rats (*Sigmodon hispidus*) experience temporal fluctuations in both humoral and cell-mediated immunity; however, these fluctuations do not correspond to seasonal specific time periods but rather reflect temporal shifts in genotypic polymorphisms within the population (Lochmiller et al. 1994).

Such seasonal variations in immunocompetence are often attributed to differential resource allocation driven by seasonal fluctuations in the energy demands of the individual (Festa-Bianchet 1989; Gustafsson et al. 1994). Energy demands most commonly cited as taxing immune system function include those imposed by growth, maintenance, and reproduction (Sinclair and Lochmiller 2000; Nelson et al. 2002). Previously, we

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evaluated patterns of resource allocation in wild deer mice in different reproductive states that were naturally infected with Sin Nombre virus (SNV), a hantavirus (Lehmer et al. 2007). In response to a general immune challenge, we found that male deer mice had significantly smaller responses to general immune challenge than females but had SNV infection rates that were nearly 1.5 times that of females (Lehmer et al. 2007). Moreover, there was an interaction between reproductive state and the immune response. These results suggested that reproductive activity may modulate the immune system of female deer mice differently from that of males and that the reduced immunocompetence of male deer mice may confer greater susceptibility to infection, including SNV, in the natural environment.

Whether sex-based differences in immune system function exist is controversial. Whereas more recent studies have found similar immune responses between sexes (e.g., Cox et al. 2009; Nunn et al. 2009), previous studies have documented sex-based differences in immune system function in a number of animal species and have shown, in general, that males tend to be more susceptible to disease and exhibit reduced immunocompetence compared with females (Billingham 1986; Saino et al. 1995; Ros et al. 1997; Klein and Nelson 1998). Such innate, sex-based differences in immunity may be influenced at least to some extent by reproductive hormones. Testosterone, the primary androgenic hormone in males, decreases both humoral and cell-mediated immune responses by inhibiting production of antibodies, T cells, and antiviral gene expression (Klein et al. 2002; Hannah et al. 2008), whereas estradiol, the primary female androgen, enhances humoral immunity by promoting antibody production and the expression of antiviral, proinflammatory, and major histocompatibility complex genes (Klein et al. 2002; Easterbrook and Klein 2008; Hannah et al. 2008; Schönrich et al. 2008). For males of many species, this represents an evolutionary trade-off, as testosterone promotes the expression of secondary sex characteristics and aggression, which may improve the likelihood of mating for an individual male while reducing immunocompetence (Hamilton and Zuk 1982). This relationship, referred to by Folstad and Karter (1992) as the immunocompetence handicap, has been documented in a number of animal species (e.g., Hart 1990; Weedkind 1992). However, most studies have only indirectly assessed the relationship between immunocompetence and reproduction in wild animals by focusing on indicators of immunocompetence such as parasite load and inflammation following immune challenge, and they have inferred the animal's reproductive state by quantifying their expression of an assortment of secondary sex characteristics such as plumage, coloration, and dominance behaviors (reviewed by Roberts et al. 2004). Because few studies have directly measured both immune system function and levels of circulating hormones, it is difficult to characterize the extent to which immunocompetence is influenced by reproduction in free-ranging animals.

Although the immunological literature points to an inextricable link between the endocrine and immune systems, ecological literature has equally emphasized the link between sea-

sonal energy demands and immunity. We thus attempted to bridge these contrasting views of immunity by evaluating seasonal patterns of immune response and reproduction in wild populations of deer mice exposed to SNV, a common pathogen. The relationship between SNV and deer mice is a long-term one (Yates 2002). Deer mice infected with SNV appear asymptomatic (O'Connor et al. 1997; Botten et al. 2002, 2003), although histopathological changes have been observed (Netski et al. 1999). Some have suggested the depressed immune response observed in infected deer mice represents a "mutually co-adaptive evolutionary event" that permits the persistence of the virus (Schountz et al. 2007). In contrast, several other studies have demonstrated consequences of SNV infection in deer mice under natural conditions, as seropositive animals gain less mass than uninfected animals (Douglass et al. 2007) and have lower survival rates than uninfected deer mice (Douglass et al. 2001; Adler et al. 2008). Others have shown that female deer mice infected with SNV may have reduced reproductive success (Dearing et al. 2009). Once infected, deer mice produce virus-specific antibodies for life (Netski et al. 1999; Herbst et al. 2001; Botten et al. 2002). Thus, SNV infection requires that deer mice allocate resources toward immunity for extended periods of time, which may ultimately reduce survival and reproductive success. The continual production of virus-specific antibodies provides a mechanism to examine long-term patterns of immune system function in a host with a chronic infection. The overall objective of this study was to track the course of the virus-specific immune response of repeatedly captured deer mice infected with SNV. We generated the following predictions based on our understanding of the relationships between reproductive hormones, physiological stress, and immunity: (1) SNV antibody titer of deer mice would decrease during the physiologically stressful period of winter; (2) antibody production would fluctuate in accordance with body mass, with larger animals presumably being more fit and, therefore, capable of producing higher titers; (3) reproductive activity would suppress the immune response of deer mice; and (4) antibody production would decline over the course of SNV infection.

Methods

Study Sites

Deer mice were nondestructively sampled from two sites (3.14 ha each) near the West Tintic Mountains in the Great Basin Desert of central Utah (Juab County) on lands administered by the U.S. Department of Agriculture. These sites have been sampled twice annually since 2002 as part of a larger, ongoing SNV research program and were selected because of their high deer mouse densities. Average ambient temperature for the fall of 2005 on our sites was 8.5°C, and average ambient temperature for the spring of 2006 was 7.2°C (U.S.D.A. Natural Resources Conservation Service data: <http://www.wcc.nrcs.usda.gov/snotel>). Average monthly temperatures during the sampling periods were within 1 SD of monthly temperatures averaged from 2002 to 2008. Vegetative communities of both sites were similar and were dominated by big sagebrush (*Artemisia*

tridentata) and Utah juniper (*Juniperus osteosperma*). To ensure independence, study sites were separated by approximately 3 km.

Deer Mouse Sampling

Sites were sampled for deer mice in September, October, and November of 2005 and March, April, and May of 2006 during three night periods that coincided with the new moon. At each site, animals were livetrapped (H. B. Sherman Traps, Tallahassee, FL) on a web of 148 traps (Mills et al. 1999). After capture, animals were identified by species and sex; all animals other than deer mice were released to their location of capture. Deer mice were anesthetized using isoflurane, weighed, and marked with uniquely numbered ear tags. We collected ~0.3 mL of blood from the retro-orbital sinus of all adult deer mice on initial capture within the 3-d sampling period. Deer mice were classified as adults if their body mass was ≥ 14.0 g (Mills et al. 1997; Borucki et al. 2000; Calisher et al. 2001). Blood was immediately stored on dry ice until transfer to a -80°C freezer. Blood was not collected from juvenile deer mice (body mass ≤ 14.0 g), and all analysis and interpretation in this study is focused on adult deer mice. Following processing, deer mice were released at their location of capture. All personnel involved in trapping and handling rodents took precautions for working with animals potentially infected with hantavirus (CDC 1995), and general techniques for capturing and processing animals were approved by the Institutional Animal Care and Use Committee at the University of Utah.

SNV Antibody Detection

In a Bio Safety Level-3 laboratory at the University of Nevada, Reno, we performed enzyme-linked immunosorbent assays (ELISA) for antibodies (IgG) against SNV in deer mouse blood as described by Otteson et al. (1996) and Feldman et al. (1993). To describe this briefly, 96 well plates were coated with recombinant SNV nucleocapsid (N) antigen at 1 : 400 in phosphate buffered saline (PBS) and stored overnight at 4°C . After the wells were washed in PBS with 0.5% Tween-20 (PBS-T), serum samples were diluted 1 : 50 in PBS with 0.5% Tween-20 and 5% skim milk (milk diluent) and plated in duplicate for 60–120 min at 37°C . Goat anti-*P. leucopus* HRP secondary antibody was diluted to 1 : 1,000 in milk diluent and incubated for 60 min at 37°C . To develop the assay, 2-2'-asino-di-(3-ethyl-benzthiozoline-sulfonate) peroxidase substrate (Kirkegaard and Perry Laboratories) was incubated for 30 min at 37°C . Plates were read using Gene 5 software on a Biotek plate reader at 405 nm. Samples were considered positive if the optical density reading was three times higher than that of the known negative serum controls on a plate.

SNV Antibody Titers

Seropositive animals were further screened for SNV antibody titer. Titer is defined as the last dilution with a positive ELISA

result. Initially, samples were diluted from 1 : 50 to 1 : 6,400. Samples that were still positive after a 1 : 6,400 dilution were diluted up to 1 : 50,000 as necessary. Antibody titers are reported herein as the geometric mean titer (GMT). Differences between groups were considered to be statistically significant if $P < 0.05$.

SNV Avidity Assays

A subsample of deer mice SNV seropositive during the March, April, or May sampling periods was selected for avidity ELISA ($n = 19$). Limited volumes of sera precluded us from performing these assays on the remaining SNV seropositive deer mice. Avidity is a measure of the combined strength of bond interactions within an antibody; the avidity of antibodies typically increases as the infection matures (Safronetz et al. 2006). In the context of this study, avidity ELISA was used to discriminate between deer mice in the early (< 30 d, low-avidity antibodies) and later (> 30 d, high avidity antibodies) stages of SNV infection (Safronetz et al. 2006). Following the avidity ELISA protocol outlined by Safronetz et al. (2006), SNV seropositive samples were tested using four wells each (two sets of duplicates). After serum incubation, 35 mM diethylamine was added (100 μL per well) to one set of duplicates to act as a denaturing solution for the antibodies bound to the N antigen. The second set of duplicates received 100 μL of PBS-T, added as a control. Plates were incubated three times for 5 min each; the wells were washed with PBS-T between each incubation. After three rounds of treatment, all wells were washed an additional six times with PBS-T before adding the secondary antibody and developing substrate. The relative avidity index (RAI) was calculated as a ratio of the optical density of the treated sample to the optical density of the untreated wells. For antibodies, samples with an RAI greater than 0.5 were classified as older infections, while those less than 0.5 were categorized as younger infections.

Reproductive Hormone Assays

In each sampling period, a subsample of repeatedly captured deer mice that were SNV seropositive was selected for measurement of levels of circulating reproductive hormones ($n = 36$ males; $n = 34$ females). Limited volumes of sera precluded us from performing these assays on all deer mice. Concentrations (ng/mL) of plasma estradiol (females) and testosterone (males) were measured using commercially developed kits following the manufacturer's protocol (Neogen, Lexington, KY). These kits are not species specific and, thus, are cross-reactive with deer mouse androgens. In accordance with the manufacturer's specifications, all samples were assayed in duplicate, and reported hormone concentrations represent the mean value of the two samples. Mean variation between the two samples was 10.76% ($\text{SE} = \pm 1.23$).

Analyses

SNV antibody titers are measured at fixed intervals and thus represent categorical data that must be analyzed with non-parametric statistical tests. To determine longitudinal patterns of change in antibody titer among repeatedly sampled deer mice, we used Kruskal-Wallis tests to measure differences in antibody titers across sampling periods. Wilcoxon signed rank tests were used to measure cross-seasonal changes in antibody titers among repeatedly captured deer mice. These analyses were used to measure both long-term changes in titer that occurred between nonconsecutive sampling periods (September to November and November to May) as well as short-term changes in titer that occurred between consecutive sampling periods (e.g., September to October, March to April). Differences in antibody titer between male and female deer mice were assessed with Mann-Whitney tests. Differences between groups were considered to be statistically significant if $P \leq 0.05$.

Because both SNV infection rates and pathology in deer mice can be influenced by an animal's age (Mills et al. 1997; Netski et al. 1999), we used ANOVAs to assess possible changes in the age structure of our study population across sampling periods. Body mass is commonly used to assess the age of rodents (Mills et al. 1997); thus, body mass was treated as the continuous dependent variable in these models. To determine whether antibody titer was related to body mass, we assigned deer mice to one of three body mass categories. Mass categories were assigned by dividing the range of body masses at the thirty-third and sixty-sixth percentiles, resulting in approximately equal numbers of deer mice in each class (Mills et al. 1997). Body mass classes were 14.0–19.5 g, 19.6–21.7 g, and 21.8–34.2 g. Differences in antibody titer among body mass classes were determined with Kruskal-Wallis tests ($\alpha = 0.05$).

Because concentrations of estradiol and testosterone are inherently different, all analyses involving reproductive hormones were run independently for male and female deer mice. We used ANOVA to measure differences in levels of circulating reproductive hormones across sampling periods ($\alpha = 0.05$). Pairwise differences were assessed using least squares means comparisons with Tukey-Kramer adjustments for multiple comparisons. Spearman correlations were used to determine relationships between reproductive hormone concentrations and antibody titers ($\alpha = 0.05$).

To assess the antibody response of deer mice with recently acquired SNV infections versus older infections, we used the information from both the collection time and the avidity assay. First, we selected a subset of animals known to have seroconverted (i.e., to have gone from seronegative to seropositive) during the course of the study. We used Mann-Whitney tests to compare antibody titers between male and female deer mice in this category. Mann-Whitney tests were also used to determine whether there was a difference in antibody response between new infections that occurred during the fall sampling periods (September, October, November) and the spring sampling periods (March, April, May). To further assess the change in antibody response across the course of SNV infection, we

compared titers of deer mice with recently acquired SNV infections to those with older infections. Deer mice with older infections were those that tested positive during all sampling periods in which they were captured. Because the first capture of these animals would not necessarily guarantee that seroconversion occurred >1 mo earlier, we omitted their titer at first capture and used titers of the second and subsequent captures in these analyses. As with the previous assessments, Mann-Whitney tests were used to determine whether antibody titers differed between deer mice with old and new SNV infections. Mann-Whitney tests were also used to assess differences in antibody titers of deer mice with recent versus old infections based on the avidity assays. Differences between groups were considered to be statistically significant if $P \leq 0.05$.

Results

Over the course of six sampling periods (September, October, November 2005 and March, April, May 2006), we collected 633 deer mice (318 males, 315 females). Of these, 41 (24 males, 17 females) deer mice collected during more than one sampling period were seropositive (Fig. 1). Table 1 shows the number of male and female deer mice that were recaptured across two to five sampling periods. While many deer mice were captured repeatedly across sampling periods, there were no individual deer mice that were recaptured during all six sampling periods.

Variation in Antibody Titer across Seasons

In all sampling periods, seropositive deer mice had a range of antibody titers (Fig. 1). Antibody titers differed across sampling periods (Fig. 2), with titer declining from September to November (September GMT = 5,545, November GMT = 420, $U_1 = 16.5$, $P = 0.02$) and increasing from November to May (May GMT = 3,582, $U_1 = 155.0$, $P = 0.03$). Individual deer mice who were repeatedly sampled in these seasons showed similar patterns, as individuals sampled in both September and November ($n = 2$) exhibited significant declines in SNV antibody titers ($Z = 1.87$, $P = 0.04$; Fig. 3), whereas deer mice sampled in both November and May ($n = 6$) had significant increases in titers ($Z = 1.98$, $P = 0.04$; Fig. 3). However, there was no detectable change in mean titer from one month to the next (e.g., March to April, April to May) among deer mice that were trapped in two consecutive sampling periods ($n = 41$; $Z = 2.53$, $P = 0.12$; Fig. 3), suggesting that changes in antibody titers within individuals occurred relatively slowly, over a period longer than 1 mo.

Body Mass and Titer

Mean body mass of deer mice in this study was 20.4 g (± 3.5). Body mass did not vary across sampling periods ($F_{5,73} = 0.29$, $P = 0.92$) and did not differ between males and females (male mean = 20.51 \pm 2.29, female mean = 20.35 \pm 4.85; $F_{1,73} = 0.21$, $P = 0.65$). Similarly, there was no significant season \times sampling period interaction ($F_{5,73} = 0.66$, $P = 0.66$).

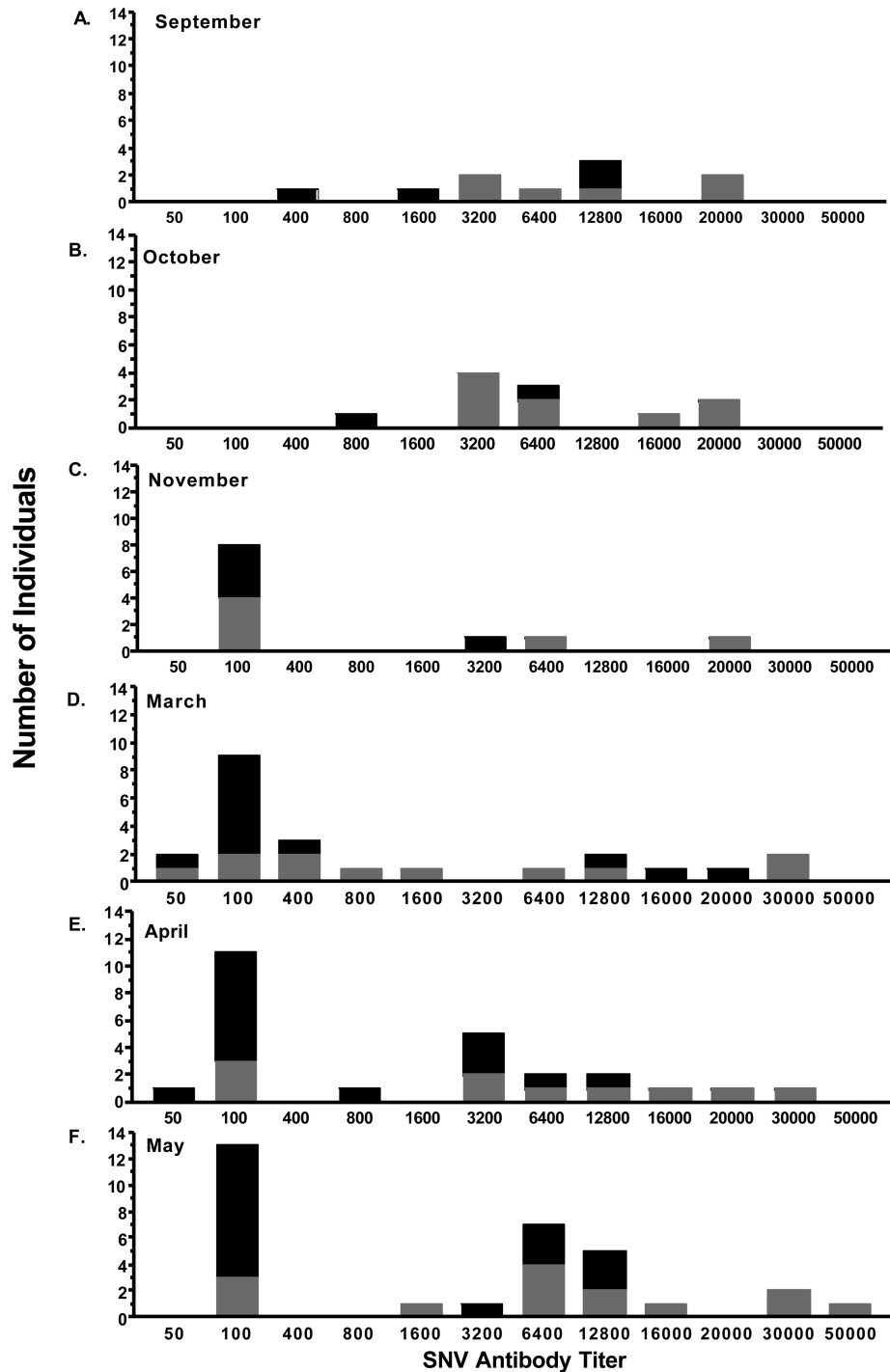


Figure 1. Frequency of antibody titers to Sin Nombre virus (SNV) measured in individual deer mice *Peromyscus maniculatus* over six monthly sampling periods. Antibody titers were determined with titration enzyme-linked immunosorbent assay performed on serums of wild-caught deer mice. Gray filled bars represent male deer mice; black filled bars represent females.

Across sampling periods, we found no relationship between body mass and antibody titer of SNV seropositive female deer mice ($U_2 = 3.97, P = 0.14$). However, in seropositive males, there was a relationship between titer and body mass ($U_2 = 7.38, P = 0.03$), with heavier males producing higher titers.

Sex and Titer

Across sampling periods, there were significant sex-based differences in antibody titers among deer mice testing positive for antibody to SNV ($U_1 = 658.50, P = 0.04$; Fig. 2). Specifically,

Table 1: Number of recaptured deer mice (*Peromyscus maniculatus*) infected with Sin Nombre virus

	2 Sampling Periods	3 Sampling Periods	4 Sampling Periods	5 Sampling Periods
No. males	17	4	2	1
No. females	12	3	2	0
Total	29	7	4	1

Note. We attempted to sample deer mice during six sampling periods (September, October, November 2005 and March, April, May 2006); however, not all deer mice were recaptured during each sampling period.

when averaged across sampling periods, male antibody titers (GMT = 3,333) were more than two times higher than those of females (GMT = 1,477). Plasma estradiol concentrations did not vary across sampling periods in female deer mice ($F_{5,29} = 1.84$, $P = 0.14$). There was no correlation between plasma estradiol concentrations and SNV antibody titer in female deer mice ($r^2 = 0.26$, $P = 0.19$). Plasma testosterone concentrations of male deer mice varied across sampling periods ($F_{5,44} = 21.83$, $P < 0.01$), declining monthly (~50%) from September to November ($P < 0.01$) and then increasing by 33% from March to April ($P < 0.01$) but remaining unchanged from April to May ($P = 0.75$). There was no significant relationship between levels of circulating testosterone and SNV antibody titers in male deer mice ($r^2 = 0.28$, $P = 0.07$).

Titer over Time

During the six sampling periods, we observed 11 deer mice (six males, five females) that seroconverted, indicating that they acquired an SNV infection during the study. Of these 11, three deer mice (one male, two females) seroconverted between the November and March sampling periods. Thus, because their exact month of seroconversion cannot be determined, these deer mice were excluded from the subsequent analyses. For deer mice that converted from one month to the next, the mean (GMT) antibody titer was 2,588. We found no difference in the titers of newly infected male and female deer mice (male GMT = 3,454, female GMT = 1,600; $U_1 = 6.00$, $P = 0.65$), although our sample size of only eight animals could limit our ability to detect differences. Similarly, we found no difference in the titers of recently converted deer mice from September to November compared with those from March to May (fall GMT = 5,472, spring GMT = 1,652; $U_1 = 8.00$, $P = 0.88$). However, small sample sizes could have precluded our ability to detect differences between groups.

The relationship between antibody titer and age of infection varied with analysis. There was no difference between SNV antibody titers of deer mice with old infections (determined from time of capture) and those with recent infections (old infection: $n = 37$ mice, GMT = 1,425; new infection: $n = 8$ mice, GMT = 2,822; $U_1 = 185.5$, $P = 0.39$). However, antibody titers differed between animals with recent infections and old infections as determined with the avidity assay ($U_1 = 73.5$, $P < 0.01$). Deer mice with older infections had titers more than 11 times that of deer mice with recent infections (old GMT = 13,869, new GMT = 1,189). These estimates were

based on low-avidity antibodies being detected in seven deer mice (three males, four females) and high avidity antibodies detected in 13 deer mice (nine males, four females), all of which were collected during the March, April, or May sampling periods.

Four of the animals who were used in the age-of-infection analyses had different age-of-infection classifications using the avidity assay versus the recapture data, and their classifications as old or new infections are likely to have affected GMT estimates for these categories. Specifically, three animals known to have older infections as validated by recapture data were classified as new infections based on avidity scores, and the GMT of these three animals was 400. Their omission from the older infection category in the avidity estimates inflates the average titer of that category. In addition, the age of infection of one animal with an extremely high titer (30,000) was classified as an old infection based on the avidity, whereas it tested negative in its previous capture 30 d before, suggesting that it could have been a recent infection (i.e., within the past 30 d). The inclusion of this titer in the old infection category further increases the GMT for old infections as estimated by avidity.

Discussion

Our results indicate that the immune response of deer mice to SNV has pronounced variations with respect to season, as antibody titers declined during the fall sampling periods and then increased during the spring sampling periods. Although these changes in antibody production occurred slowly, this seasonality is reflected in the general trend of the population as well as within individual deer mice that were tracked over winter. The reduction in titer may be the result of reduced immunocompetence induced by winter conditions. Winter has been associated with increased susceptibility to infectious disease, elevated disease prevalence, and higher rates of mortality in a number of mammalian species (John 1994; Lochmiller et al. 1994; Nelson and Demas 1996). For example, male Siberian hamsters show increased rates of sickness during short-day, winterlike periods compared with long-day, summerlike periods (Bilbo et al. 2002). Demas and Nelson (1996) found that deer mice maintained at 8°C had reduced spleen masses and lower levels of circulating antibodies than deer mice maintained at 20°C. Reduced immunocompetence during winter is likely the result of increased physiological stress during this period. Stress imposed by the poor resource conditions typical of winter, such as low ambient temperatures and reduced food and

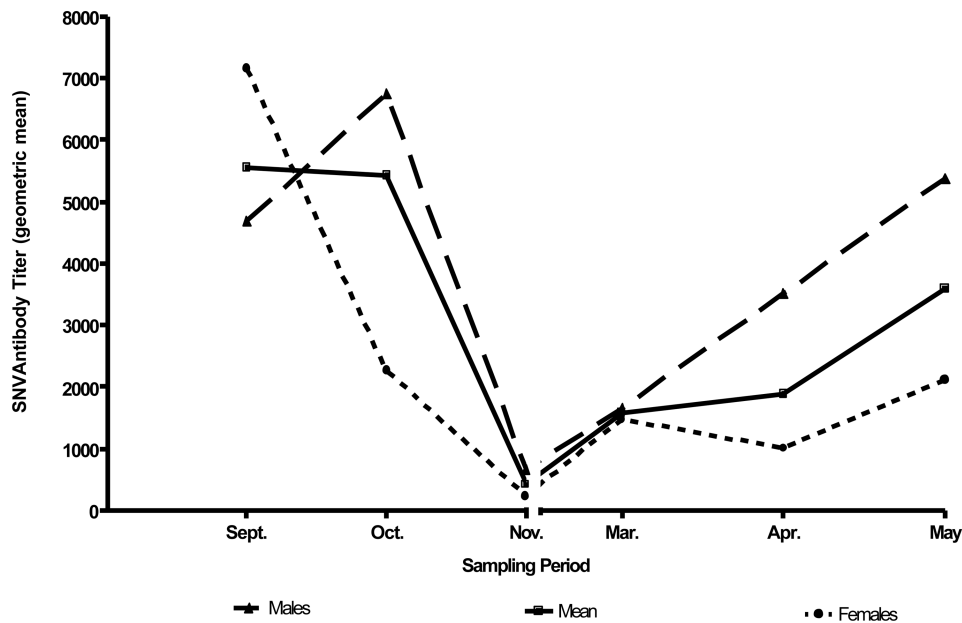


Figure 2. Changes in geometric mean antibody titers of Sin Nombre virus (SNV) measured in wild-caught male and female deer mice *Peromyscus maniculatus* over six sampling periods. Antibody titers were determined with titration enzyme-linked immunosorbent assay.

water availability, have a demonstrated effect on disease and death rates of wild animals (Pucek et al. 1993; Tkadlec and Zejda 1998; Peach et al. 1999).

Physiological condition has been cited as an important determinant of an animal's ability to mount a strong immune response (Folstad and Karter 1992; Poiani et al. 2000). However, in our study system, body mass appeared to affect the ability of males to produce SNV antibodies but did not appear to affect antibody production in female deer mice. It was noteworthy that whereas antibody titers of deer mice declined during fall and increased during spring, body masses remained relatively constant across seasons. These results support the general tenets of the resource allocation hypothesis, which predict that during winter, immunocompetence is compromised to offset the energetic costs of maintenance and thermoregulation. Because SNV does not have an obvious acute effect on deer mice (Hjelle and Yates 2001; Yee et al. 2003), it is possible that maintaining relatively constant body mass across seasons is, in a sense, a higher energetic "priority" for deer mice than the production of SNV antibodies. It is possible that a relationship between immune investment and body condition in deer mice exists for more virulent pathogens. This potential trade-off between resistance and tolerance has been recently proposed (Råberg et al. 2007; Boots 2008). Råberg and colleagues (2007) suggest that by reducing the response to non-lethal pathogens, animals increase fitness, because tolerating a mild disease caused by nonlethal pathogens may be less physiologically demanding than the cost of mounting immunological resistance. Mediating the fitness costs of SNV infection may have promoted the long and relatively stable coevolutionary history that deer mice have with SNV.

Our results indicate that deer mice show pronounced sex-

based differences in SNV antibody production. Across seasons, male deer mice produced significantly higher antibody titers than female deer mice. These results, in conjunction with those of our previous study, imply that deer mice may experience trade-offs in immune function. Although male deer mice invest in SNV antibodies to a greater extent, they have a decreased ability to respond to a novel immune challenge compared with females (Lehmer et al. 2007). Whereas several laboratory-based studies have found that females generally have higher levels of circulating antibodies and typically mount greater antibody-mediated immune responses than males (Stoeger et al. 1988; Schuurs and Verheul 1990; Olsen and Kovacs 1996; Klein et al. 2000, 2004; Nilsson et al. 2007), these patterns may not reflect those of wild mammals responding to infection with naturally occurring pathogens. Male deer mice produce robust antibody responses to SNV infection; however, they typically have higher rates of SNV infection than females (Mills et al. 1997; Douglass et al. 2007; Lehmer et al. 2007). This follows a general pattern observed in other host-pathogen systems in which males have a greater susceptibility to infection than females (Billingham 1986; Zuk and McKean 1996; Klein et al. 2000; Klein and Calisher 2007), including infections caused by parasites, bacteria, and viruses (Grossman 1985; Møller et al. 1998; Klein et al. 2000; Moreno et al. 2001). However, it is noteworthy that despite a high production of virus-specific antibodies, male deer mice are unable to fully clear SNV infection. Over time, this continued immune investment could translate into a higher fitness cost for male deer mice compared with female deer mice. One other possible explanation for these results involves differences in residual reproductive value between males and females. When an individual's future reproductive opportunities decline, organisms are predicted to in-

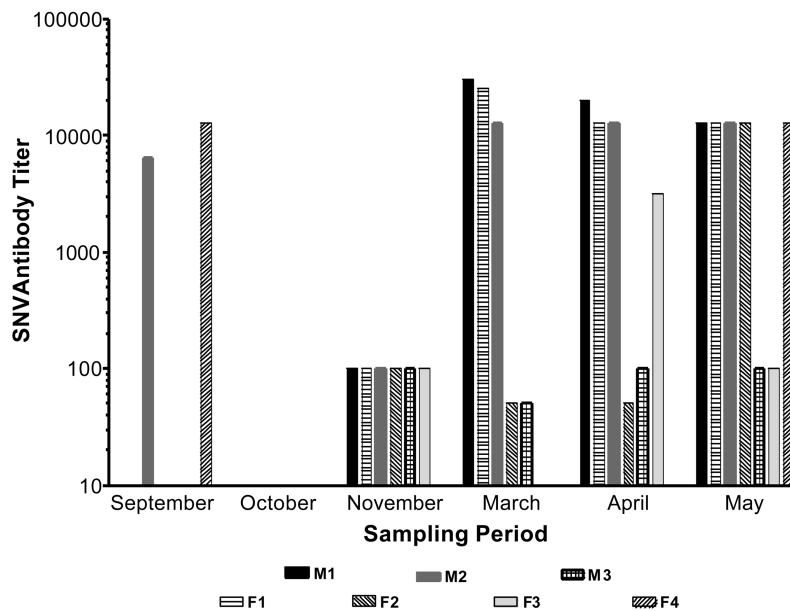


Figure 3. Geometric mean antibody titer of Sin Nombre virus (SNV) measured in wild male ($n = 3$) and female ($n = 4$) deer mice *Peromyscus maniculatus* that were captured both before winter (e.g., during the September, October, or November sampling periods) and following winter (e.g., during the March, April, or May sampling periods). Antibody titers were determined with titration enzyme-linked immunosorbent assay.

crease current reproductive investment (Minchella and LoVerde 1981; Clutton-Brock 1984). Given that the life span of female deer mice is shorter than that of males, particularly when they are infected with SNV (Adler et al. 2008), females infected with SNV may divert energy away from immunity in favor of increasing their current reproductive success. Although such fecundity compensation has rarely been documented in mammals, Schwanz (2008) found that female deer mice infected with the parasitic trematode *Schistosomatium douthii* have significantly higher reproductive outputs compared with uninfected females. Conversely, trade-offs in terminal reproductive investment have been proposed as a mechanism to explain lower parental investment among tropical birds with high adult survival rates (Wikelski and Ricklefs 2001).

It is noteworthy that we found no apparent relationship between reproductive hormone levels and antibody production in either male or female deer mice. A large body of research indicates that testosterone can have a pronounced inhibitory effect on antibody production (e.g., Grossman 1985; Alexander and Stimson 1988; Stoege et al. 1988; Schuurs and Verheul 1990; Olsen and Kovacs 1996). However, a handful of studies have demonstrated that the effects of reproductive hormones, namely testosterone, may be much less dramatic than previously recognized. For example, Klein and Nelson (1998) found that male meadow voles *Microtus pennsylvanicus* had more pronounced immune responses than did male prairie voles *Microtus orchogaster* despite having higher levels of circulating testosterone concentrations and higher reproductive organ mass. Similarly, testosterone supplementation does not suppress the production of either antibodies or eosinophils in male barn swallows (Saino et al. 1995). Wikelski and Ricklefs (2001) ad-

judicate these seemingly opposing effects of testosterone on immunocompetence by suggesting that this immune-endocrine relationship can be complicated by differences in resource availability. For example, Wikelski and Ricklefs propose that under favorable environmental conditions, individuals with elevated levels of testosterone might be capable of overresponding to immune challenges, but when resource conditions are poor, these same individuals may suffer suppressed immunity (Wikelski and Ricklefs 2001). Thus, if the trade-off between reproduction and immunity is obscured by other factors when resources are limited, our ability to detect a relationship between androgen levels and antibody production could have been limited. Similarly, it is also important to emphasize that because we did not measure and control for variation in corticosterone levels in our hormone assays (Martin et al. 2008), the lack of effect of testosterone on antibody production should be interpreted with caution, particularly because antibody production relates to seasonal stressors. Future studies should continue to explore the relationship between androgens and antibody production in natural host populations.

We did not find a consistent relationship between age of infection and antibody titer using two methods to age infection. There was no difference between the titers of animals that seroconverted within the last month and those with older infections, with age of infection estimated from recapture data. However, we did find a significant difference in titer when age of infection was estimated with an avidity ELISA. Deer mice with older infections had titers more than 11 times higher than those with recent infections. It is possible that this discrepancy arises from differences between the two methods of analysis, as the recapture method estimates age of infection by noting

seroconversions between sampling periods whereas the avidity assay uses the maturity of antibodies produced by individual animals as a proxy for the age of infection. However, it is also possible that antibody titer may directly influence the results of the avidity assay. The age of infection was incorrectly classified using the avidity score for three of 19 animals in the presence of recapture information, and the GMT for these animals was 400. In addition, one animal with a very high titer was classified as an old infection by avidity, but it tested negative in its previous capture ~30 d before, suggesting that it could have been a recent infection (i.e., within the past 30 d). It is possible that this animal could have had an older infection if it had been infected before its initial capture and if the antibodies had not yet reached a detectable level, which can take 14–21 d after infection (Botten et al. 2000). In this scenario, the resolution of our sampling regime may be the source of the discrepancy rather than the avidity assay. However, this animal also had a very high titer of 30,000. Thus, if titer indeed influences avidity, the avidity score of this animal could have been artificially elevated by its high titer. In summary, the lack of congruence between the two methods to age infections coupled with the titer data suggest that titer may confound the results of the avidity assay, particularly in the case of low-titer serums. Older infections with low-antibody titers may be misclassified as low-avidity samples, resulting in an overestimation of the number of recent infections. In addition, if low-titer samples are rarely classified as high avidity, the average titer of old infections may also be artificially inflated, further exaggerating the difference between the titers of high- and low-avidity samples. More study is necessary to evaluate the effect of titer on the avidity assay.

Our study underscores the complexity of natural host-pathogen systems and illustrates the multitude of factors that contribute to immunological response of wild animals to infectious agents. The results indicate that immune system function of deer mice is seasonally variable. While this variation in immunocompetence may be dependent on seasonal stressors, our results do not suggest that the energetic cost of reproduction is the primary stressor responsible for this variation. Our results support the general tenets of the resource allocation hypothesis because deer mice may compromise immunocompetence to offset the energetic costs of maintenance during winter. Because the immune investment of deer mice remains elevated for extended periods following infection with SNV, this investment may result in other long-term energetic trade-offs.

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