Fine-scale genetic structure of woodrat populations (Genus: *Neotoma*) and the spatial distribution of their tick-borne pathogens

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Abstract

Dusky-footed woodrats are territorial cricetid rodents that individually occupy large stick houses from which they foray to gather food, find mates, and engage in other activities. These rodents are often bitten by *Ixodes* spp. ticks and are reservoirs of some strains of tick-borne bacterial pathogens such as *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. Limited dispersal by hosts and vectors could create fine-scale population structure where related hosts and pathogen exposure are co-distributed in space. To quantify population genetic structure and infection status, we genotyped 167 woodrats using a panel of 15 microsatellite loci from three northern California study sites: Soquel (SD), Cold Canyon (CC), and Quail Ridge (QR). We used

quantitative PCR and serology to test for infection with *A. phagocytophilum* and *B. burgdorferi*. All three populations maintained similar, moderately high levels of genetic variation. For *A. phagocytophilum*, the PCR-prevalence was higher at SD (30.0%) than CC (13%) and QR (7%) whereas the seroprevalence was similar at all three sites (13-18%). The *B. burgdorferi* PCR-prevalence at CC was 11%, no woodrats were PCR-positive at QR, and none were tested at SD. We found a negative correlation between pairwise genetic relatedness and spatial distance with the majority of highest order relatives occurring within 200 meters of one another. Related dyads were more likely to be adult females than males, suggesting that adult female residents are the primary source of spatially proximate, high-order relatives in woodrat populations. Despite spatial genetic clustering of hosts, our spatial window test found no significant clustering of pathogens. Woodrats that were seropositive for *A. phagocytophilum* had higher heterozygosity than seronegative woodrats, which could be consistent with genetically diverse individuals having greater capacity to mount an immune response. Overall, our analyses show that limited dispersal of individual woodrats leads to fine-scale genetic structure within populations. Genetic structure, coupled with the limited dispersal of *I. pacificus* ticks could result in disease dynamics that are uniquely restricted to small spatial scales. By combining host genetic and disease studies we are able to infer limited dispersal and structured populations among hosts which affect infectious disease clustering and dynamics.

Introduction

The dusky-footed woodrat, *Neotoma fuscipes* Baird, is a large cricetid rodent found conspicuously and at sometimes high density in many coastal areas from Oregon through the San Francisco Bay Area to Monterey Bay, through the Diablo Range on the west side of the Central Valley and along the foothills of the northern Sierra Nevada Mountains (Carraway and Verts 1991). This species is territorial, aggressive, and solitary with the exception of females with young

1 (Linsdale and Tevis 1951, Cameron 1971). Each individual woodrat occupies a “house” which may
2 consist of chambers among boulders or in downed logs or stumps, large above-ground stick-
3 structures sometimes a meter high, or even stick-houses in tree canopies (English 1923). Particular
4 houses seem to be preferred by virtue of location, size, protection from elements etc., and turnover
5 in house occupation occurs when a resident dies or emigrates and another moves in or when a
6 more dominant individual drives a resident from its house (Linsdale and Tevis 1951).

7 *Neotoma* populations are moderately kin-structured (Matocq and Lacey 2004). Although
8 both males and females are known to disperse, females are the more philopatric sex and typically
9 remain within 100-200 meters of their natal house (Matocq and Lacey 2004). Spatial structuring of
10 woodrat populations and patterns of individual movement are of interest in part because they help
11 determine the spatial structure and epidemiology of woodrat ectoparasites such as ticks and their
12 tick-borne pathogens. Understanding spatial genetic patterns of hosts is helpful for two reasons.
13 Such patterns arise from ecological history, particularly in a spatially structured species like dusky-
14 footed woodrats, and interpreting these patterns helps us understand how ecological history may
15 have influenced disease exposure and transmission patterns.

16 Throughout most of their range, dusky-footed woodrats are exposed to the Pacific black-
17 legged tick, *Ixodes pacificus* (Furman and Loomis 1984). This tick is the main vector of two
18 obligately tick-transmitted bacteria: *Borrelia burgdorferi* which causes Lyme disease and
19 *Anaplasma phagocytophilum* which causes granulocytic anaplasmosis (Lane and Loye 1989, Richter
20 et al. 1996). Larval and nymphal *I. pacificus* feed preferentially on small mammals or lizards
21 whereas adult ticks prefer larger hosts such as deer (Furman and Loomis 1984). In general *I.*
22 *pacificus* ticks have limited dispersal ability except during the few days when they are attached to a
23 host, which occurs once per developmental stage. One study showed that nymphal *I. pacificus*
24 moved no more than three meters unless on a host (Lane et al. 2009).

Host populations may exhibit genetic and spatial heterogeneity in their immune response to parasites and pathogens. Woodrats are reservoir hosts for some strains of *B. burgdorferi* and *A. phagocytophilum* (Brown and Lane 1992, Foley et al. 2008). Genetic studies have shown that these pathogens each comprise a genospecies complex and woodrats commonly carry strains that are closely related to but different from the genospecies that infect humans or domestic animals (Brown et al. 2006, Rejmanek et al. 2012). In fact the reproducible findings of relatively specialized ticks and tick-borne pathogens within woodrat populations suggest that there has been possible coevolution among the hosts, ticks, and pathogens (Foley et al. 2009b, Rejmanek et al. 2013).

The specific goals of this study were to characterize spatial genetic structuring of woodrats in three different study sites and to associate the spatial distribution of the host with the spatial distribution of two species of tick-borne pathogens. We hypothesized that the woodrat population will be genetically structured in such a way that reflects relatively minimal dispersal capacity, leading to the local accumulation of relatives, especially females, on the landscape. In conjunction with their hosts/reservoirs, we hypothesized that *Anaplasma* and *Borrelia* prevalence will demonstrate a clustered spatial distribution.

Materials and Methods

Study sites and sample collection

Woodrats were trapped and sampled at three sites: 1) Quail Ridge (QR) University of California Reserve, located near Lake Berryessa in Napa County, 2) Stebbins Cold Canyon (CC) University of California Reserve in western Yolo County, and 3) Soquel Demonstration Forest (SD) in Santa Cruz County. Vegetation at QR where trapping was conducted consists of riparian woodlands and dense oak woodland, including valley oak (*Quercus lobata*), interior live oak (*Q. wislizeni*), blue oak (*Q. douglasi*), gray pine (*Pinus sabiniana*), poison oak (*Toxicodendron diversilobum*), redbud (*Cercis occidentalis*), California wild grape (*Vitis californica*), toyon

1 (*Heteromeles arbutifolia*), whiteleaf manzanita (*Arctostaphylos manzanita*), hairy ceanothus
2 (*Ceanothus oliganthus*), and sticky monkeyflower (*Mimulus aurantiacus*). Trapping occurred at two
3 sites, 8.57 and 3.6 ha, respectively. At CC, woodrat trapping was conducted primarily across 12 ha
4 on a steep north-facing slope where vegetation comprises gray pine, interior live oak, grasses,
5 toyon, yerba santa (*Eriodictyon glutinosa*), and poison oak. SD is a managed forest with
6 heterogeneous vegetation regimes. Woodrats were captured in discontinuous stands of second-
7 growth Douglas fir (*Pseudotsuga menziesii*) and coast redwood (*Sequoia sempervirens*), and mature
8 coast live oak (*Quercus agrifolia*) over a combined area of 8.3 ha.

9 Trapping started in February 2010 and extended through 2012. Two extra-large Sherman
10 (HB Sherman, Tallahassee, FL) or small Tomahawk (Hazelhurst, WI) live traps were baited with
11 peanut butter and oats and set at each active woodrat house. Woodrat houses were identified by
12 the typical stick structure or excavation under rock or in downed logs with open entrances, fresh
13 dirt, and fresh feces. GPS coordinates were recorded at each successful capture site using a hand-
14 held device (Garmin 62S GPS, Garmin International, Olathe, KS). Upon capture, each animal was
15 anesthetized with approximately 15 mg ketamine and 1.5 mg xylazine per kg body weight. A
16 physical examination was performed, the animal was weighed, and sex and body condition score
17 (Ullman-Cullere and Foltz 1999) were determined. A permanent, individually-numbered metal ear
18 tag (1005-1 Monel, National Band and Tag Co., Newport, KY) was placed. A blood sample was
19 collected into EDTA via abrasion of the retro-orbital sinus. We used scissors to collect a small
20 sample of ear tissue via sterile technique and preserved this tissue in 70% ethanol. Any ticks were
21 removed into 70% ethanol. The animal was released into its house upon recovery from anesthesia.
22 All animals were handled under the authority of the UC Davis Campus Veterinarian and IACUC.
23 Trapping was subject to approved permits from the California Department of Fish and Wildlife, UC
24 Reserve System, and Calfire.

Woodrat genetics

DNA for genetic testing was extracted from 50 µl blood using a Qiagen blood and tissue kit (Qiagen, Valencia, CA), following manufacturer's guidelines. We genotyped the following 15 microsatellite loci: Nfu1, Nfu4 (Matocq 2001); Nlep1, Nlep3, Nlep7, Nlep11, Nlep12, Nlep13, Nlep16, Nlep17 (Sousa et al. 2007) and Nma04, Nma10, Nma11, Nma14, Nma15 (Castleberry et al. 2000). Reactions consisted of 100-200 ng of DNA, 3 µL of 2x Qiagen Multiplex PCR Master Mix (Qiagen), 0.5 µL of 10 µM forward and reverse primers, and distilled water in a total reaction volume of 15 µL. We multiplexed samples in 4 panels containing 3-4 loci each. All forward primers were 5' modified with 6-FAM, HEX, NED, or PET fluorescent labels (Applied Biosystems) and used under the following conditions: initial denaturation at 95°C for 15 min; 35 cycles of 94°C for 30 s, 56-67°C for 1 min 30 s, and 72°C for 1 min; and final extension at 60°C for 30 min. We diluted amplified PCR products (1:100 to 1:500 depending upon the locus) and combined 1 µL of diluted PCR product with 10 µL of Hi-Di Formamide (Applied Biosystems), 0.005 µL of GeneScan 500 LIZ Size Standard (Applied Biosystems), and 9 µL of distilled water. We resolved fragments on an ABI 3730 DNA Analyzer (Applied Biosystems) in the Nevada Genomics Center, University of Nevada, Reno. We scored genotypes using GeneMarker v 1.95 (SoftGenetics, State College, PA). Any sample with ambiguous peaks or rare alleles was re-genotyped in addition to ~30% of the total sample to ensure scoring consistency.

Pathogen testing

Blood and ear tissue samples were not available for every animal, so pathogen testing was done where possible and the results were reported for each pathogen as the percentage of infected (or seropositive) individuals. Blood sample DNA for testing of *A. phagocytophilum* was extracted as described above. Ear tissue for testing of *B. burgdorferi* was surface-sterilized with 10% bleach for 5 min and then finely minced with a sterile blade. 25 mg samples were digested overnight in

1 proteinase K and buffer ATL and the Qiagen blood and tissue spin column kit was used to complete
2 DNA extraction following the manufacturer's instructions. We screened samples for *A.*
3 *phagocytophilum* DNA using TaqMan real-time PCR (RT-PCR) in a combined thermocycler/
4 fluorometer (ABI Prism 7700, Applied Biosystems, Foster City, CA). We modified the RT-PCR for *A.*
5 *phagocytophilum* (Drazenovich et al. 2006) to have the following thermocycling conditions: 60 °C
6 for 30 s, 95 °C for 10 min, and 40 cycles at 95 °C for 30 s, followed by 60 °C for 30 s. We considered
7 PCR reactions to be positive if they had a cycle threshold value < 40 and characteristic amplification
8 plots. We performed RT-PCR for *B. burgdorferi* as previously described (Barbour et al. 2009), also
9 using a cycle threshold value < 40 as a cutoff for a positive test result. To assess for *A.*
10 *phagocytophilum* antibodies, we modified an indirect immunofluorescent antibody (IFA) assay
11 (Asanovich et al. 1997) as follows. Serum samples were diluted 1:75 in phosphate buffered saline
12 (PBS) and applied to commercially available IFA slides (VMRD, Pullman, WA), allowed to incubate
13 in moisture for 30 min, and then washed three times with PBS. We applied an FITC-conjugated goat
14 anti-rat heavy and light chain IgG secondary antibody (KPL, Gaithersburg, MD) diluted 1:100 in
15 PBS, incubated as before, washed three additional times, and added 3 drops of eriochrome black as
16 a counterstain during the last wash. Slides were assessed under UV fluorescence for bright green
17 characteristic morulae within cells. Each run contained negative (water) and positive (sera from
18 previously experimentally infected woodrats) controls. Although weak green fluorescence can be
19 seen in some false-positive samples and at high dilutions of true positive samples, use of a high
20 dilution (1:75), comparison to known positive controls, and the requirement for strong
21 fluorescence targeted only to morulae contribute to maximum specificity for this assay.

22 23 *Statistical analysis*

24 Data were maintained in a database (FileMaker, Santa Clara, CA) and analyzed with the
25 statistical program R (R Core Team 2014). For hypothesis testing, a P-value of 0.05 or less was

1 considered evidence of statistical significance. Deviation in sex ratio from 1 female: 1 male was
2 assessed at each site with a chi-square goodness of fit test. Population sizes were estimated from
3 recaptures of tagged woodrats using a Lincoln-Peterson estimator (Braun 2005) implemented in
4 the R package “mra”, and density calculated for the area that encompassed all points in which
5 woodrats were detected.

6 We estimated standard diversity indices and tested for Hardy-Weinberg equilibrium in
7 GenAlEx (Peakall and Smouse 2006, Peakall and Smouse 2012). We quantified the amount of
8 genetic divergence among the three sampling localities using pairwise F_{ST} as implemented in the
9 AMOVA (codominant-allelic) subroutine of GenAlEx, with 999 permutations to assess significance.
10 We estimated genetic and geographic distances among individuals and conducted Mantel tests (999
11 permutations) in GenAlEx. Because the SD locality was sampled at a much broader spatial scale and
12 using transects that were spatially distant from one another, we limited our analysis of the
13 relationship between genetic and geographic distances to CC and QR that were sampled in a more
14 spatially continuous fashion. To quantify patterns of relatedness within each population, we
15 estimated ‘r’ using the approach of Queller and Goodnight (Queller and Goodnight. 1989) as
16 implemented in GenAlEx. For relatedness estimates in CC, locus Nma14 was removed prior to
17 analysis because it was monomorphic in this population. As the larger sample sizes at the CC site
18 allowed even more thorough assessment of fine-scale genetic patterns, we also conducted sex-
19 specific genetic spatial autocorrelation analyses of this site as implemented in GenAlEx using the
20 Multiple Dclass subroutine. We binned individual comparisons by 10 meter increments since
21 immediate neighboring woodrats are typically within a 10 meter radius of a focal woodrat house.
22 We estimated the autocorrelation statistic ‘r’ within each spatial bin for males and females and
23 report the mean and 95% bootstrap confidence intervals, with significance assessed using 999
24 permutations. Finally, as a measure of genetic diversity at the individual level, we estimated
25 individual heterozygosity as the proportion of loci at which an individual is heterozygous.

We visualized the fine-scale spatial distribution of individual heterozygosity and relatedness by mapping these surfaces as done previously (Vandergast et al. 2011), except that our samples constituted individual animals instead of populations. We used inverse distance weighting to interpolate values for unknown locations using a power of two for all maps and considering 12, 20, and 40 neighbors for individual heterozygosity, pairwise relatedness for all individuals, and pairwise relatedness of just females using ArcGIS 10.1 (Environmental Systems Research Institute, Inc., Redlands, CA). We performed interpolations on pairs of points by generating Voronoi polygons, drawing lines connecting samples in neighboring polygons, calculating the midpoints of those lines, and using the difference between pairs of samples at the midpoint to perform the interpolation. In instances where two or more samples had the same spatial location (i.e. caught at the same trap station), we randomly jittered the position of one or more woodrats up to two meters from the site of capture to allow visualization of multiple individuals and their associated genetic parameters.

Differences in numbers of animals that were disease test-positive between the sexes were evaluated by chi-square tests and among sites with Fisher's exact tests. During analysis, we observed that there was a low correspondence between the serology and qPCR data for *A. phagocytophilum*. We therefore calculated a kappa coefficient for all woodrats combined and for each site separately, with interpretations of values as previously described (Martin et al. 1987). Latitude and longitude for each woodrat house were plotted using qGIS (www.qgis.org) to create maps of woodrats by genotype and infection status. Spatial clustering in pathogen infection (PCR status) or exposure (serostatus) among woodrats was evaluated using SaTScan (Version 9.3) to calculate a scan statistic by considering overlapping cylinders as possible scanning windows of coordinate points (Kulldorff et al. 2005). A maximum spatial cluster size of 50% of the population at risk was used. Clusters were evaluated by comparing the number of expected positive results if randomly distributed in space with the observed number of positive results. P-values were generated by performing 999 random Monte Carlo simulations under the null hypothesis.

We compared mean individual heterozygosities across sites (where possible) among woodrats that were test-positive or negative by PCR for *A. phagocytophilum* or *B. burgdorferi* or seropositive for *A. phagocytophilum* by two-way ANOVA including site, pathogen status, and interaction terms, followed by a Tukey's test to assess for post-hoc differences. We also determined whether sero- or PCR-prevalence was associated with site average heterozygosity by linear regression. In order to determine whether there was a co-association of genotype and pathogen status in space, we ran a series of logistic regressions. The response variables were the results of each pathogen test, and predictor variables were relatedness of each focal woodrat and its nearest neighbor, pathogen test status of the nearest neighbor, and the interaction term.

Results

Woodrat numbers and density

We captured 173 individual woodrats across three sites, including 30 at QR, 97 at CC, and 46 at SD. The proportions of captures across the sites that were male woodrats were 0.3 at QR, 0.47 at CC, and 0.37 at SD. Deviations from a 1:1 male:female sex ratio were not statistically significant in any population. Estimates of population sizes in the areas we studied, averaged over sampling periods, were 37 for QR, 53 at CC, and 57 at SD. The calculated densities of woodrats at these sites were 3.0 woodrats per ha at QR, 4.4 per ha at CC, and 6.9 per ha at SD.

Genetics of woodrat populations

We obtained multi-locus genotypes for 167 individuals (QR N = 25; CC N = 96; SD N = 46) and found a high level of consistency in scores among multiple runs with less than 1% error rate. No loci showed significant deviations from Hardy-Weinberg equilibrium in more than one locality, thus, we retained all loci in further analyses. Consistent with their geographic proximity, CC and QR were significantly but minimally differentiated from one another with a pairwise F_{ST} of 0.02 ($p =$

0.001). Both of these northern populations were substantially differentiated from the SD population ($F_{ST} = 0.17$ SD vs. CC; $F_{ST} = 0.14$ SD vs. QR; $p < 0.001$). All three populations maintained moderately high and similar numbers of alleles and average heterozygosity (Table 1). QR had the lowest average number of effective alleles (5.1) and SD the highest (6.5), while CC had the lowest average unbiased heterozygosity (0.69) and SD the highest (0.79).

There was a negative correlation between pairwise genetic relatedness and spatial distance in both the QR ($z = -9.02$, $r = -0.342$, $p < 0.001$) and CC populations ($z = -16.21$, $r = -0.192$, $p < 0.001$; Figure 1; only QR shown). At QR, the highest order relatives ($r > 0.25$) were always located within approximately 200 meters of one another. Of the 17 dyads with relatedness greater than 0.25 and for which sex of both animals was known, 7 (41%) were composed of two females and only one dyad (5.8%) was composed of two males but this result was not significant (chi-square test, $p = 0.117$). CC was characterized by a similar pattern of declining genetic relatedness with spatial distance; again, the most highly related individuals ($r > 0.25$) typically occurred within 200 meters. At CC, of the 264 dyads of known sex with relatedness > 0.25 , approximately equal proportions included two females ($N = 68$, 26%) and two males ($N = 64$, 24%). This balanced pattern remained consistent at the first-order relative level ($r > 0.5$) with female-female dyads accounting for 21% of the total (9 of 43) and male-male dyads accounting for 23% (10 of 43).

Our genetic spatial autocorrelation analyses of the CC locality show that both males and females have significantly positive genetic structure at fine spatial scales (r values above the upper limits of random distribution of genetic variation; Figure 2). At the smallest distance classes, females tend to be surrounded by more highly related individuals than males (e.g. female $r = 0.39$ versus male $r = 0.05$ within 10 meters; not significantly different than random for males), and this positive genetic structure in females declines monotonically with distance. Positive spatial structure in the female population extends the entire sampling distance taken at CC ($p < 0.05$

through ~280 meters), while male positive genetic structure extends to ~ 200 meters (with exception of the first distance bin < 10 meters).

Most woodrats at CC had moderate levels of individual genetic diversity, although the northeastern and southeastern quadrants of the study site seem to have mild aggregations of individuals with relatively low heterozygosity (Figure 3). In terms of pairwise relatedness across the site, we find that the major subdivisions in relatedness (blue areas, Figure 3C and D) are similar whether we consider all woodrats (Figure 3C) or just females (Figure 3D).

Spatial ecology of pathogens in woodrats

There were no differences between male and female woodrats for *A. phagocytophilum* or *B. burgdorferi* serology or PCR (all $p > 0.1$) and there were not enough juvenile animals tested to assess for differences by age. Woodrats tended to be either both sero- and PCR-negative for *A. phagocytophilum* or positive on one test but not the other, based on kappa coefficients which were 0.112 for all sites combined, 0.055 for CC, 0.315 for SD, and 0 for QR. Significantly more woodrats were *A. phagocytophilum* PCR-positive at SD (30%), compared with 13% at CC and 7% at QR ($p = 0.01$, Table 2). Serological results were very similar at all three sites, from 13-18% prevalence ($p = 0.69$). For *B. burgdorferi*, testing was not available for SD. Although the *B. burgdorferi* PCR-prevalence at CC was 11% and no woodrats were *B. burgdorferi* PCR-positive at QR, this difference was not significantly different ($p = 0.12$).

A SaTScan spatial window test was run to assess for clusters at each site for each pathogen test and no significant tests were detected. Nevertheless visual inspection highlighted some interesting patterns. Woodrats at SD clustered into five discrete locations and our unpublished data show that woodrat houses continue in patches in all directions beyond those targeted for trapping in this study. One *A. phagocytophilum* PCR-woodrat was found just outside the forest near an

entrance gate in a very steep site of chaparral habitat (Figure 4A). There were 13 woodrats (five of which, 38.5%, were *A. phagocytophilum* PCR-positive) in a relatively small fragment of mature coast live oak forest (Amaya transect) in the northwest corner where woodrat houses are very dense. Another cluster of eight woodrats (*A. phagocytophilum* PCR-prevalence of 12.5%) was found in an ecotone transitioning from mixed redwood to a large managed meadow of primarily Mediterranean weeds (LZ transect). Woodrats from Braille Trail, gulches on Corral Road, and at the Corral Road extension spur occurred in mixed conifer sites with very steep hillsides, relatively closed canopy with Douglas fir, coast redwood, madrone, and a small amount of coast live oak: *A. phagocytophilum* PCR-prevalence among these woodrats was 31.8%. Woodrats in live oak had an *A. phagocytophilum* seroprevalence of 20%, while those in mixed conifer forest had 14.3% and those at the ecotone 0.0% (Figure 4B).

At the CC site, woodrats were distributed almost homogenously across a steep (approx. 45% slope) north-facing hillside where the predominant vegetation was Mediterranean weed understory in a mixed oak-pine grassland. Uphill of this area is dense chaparral and downhill is a creek canyon, neither of which support woodrats. Spatial analysis revealed two interesting phenomena: *A. phagocytophilum* PCR-positive woodrats tended to predominate to the north of a trail (PCR-prevalence 24.2%) vs. south (none positive among 32). Moreover serology showed an opposite pattern (seroprevalence in north of 9.1% vs. 31.2% in south)(Figures 5A and 5B). The four *B. burgdorferi* PCR-positive woodrats were evenly divided across the trail (5C).

At the QR site, two main sets of houses were separated by a small road onto north vs. south-facing slopes: the woodrats on the north-facing slope tended to be found in a denser forest of mixed oak and grape, while the woodrats on the south-facing slope were in a much less closed canopy pine-oak grassland. All woodrats were PCR-negative for *B. burgdorferi*. Serology and PCR for *A. phagocytophilum* were discordant: in the woodrats on the south-facing slope, seroprevalence was 0

1 while PCR-prevalence was 12.5%, compared with seroprevalence of 27.3% and PCR-prevalence of
2 0% among the woodrats on the north-facing slope (Figures 6A and 6B).

3 4 *Population genetics of woodrat disease*

5 Mean individual heterozygosities did not differ significantly between *A. phagocytophilum*
6 PCR-positive and negative woodrats at all sites combined, but were significantly different for
7 serology for that pathogen ($p = 0.02$) and *B. burgdorferi* PCR ($p = 0.05$, Table 3). There were only
8 two sites tested by PCR for *B. burgdorferi* of which QR had all negative woodrats, so comparison
9 between sites could not be performed because of confounding. The most significant finding was
10 that woodrats at all sites that were *A. phagocytophilum* seropositive tended to have higher
11 heterozygosity than seronegative woodrats, with no significant interaction term (site $F = 3.8$, $df = 2$,
12 $p = 0.025$; serostatus $F = 5.3$, $df = 1$, $p = 0.022$; interaction $F = 0.3$, $df = 2$, $p = 0.73$). Post-hoc testing
13 revealed a significant difference (adjusted $p = 0.021$) in heterozygosity between SD and CC, with the
14 adjusted p for serostatus = 0.022. At Cold Canyon where we had the greatest number of woodrats
15 and results from all three tests, we found a higher mean heterozygosity for *A. phagocytophilum*
16 PCR-negative animals and a higher value for seropositive animals. At both QR and SD, *A.*
17 *phagocytophilum* sero- and PCR-positive animals tended to have increased heterozygosity than
18 corresponding negative animals. Visual inspection of the *A. phagocytophilum* antibody status of
19 individual woodrats overlain on the spatial patterns of heterozygosity and pairwise relatedness
20 does not suggest any obvious pattern (Figure 3). At the site level, linear regression of mean
21 heterozygosity among woodrats and PCR- or seroprevalence for either pathogen was not significant
22 ($p > 0.3$ for all). Logistic regressions using pairwise relatedness and pathogen test status of nearest
23 neighbor to predict *A. phagocytophilum* serostatus, PCR status, or *B. burgdorferi* PCR status were
24 not significant ($p > 0.4$ for all).

Discussion

Dusky-footed woodrats are perhaps best known for the ostentatious stick-houses they construct across landscapes with suitable vegetational and abiotic conditions, although perceptive natural historians can often find additional houses under logs and rocks and even in the tree canopy. Their aggressive territorial defense of the houses, solitary behavior except with young, ectoparasite fauna within houses, and dispersal behavior all constrain the ecology of vector-borne pathogens to which they are exposed. Dusky-footed woodrats are reservoirs for at least some genospecies of the tick-transmitted pathogens *B. burgdorferi*, *A. phagocytophilum*, and *Theileria youngi* (Kjemtrup et al. 2009), and probably important hosts for other vector-borne pathogens including *Yersinia pestis*, the agent of plague (McCoy 1910), *Trypanosoma cruzi* which causes Chagas disease (Bern et al. 2011), and other pathogens as well. They are parasitized by generalist ticks such as *I. pacificus* and specialist, nidicolous (nest-dwelling) ticks including *I. spinipalpis* and *I. woodi* which the rodents encounter in their nest cups (Furman and Loomis 1984). In this study we explored spatial patterns of woodrat occupancy at three northern California study sites, genetic relatedness among the woodrats at these sites, and how the spatial distribution of woodrats might influence the pathogen distribution. Although strong influences were not detected, animals with higher individual heterozygosity were more likely to be seropositive for *A. phagocytophilum*. Our findings also supported earlier work showing limited dispersal and intermediate philopatry in the related large-eared woodrat (Matocq and Lacey 2004), and studies across northern California of variable prevalence of pathogen exposure and infection (Foley et al. 2009a). The limited dispersal of individual woodrats, coupled with the inability of *I. pacificus* ticks to move more than a few meters unless on a host, would serve to limit broad dispersal of woodrat tick-transmitted pathogens and could support disease dynamics that are uniquely restricted to small spatial distances defined by daily individual woodrat activity.

Superimposed on the spatial patterns affecting the opportunity to acquire tick-borne infections is the host response to infections once exposed. We found that woodrats with higher heterozygosity tended to have higher probabilities of being *A. phagocytophilum* seropositive, which could suggest that they are more likely to mount robust, detectable antibody responses after exposure, given that the ecological data support broad pathogen distribution. This result also suggests that more fine-tuned genetic evaluation, such as genotyping particular genes associated with immunity (such as MHC, cytokine, or other related genes), could provide greater insight into population patterns of innate host responsiveness. Association of outbreeding with parasitism has received attention previously across a range of disease systems (Schmid-Hempel 2011). Of studies that did examine pathogen load or prevalence depending on inbreeding, most either found that low heterozygosity or inbreeding were associated with higher pathogen load or susceptibility, or that there was no effect. Interestingly, various populations of Galapagos hawk on different islands showed evidence of low heterozygosity that was a function of small island size and populations with lower average heterozygosity had higher amblyceran louse abundance (Whiteman et al. 2006). However, innate antibody production varied in a way not directly related to inbreeding, with more outbred populations having intermediate titers, which may have some similarities to the woodrats in this study. The interpretation of test agreement based on kappa coefficient may be relevant. It appeared that actively infected (*A. phagocytophilum* PCR-positive) woodrats actually tended not to be seropositive which is unexpected. One possible explanation is that actively infected animals are somehow more tolerant of the bacterium (which may be a commensal in this host species), resulting in lower or negative serotiters. More work is needed to examine woodrats and other reservoir small mammal reservoirs of tick-borne pathogens, but it appears that there is often little or no fitness impact of these pathogens on the host (Brown et al. 2005). As an example, *B. burgdorferi* can have a spectrum of impacts on small mammals, from no influence on health or survival, to differential antibody responses across different reservoir species (Kurtenbach et al.

1994, Schwanz et al. 2011, Voordouw et al. 2015). These results highlight that host antibody production may be altered in inbred animals and may be difficult to predict for reservoir hosts.

Spatial epidemiology is crucial to understand spatial heterogeneity in disease transmission (Ostfeld et al. 2005) and literally thousands of studies have used spatial tools to understand pathogen distribution, risk, and related questions. More uncommon is exploration of spatial genetic epidemiology, although it has been done for a diversity of pathogens including influenza, chronic wasting disease, and rabies (Archie et al. 2009). Much rarer still is spatial genetic epidemiology of spatially structured host populations, even though such studies can show how host spatial structuring (e.g. by philopatry) could reflect ecological factors that created patterns that influence hosts *and* their diseases as well, and could indicate spatial heterogeneity in host susceptibility. A study of hookworms in Africa incorporated demographic and human host genetic risks to show that the factors affecting infestation did in fact include host relatedness but these were less important than presence of mud flooring in home, education level of head of household, and unidentified household factors (Pullan et al. 2010).

Our study focuses on spatial genetic epidemiology of reservoir hosts. Numbers of alleles, individual heterozygosity and relatedness, and population mean heterozygosity and other genetic parameters were characteristic of a moderately out-bred small mammal with limited dispersal and large effective population sizes. The data are consistent with previous work on the large-eared woodrat which demonstrated that young animals, particularly females, dispersed away from natal houses but typically only a few houses beyond, creating local zones of line-bred, related families (Matocq and Lacey 2004). Based on telemetry, woodrats may disperse less than < 50 m on occasion (further if in disturbed habitat), but then animals commonly returned to their original site (Sakai and Noon 1993). However, unlike previous genetic studies of woodrats, the populations we studied occurred in highly vegetatively heterogeneous habitats, particularly at SD. We would expect such

1 heterogeneity to be reflected in genetic (and disease) patterns but think that the larger scale of
2 sampling at SD relative to CC and QR makes such comparisons difficult.

3 Male-biased dispersal in other woodrat populations results in local populations that are
4 composed of largely unrelated males (Matocq and Lacey 2004). At CC, we found significantly
5 positive genetic structure among males, albeit to a lesser spatial extent and lesser magnitude
6 (lower r values) than in females. It is possible that male dispersal tendencies are consistently
7 different at CC in comparison to other woodrat populations, but we suspect that the relatively high
8 estimates of relatedness, especially among males, were caused by the capture of pre-dispersal
9 individuals during our sampling. Indeed, the 1:1 sex ratio in our sample of CC suggests that pre-
10 dispersal animals remained on site since sex ratios of adult residents are typically female biased
11 (Matocq and Lacey 2004). As found in other woodrat populations, and consistent with our results
12 for CC, most female relatives are found spatially proximate to one another, although any one female
13 also has several unrelated neighbors (Matocq and Lacey 2004). How levels of relatedness among
14 neighboring animals determine contact rates and potential for pathogen transmission remains to
15 be studied. Likewise, the spatial extent of relatively genetically “homogenous” hosts may play a role
16 in the local transmission and maintenance of disease on the landscape. The role of sex-specific
17 differences in the spatial extent and magnitude of genetic homogeneity in pathogen dynamics needs
18 further attention.

19 Daily activities within stable territories contribute to the patterns we observed in infection
20 status. Woodrats do forage outside their houses and during such times territories may overlap,
21 serving as a means by which woodrats can share parasites and disease. Male woodrats may alter
22 their home ranges, which are significantly larger than those of females (2300 m² vs. 1900 m²), to
23 promote overlap with particular females in estrous (Cranford 1977). Woodrats (and some
24 sympatric *Peromyscus* spp.) may occupy multiple houses simultaneously or serially, which
25 represents a nidus of infection for parasites. Mean residence time per house is 34 days (Cranford

1977), although we have telemetry data in SD showing one woodrat occupying three different houses over three sequential nights (Foley, unpub. data). Movement patterns change by season, year, habitat, food availability, and population size, so all of these things would change the tick-borne disease risk landscape. No statistically significant clusters of PCR- or seropositive woodrats were detected in the present study, consistent with the null hypothesis that *A. phagocytophilum* and *B. burgdorferi* are relatively homogeneously distributed in woodrat populations in northern California, although we did find areas with higher densities of pathogen infection and exposure in SD and QR. Because the pathogens we evaluated are obligately tick-transmitted, spatial spread of the pathogens is determined by the spatial spread of the ticks, which may include questing *I. pacificus* as well as nidicolous ticks that remain in the nest except when on a host (e.g. *I. spinipalpis* and *I. woodi*). The interplay of subadult male woodrat dispersal and female tendency to stay near but not at natal houses distributes infected ticks to a limited extent. However, hosts of the adult ticks, such as deer, and hosts like birds which carry juvenile ticks long distances (Dingler et al. 2014) would obviously have greater ability to disperse ticks and could disrupt tighter spatial patterns driven by reservoir host spatial structure. However, neither *A. phagocytophilum* nor *B. burgdorferi* have transovarial transmission, so adult tick dispersal is irrelevant for the distribution of infective ticks. It would also be interesting to consider how patterns in endemic disease might differ from those early after introduction of a pathogen in a landscape. Additional study of pathogen spatial structure *per se* could reveal interplays of host, tick, and tick-host interactions, as pathogens spread after introduction.

In total now, three populations of dusky-footed woodrats and one of large-eared woodrats have been shown to have population genetic characteristics of intermediate philopatry. The intriguing result of increased heterozygosity in seropositive woodrats raises questions of how genetic status impacts disease response in other populations of woodrats and to pathogens that might be directly transmitted or have greater fitness impacts on the host. Additional studies should

1 assess more populations, directly transmitted pathogens that can cause disease in woodrats, and
2 genes known to have a direct influence on host response to infection. We are enthusiastic about
3 woodrats as subjects for such studies because individuals are relatively limited in their use of space
4 and populations are genetically structured, allowing for insights into interactions of genetic and
5 ecological influences on various pathogen ecologies.

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Table 1. Genetic diversity of three dusky-footed woodrat populations in northern California based on 14 microsatellite loci. N_A = average number of alleles per locus; N_E = average effective number of alleles per locus; H_O = observed heterozygosity; uH_E = unbiased expected heterozygosity. Standard errors are shown in parentheses.

Locality	N	N_A	N_E	H_O	uH_E
Cold Canyon	96	8.9 (1.5)	5.8 (1.0)	0.69 (0.07)	0.69 (0.08)
Quail Ridge	25	7.3 (1.2)	5.1 (0.7)	0.72 (0.07)	0.71 (0.07)
Soquel Demonstration Forest	46	11.4 (1.3)	6.5 (0.9)	0.74 (0.06)	0.79 (0.05)

Table 2. Site-specific prevalence (and 95% confidence intervals) of *Anaplasma phagocytophilum* real-time PCR-positive dusky-footed woodrats, *Borrelia burgdorferi* real-time PCR-positive woodrats, and *A. phagocytophilum* seropositive woodrats at three sites in Northern California.

	Soquel Demonstration Forest	Cold Canyon	Quail Ridge
<i>A. phagocytophilum</i>	0.30 (0.18-0.46)	0.13 (0.07-0.22)	0.07 (0.01-0.24)
PCR			
<i>B. burgdorferi</i> PCR	N/A	0.11 (0.05-0.19)	0 (0-0.14)
<i>A. phagocytophilum</i>	0.13 (0.04-0.46)	0.18 (0.11-0.28)	0.13 (0.03-0.35)
IFA			

N/A indicates samples were not available to perform testing at that site.

Table 3. Mean individual heterozygosities of dusky-footed woodrats from three sites in northern California that tested positive or negative for *Anaplasma phagocytophilum* by PCR, for exposure to *A. phagocytophilum* by serology, and for *Borrelia burgdorferi* by PCR.

	Test result	Soquel Demonstration Forest	Cold Canyon	Quail Ridge	Total	P
<i>A. phagocytophilum</i> PCR	Negative	0.839	0.813	0.824	0.821	0.64
	Positive	0.858	0.799	0.857	0.833	
<i>B. burgdorferi</i> PCR	Negative	N/A	0.815	0.827	0.817	NS
	Positive	N/A	0.782	N/A	0.782	
<i>A. phagocytophilum</i> IFA	Negative	0.838	0.806	0.819	0.815	0.02
	Positive	0.845	0.835	0.853	0.839	

N/A indicates either test was not performed for that population or that all animals were test-negative.

Figure Legends

Figure 1. Negative correlation between pairwise genetic relatedness and spatial distance in a population of dusky-footed woodrats at the Quail Ridge University of California Reserve in northern California.

Figure 2. Fine-scale genetic structure in male woodrats (A) and female woodrats (B) of the Cold Canyon population of dusky-footed woodrats in northern California. Mean genetic autocorrelation values (r) are shown with black bars for each distance class (vertical bars represent 95% confidence interval). The upper and lower bounds of the random spatial distribution of genetic variation are shown with gray bars.

Figure 3. Patterns of genetic structure and *Anaplasma* antibody-positive status in the Cold Canyon population of dusky-footed woodrats in northern California. (A) Spatial distribution of male and female woodrats across the study site. (B) Spatial distribution of individual woodrat heterozygosity and *Anaplasma* antibody-positive status. (C) Spatial distribution of average pairwise relatedness among all woodrats and individual *Anaplasma* antibody-positive status. (D) Spatial distribution of average pairwise relatedness among female woodrats and individual *Anaplasma* antibody-positive status.

Figure 4. Geographical distribution of woodrats at Soquel Demonstration Forest testing positive or negative by PCR for *Anaplasma phagocytophilum* (A) or by serology for *A. phagocytophilum* (B).

1 Figure 5. Geographical distribution of woodrats at Cold Canyon Reserve testing positive or negative
2 by PCR for *Anaplasma phagocytophilum* (A), serology for *A. phagocytophilum* (B), or PCR for
3 *Borrelia burgdorferi* (C).

4

5 Figure 6. Geographical distribution of woodrats at Quail Ridge Reserve testing positive or negative
6 by PCR for *Anaplasma phagocytophilum* (A), and serology for *A. phagocytophilum* (B).

Figure 1

Figure 2

Figure 3a-d

Figure 4a

Figure 4b

Figure 5a

Figure 5b

Figure 5c

Figure 6a

Figure 6b