Effects of rodent community diversity and composition on prevalence of an endemic bacterial pathogen - *Bartonella*

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Abstract. By studying *Bartonella* prevalence in rodent communities from 23 geographic sites in the western United States and one site in northern Mexico, the present study focused on the effects of rodent community diversity (measured by richness and Shannon index) and composition on prevalence of *Bartonella* infections. The analysis showed negative correlations of *Bartonella* prevalence with rodent richness and Shannon index. Further, *Bartonella* prevalence varied among rodent genera/species. Three models were applied to explain the observations. (1) Within-species/genus transmission: *Bartonella* strains usually are host-specific and adding non-host species would decrease *Bartonella* prevalence in its principal host through reduction of host contact (encounter reduction); (2) Frequency-dependence: Adding hosts would decrease the proportion of all infected individuals in the community, resulting in a reduction in the number of contacts between susceptible and infected individuals that usually leads to transmission (transmission reduction); and (3) Dominant species effect: Dominant species, if not susceptible to *Bartonellae*, can constrain the abundance of susceptible hosts (susceptible host regulation). These mechanisms work in concert; and the level of *Bartonella* prevalence is an outcome of regulation of all of these mechanisms on the entire system.

Key words. *Bartonella*; community diversity; community richness; dilution effect; dominant species; rodents; Shannon diversity index.

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INTRODUCTION

Within an ecological community, the general importance of diversity for performance of ecosystem functions has been widely discussed (Ostfeld *et al*. 2000a; Ostfeld *et al*. 2000b; Schmidt *et al.* 2001; Keesing *et al.* 2006; Mills 2006). More specifically, a potential connection between species diversity and disease transmission has long been recognized. For example, medical entomologists suggested a connection between species diversity and transmission of vector-borne diseases in humans decades ago, when they applied the concept to malaria and malarial transmission dynamics, and argued that malaria transmission might be reduced if alternative hosts for mosquito vectors (e.g. livestock) were placed at areas around human habitation, an approach termed "zooprophylaxis" (Garrett-Jones 1964; Molineaux *et al*. 1978).

More recently however, the link between diversity and disease prevalence has received greater attention following detailed studies of the tick-borne Lyme disease that is caused by a spirochete (*Borrelia burgdorferi*) with the Black-legged Tick (*Ixodes scapularis*) and the White-footed Mouse (*Peromyscus leucopus*) as its primary vector and vertebrate hosts, respectively. In the northeastern US, the White-footed Mouse is the most competent host for the Lyme spirochete and this host also is the most abundant in species-poor host communities. Other non-mouse hosts are relatively poor reservoirs for the Lyme spirochete, as they are fed on but rarely become infected via ticks. Within communities of high host species diversity, Lyme disease prevalence is therefore lower than in low diversity communities, because fewer ticks become infected (LoGiudice *et al*. 2003). Lyme disease, being well understood, has been used as the classical model to study the effect of biodiversity on disease risk (Ostfeld *et al.* 2000a).

Several recent theoretical studies have attempted to delineate under what general conditions host diversity might increase or decrease disease prevalence (Holt *et al*. 2003; Dobson 2004; Rudolf *et al*. 2005; Keesing *et al*. 2006). Ostfeld and others (Ostfeld *et al*. 2000a; Ostfeld *et al*. 2000b; Schmidt *et al*. 2001) have proposed a general theoretical model for vector-borne diseases, describing a mechanism by which increasing diversity of potential vertebrate host species would result in lower prevalence of infection in the vector and lower risk of infection of humans, referred to as the 'dilution effect'. Based on the Lyme disease model, Ostfeld *et al.* (2000b) defined four conditions, which are necessary for applying the concept of dilution effect to other vector-borne zoonoses: 1) the feeding habits of the vector must be generalized (generalist vector); 2) the pathogen is acquired by the vector through oral route (oral acquisition); 3) the ability of a particular host species to infect a vector varies among host species (competence variation); 4) the most competent reservoir host tends to be a community dominant. According to the dilution-effect model, adding species to the host community will either diminish the population density of the primary reservoir host, e.g., via predation or resource competition, or reduce the absolute vector burden on the reservoir host, e.g., by diverting vector meals from the reservoir host to incompetent reservoirs (Schmidt *et al*. 2001; Keesing *et al*. 2006). Moreover, the presence of alternative host species with low reservoir competence may reduce the effective reservoir competence

of hosts of other species by reducing encounter rates between infected vectors and susceptible hosts (Schauber *et al*. 2002). Together, these would result in lower infection prevalence in the vector population, which would in turn decrease human disease risk (Ostfeld *et al*. 2000b; Schmidt *et al*. 2001).

Besides Lyme disease, dilution effects have also been observed in hantavirus pulmonary syndrome (Ruedas *et al.* 2004), rodent-borne illness caused by arenaviruses (Mills 2006), and West Nile (Ezenwa *et al*. 2006; Swaddle *et al*. 2008; Allan *et al*. 2009). These observations indicated the generality of dilution effect in application to other zoonoses. Here, we explore whether similar effects can be observed in prevalence of *Bartonella* infection in diverse rodent populations.

The genus *Bartonella* includes a variety of species that are widely distributed among many rodent species and other mammalian hosts, including humans (Birtles *et al*.1994; Kosoy *et al*. 1997; Welch *et al*. 1999; Chang *et al*. 2000; Ying *et al*. 2002; Kosoy *et al*. 2003; Jardine *et al*. 2005). Infection rates vary substantially among rodent species, and some observations demonstrated that dominant species determine the overall prevalence of *Bartonella* infection in a rodent community (Kosoy *et al*. 2003). In North America, it has been shown that a specific *Bartonella* strain commonly infects rodents of one species or their close relatives (Kosoy *et al*. 1997; Jardine *et al*. 2006; Bai *et al*. 2008), suggesting a possibility of co-speciation of *Bartonella*e with their natural hosts. Although transmission mechanisms by which individual rodents acquire *Bartonella* infections are not fully understood, arthropods, such as fleas, have been implicated as potential vectors (Breitschwerdt *et al*. 2000; Stevenson *et al*. 2003) and experimental studies have demonstrated that fleas can transmit *Bartonella*e between rodents (Bown *et al*. 2004). Other routes, such as vertical transmission (Kosoy *et al*. 1998), are possible.

Bartonella infections have been increasingly associated with human illnesses. Rodents of some species have been found to be reservoir hosts of some *Bartonella*e that are presumed human pathogens (Birtles *et al*. 1995; Ellis *et al*. 1999; Welch *et al*. 1999; Kosoy *et al*. 2003; Iralu *et al*. 2006). Although some studies demonstrated a potential effect of rodent community structure on *Bartonella* prevalence (Kosoy *et al*. 1997; Telfer *et al*. 2005), understanding the mechanisms of the impact of diversity on *Bartonella* infections remains unclear and is critical for evaluating a generality of the patterns, predicting net effects, and reducing risks of human exposure.

The objective of the present study is to investigate whether *Bartonella* prevalence is associated with host diversity and community composition. We compile data on rodent communities and *Bartonella* prevalence from over 20 study sites across the western USA. Specifically, we examine the association between rodent diversity (measured as richness and the Shannon diversity index at both species and genus levels) on *Bartonella* prevalence. We also characterize variation in *Bartonella* prevalence among rodent host species to infer how community composition may influence pathogen occurrence. We discuss the possible mechanisms of how diversity of rodent community can affect *Bartonella* prevalence taking into account characteristics such as a co-speciation of a specific *Bartonella* strain with its natural host (within species/genus transmission), variation of *Bartonella* prevalence among different host species (dominant species effects), and proportion of infected individuals (frequency-dependant transmission).

MATERIALS AND METHODS

STUDY SITES

We selected 24 geographic sites that represent diverse rodent communities within various habitats, including short grassland, bush shrubland, sagebrush shrubland, montane shrubland, conifer forest, and broadleaf woodland. Except for one site (Janos) located in northern Mexico, the other 23 sites were located in nine states of the western United States. These include four sites in Arizona (Apache, Fort Huachuca, Phoenix, and Yuma), one in California (Orange), six in Colorado (Boulder, Comanche, Fort Collins, Fort Lewis, Loveland, and Red Feather Lakes), one in Kansas (Cimarron), two in Nevada (Clark and Vya), four in New Mexico (Placitas, Rio Rancho, Sevilleta, and Socorro), two in South Dakota (Badlands and Wind Cave), two in Utah (Mojave and Pinto), and one in Wyoming (Thunder Basin) (Figure 1).

Trapping, animal processing and blood collections were performed during the years 1995 to 2006. All rodents were trapped using Sherman live traps (8 cm x 9 cm x 23 cm; H.B. Sherman Traps, Inc., Tallahassee, FL). Small mammals were identified to species in the field using a variety of morphological criteria. Captured rodents from all sites were processed following safety procedures published by the US Centers for Disease Control and Prevention (Mills *et al.* 1995a, b). Rodent bloods were collected from the retro-orbital plexus. Animals were released at their capture sites after data collection. The related procedures were approved by the Institutional Animal Care and Use Committees of local institutions.

BARTONELLA CULTURING, VERIFICATION BY PCR AND MEASUREMENT OF *BARTONELLA* PREVALENCE

Isolation of *Bartonella*e followed methods published elsewhere (Kosoy *et al.* 1997). *Bartonella*e were verified by polymerase chain reaction (PCR) amplification of a region in the citrate synthase gene (*gltA*) that is specific for *Bartonella* (Norman *et al*. 1995). Prevalence of *Bartonella* infections was measured by percentage of culture-positive rodents over tested rodents, and was measured at different levels: overall *Bartonella* prevalence in the entire rodent community; *Bartonella* prevalence at the level of rodent host genus; and *Bartonella* prevalence at the level of host species.

ESTIMATION OF RODENT COMMUNITY DIVERSITY

Diversity of the rodent community was estimated in three ways: 1) number of rodent genera or species in a community; 2) proportion of rodents of a genus or a species in a community; and 3) Shannon diversity index at the genus level (H1) or the species level (H2). The Shannon diversity was calculated from the proportional abundances (P*i*) of rodents of each genus or

species in the entire community as $H = -\sum Pi \ ln \ Pi \ (i = 1 \text{ to } s)$, where *s* is the total number of genus/species in the community, and P*i is the proportion of s comprising the ith genus/species.*

STATISTICAL ANALYSES

All statistical analyses were performed using programs within the SAS package (Statistical Analysis System, version 9.1, SAS Institute Inc., Cary, North Carolina). Statistical tests for significance of difference or correlation between compared groups were performed at the 0.05 probability level.

All datasets, including genus/species richness, Shannon indices (H1 and H2), and *Bartonella* prevalence in each community, were first tested for conformity to a normal distribution using the Wilks-Shapiro test prior to further analyses. Chi-square analyses were performed to determine whether *Bartonella* prevalence differed among geographic locations, rodent genera, and rodent species. Fisher's exact test was used if the sample size of any compared group was equal to or smaller than 60. The associations between *Bartonella* prevalence and genus/species richness, and Shannon diversity were determined by performing simple regression and correlation analyses.

RESULTS

SMALL MAMMALS

A total of 2,159 small mammals belonging to 54 species of 19 genera within 5 rodent families were captured from the 24 study sites. *Peromyscus* mice accounted for 53.9% (n = 1163) and were the most common rodents; *Onychomys* mice accounted for 11.1% (n = 239); *Neotoma*, *Chaetodipus*, and *Spermophilus* accounted for 8.1% (n = 175), 5.7% (n = 123), and 5.0% (n = 108), respectively. The other 14 mammalian genera accounted for a very small portion of total animals captured, ranging from <0.1% to 3.9%. At the species level, the Deer Mouse (*P. maniculatus*) were the most common species, accounting for 37.3% (n = 805) of the total captures; the Northern Grasshopper Mouse (*Onychomys leucogaster*) was the second most common species captured, accounting for 10.1% (n = 217). The other 52 species accounted for 52.6% of captures, with individual species ranging from 0.04% to 5.5% of total captures

COMMUNITY DIVERSITY

Species/genus richness

Community diversity varied by location. Among sites, the genus richness (number of rodent genera) ranged from two (Fort Lewis, Placitas, Vya) to nine (Fort Huachuca, Cimarron) and species richness (number of rodent species) ranged from two (Fort Lewis, Vya) to13 (Fort Huachuca) (Table 1). The distribution of both genus richness and species richness was normal (Wilks-Shapiro test, W = 0.95 and 0.95, p = 0.21 and 0.23).

Shannon index

The Shannon index ranged from 0.32 to 1.68 at the genus level (H1) and from 0.32 to 2.23 at the species level (H2) among study sites (Table 1). The community at Wind Cave had the lowest diversity at both genus and species levels (H1 = H2 = 0.32), with four genera and four species present at the site; the community at Janos had the highest diversity at the genus level (H1 = 1.68), with eight genera present at the site; and the community at Fort Huachuca had the highest diversity at the species level (H2 = 2.23), with 13 species present at the site. The Shannon indices at both genus and species levels were normally distributed (Wilks-Shapiro test, W = 0.94 and 0.96, p = 0.14 and 0.42).

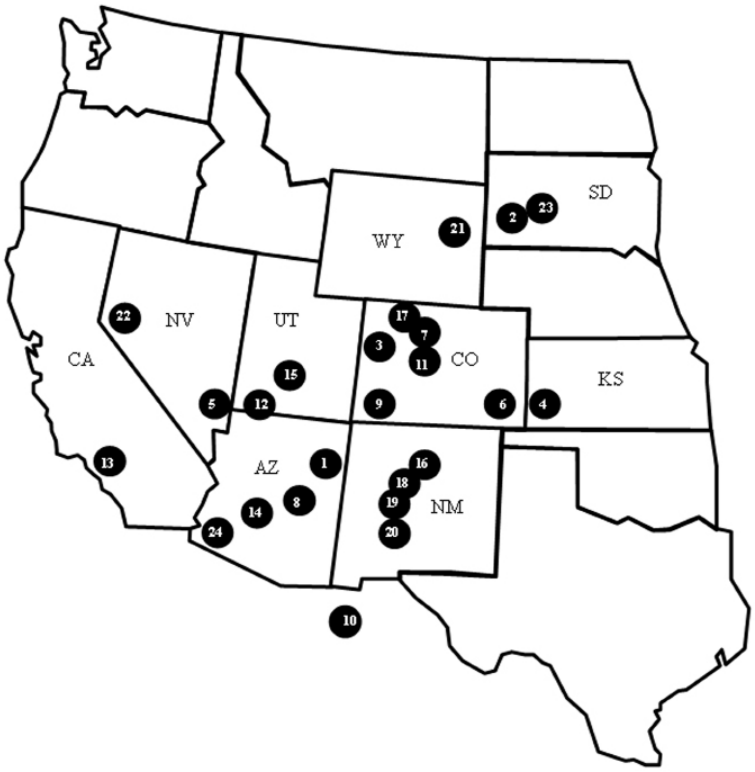

Figure. 1. Geographic location of the 24 study sites (the numbered black spots), with one site located in northern Mexico, and the other 23 located in nine western states of the United States. 1, Apache AZ; 2, Badlands SD; 3, Boulder CO; 4, Cimarron KS; 5, Clark NV; 6, Comanche CO; 7, Fort Collins CO; 8, Fort Huachuca AZ; 9, Fort Lewis CO; 10, Janos Mexico; 11, Loveland CO; 12, Mojave UT; 13, Orange CA; 14, Phoenix AZ; 15, Pinto UT; 16, Placitas NM; 17, Red Feather CO; 18, Rio Rancho NM; 19, Sevilleta NM; 20, Socorro NM; 21, Thunder Basin WY; 22, Vya NV; 23, Wind Cave SD; 24, Yuma AZ.

PROPORTION OF PEROMYSCUS MICE, *ONYCHOMYS* MICE AND OTHERS IN THE COMMUNITIES

Peromyscus mice were present at all 24 sites, with their proportions ranging from 20.9 to 93.2% per community. The number of species of this genus within a site varied from one to four. The Deer Mouse was absent in Phoenix, Placitas, and Mojave; comprised from 3.3% to 93.2% of total captures at the other 21 sites, and was the dominant rodent at 12 of the study sites (Table 1). The Pinyon Mouse (*P. truei*), found at seven sites, was the dominant rodent at Apache (64.5%). The Brush Mouse (*P. boylii*) was found at five sites and was the dominant rodent in Mojave (31.0%) and Placitas (67.4%). Cactus Mice (*P. eremicus*) and White-footed Mice (*P. leucopus*) were found at seven and six sites, respectively, and were the respective dominant rodent in Yuma (83.6%) and Rio Rancho (40.5%). Mice of another three species of *Peromyscus*, the California Mouse (*P. californicus*), the Canyon Mouse (*P. crinitus*) and the Rock Mouse (*P. difficilis*), were found only at one or two sites.

Onychomys mice were captured at nine sites, comprising from 0.8% to 47.5% of total captures per community. The Northern Grasshopper Mouse (*O. leucogaster*) was captured in eight sites and was the dominant rodent at Comanche (41.1%) and Cimarron (47.5%); the Southern Grasshopper Mouse (*O. torridus*) was captured at two sites and was the dominant rodent at Janos (21.2%) (Table 1).

Rats of three species of *Neotoma*, White-throated Woodrat (*N. albigula*), Desert Woodrat (*N. lepida*), and Mexican Woodrat (*N. mexicana*) were the dominant rodents at Sevilleta, Orange, and Loveland, respectively (Table 1). The Rock Pocket Mouse (*Chaetodipus intermedius*) was the dominant rodent at the Phoenix site (Table 1).

PREVALENCE OF *BARTONELLA* INFECTION BY RODENT GENUS AND SPECIES.

*Bartonella*e were detected in 813 of 1,952 (41.6%) rodents from all study sites. The positive rodents belonged to 36 species of 16 genera and prevalence varied by genus and species (Figure 2). Among these rodents, *Onychomys* mice were the most likely to have *Bartonella*e, with 69.7% (159/228) of individuals infected. Both species within the genus had *Bartonella* infection, with prevalence of 70.8% (148/209) in Northern Grasshopper Mice and 57.9% (11/19) in Southern Grasshopper Mice. Fisher's exact test showed that the prevalence did not significantly differ between the two species ($p = 0.30$).

Prevalence of *Bartonella* also was high among chipmunks of the genus *Tamias* (64.1%, 41/64). Rodents of four *Tamias* species tested had a high prevalence of *Bartonella* infection: 60.6% (20/33) in the Least Chipmunk (*T. minimus*), 66.7% (12/18) in the Uinta Chipmunk (*T. umbrinus*), 66.7% (2/3) in the Colorado Chipmunk (*T. quadrivittatus*), and 70% (7/10) in the Cliff Chipmunk (*T. dorsalis*). Fisher's exact test showed there was no significant difference in *Bartonella* prevalence among the four species ($p = 0.92$).

The average prevalence of *Bartonella* in mice of the genus *Peromyscus* was 44.2% (473/1070) but varied by species within the genus (Figure 2). *Bartonella*e were found in seven of eight tested *Peromyscus* species. *Bartonella* prevalence was 0% (0/1) in the Canyon Mouse, 15.8% (16/101) in the Cactus Mouse, 23.2% (13/56) in the White-footed Mouse,

Site	NC	NG	NS	DS	PPDS (%)	H1	H2	NT	NP	PR (%)
Apache	31	3	4	PETR	64.5	0.61	1.02	31	19	61.3
Badlands	138	5	5	PEMA	70.3	0.95	0.95	123	54	43.9
Boulder	169	6	9	PEMA	58.0	1.2	1.28	169	61	36.1
Cimarron	202	9	10	ONLE	47.5	1.49	1.59	190	117	61.6
Clark	111	7	11	PEMA	28.8	1.26	1.94	105	29	27.6
Comanche	112	8	9	ONLE	41.1	1.41	1.48	102	40	39.2
Fort Collins	55	4	5	PEMA	34.6	1.38	1.43	55	27	49.1
Fort Huachuca	127	9	13	PEMA	21.3	1.43	2.23	102	19	18.6
Fort Lewis	51	2	2	PEMA	54.9	0.69	0.69	51	42	82.4
Janos	99	8	12	ONTO	21.2	1.68	2.12	78	20	25.6
Loveland	31	3	3	NEME	48.4	0.98	0.98	31	12	38.7
Mojave	29	6	10	PEBO	31.0	1.41	2.01	29	17	58.6
Orange	176	6	11	NELE	23.3	1.34	2.11	144	54	37.5
Phoenix	86	6	6	CHIN	51.2	1.32	1.32	71	3	4.2
Pinto	59	4	5	PEMA	76.3	0.58	0.85	59	31	52.5
Placitas	49	2	3	PEBO	67.4	0.83	0.83	35	18	51.4
Red Feather	56	4	6	PEMA	42.9	1.18	1.32	56	26	46.4
Rio Rancho	42	5	7	PELE	40.5	1.23	1.63	42	15	35.7
Sevilleta	45	4	7	NEAL	53.3	1.05	1.5	45	21	46.7
Socorro	56	8	12	PEMA	30.4	1.4	1.96	44	5	11.4
Thunder Basin	236	7	7	PEMA	69.5	0.96	0.96	210	114	54.3
Vya	35	2	2	PEMA	74.3	0.57	0.57	35	20	57.1
Wind Cave	103	4	4	PEMA	93.2	0.32	0.32	93	43	46.2
Yuma	61	5	6	PEER	83.6	0.56	0.7	52	6	11.5

Table 1. NC = Number of rodents captured; NG = Number of rodent genus in a community; NS = Number of rodent species in a community; DS = Dominant species at the site; PPDS = Proportion of the dominant species in the entire rodent community at the site; H1 = Shannon index at genus level; H2 = Shannon index at species level; NT = Number of rodents tested for bartonella; NP = Number of rodents positive for *bartonella*; PR = bartonella prevalence in the entire rodent community at the site; PETR = *Peromyscus truei*; PEMA = *Peromyscus maniculatus*; ONLE = *Onychomys leucogaster*; ONTO = *Onychomys torridus*; NEME = *Neotoma mexicana*; PEBO = *Peromyscus boylii*; NELE = *Neotoma lepida*; CHIN = *Chaetodipus intermedius*; PELE = *Peromyscus leucopus*; NEAL = *Neotoma albigula*; PEER = *Peromyscus eremicus*.

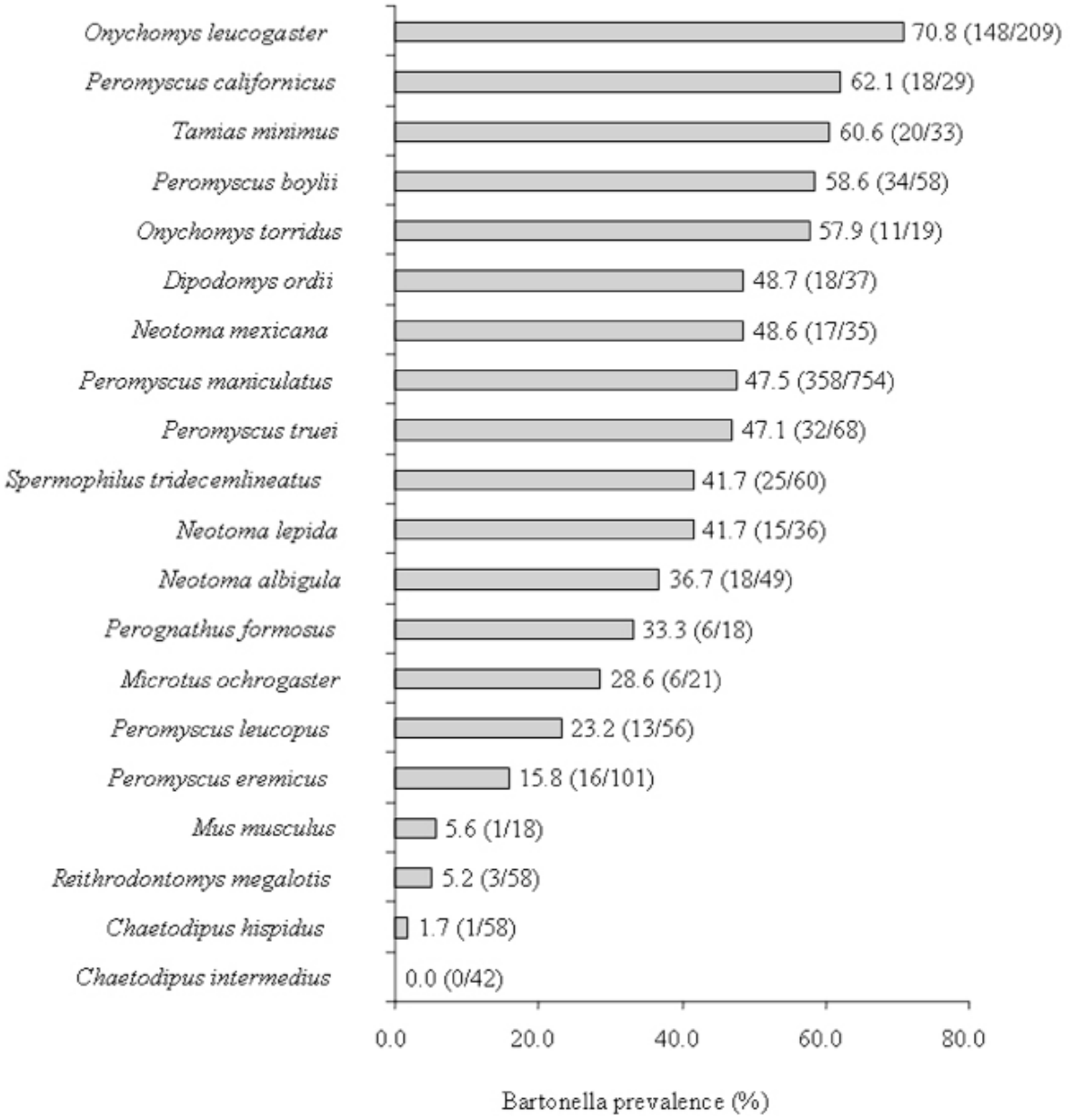

Figure 2. Variation of *Bartonella* prevalence among rodent species. *Bartonella* prevalence was measured as the percentage of culture-positive rodents of tested individuals of each species from the entire study. The numbers in parenthesis are culture positives/tested. Only species with sample sizes of 18 or greater individuals are presented.

47.1% (32/68) in the Pinyon Mouse, 47.5% (358/754) in the Deer Mouse, 58.6% (34/58) in the Brush Mouse, 62.1% (18/29) in the California Mouse, and 66.7% (2/3) in the Rock Mouse. Compared to the mean prevalence for all *Peromyscus* species, the prevalence in the Brush Mouse and the California Mouse was significantly higher (Fisher's exact test, $0.0258 < p < 0.0473$), whereas that in the Cactus Mouse and the White-footed Mouse was significantly lower (Fisher's exact test, $p < 0.001$). The prevalence in mice of the other four species did not differ from the mean (Fisher's exact test, $0.12<p<0.55$).

The prevalence of *Bartonella* in rats of genus *Neotoma* was 41.9% (54/129), with 36.7% (18/49), 41.7% (15/36), 42.9% (3/7), 48.6% (18/37) and 50% (1/2) in the White-throated Woodrat, the Desert Woodrat, the Dusky-footed Woodrat (*N. fuscipes*), the Mexican Woodrat, and the Southern Plains Woodrat (*N. micropus*), respectively. Fisher's exact test showed the prevalence variation among these four species was not significant ($p = 0.72$).

Twenty of 50 *Dipodomys* rats of three species had *Bartonella* infections, with 22.2% (2/9) in Merriam's Kangaroo Rats (*D. merriami*), 48.6% (18/37) in Ord's Kangaroo Rats (*D. ordii*), and 0% (0/4) in Banner-tailed Kangaroo Rats (*D. spectabilis*). Only 2 of 112 *Chaetodipus* mice were infected with *Bartonella*. Mice of six species of *Chaetodipus* were tested but all except two (one Hispid Pocket Mouse [*Chaetodipus hispidus*] and one Desert Pocket Mouse [*Chaetodipus penicilatus*]) were free of *Bartonella*. Prevalence was also very low in *Reithrodontomys* (3.8%, 3/79) and *Mus* mice (5.6%, 1/18). Rodents of other

genera had moderate prevalence: 25% (4/16) in *Sigmodon* rats, 26.9% (7/26) in *Perognathus* mice, 27.3% (9/33) in *Microtus* voles, and 34% (34/100) in *Spermophilus* ground squirrels.

PREVALENCE OF *BARTONELLA* INFECTION BY RODENT COMMUNITY

The *Bartonella* prevalence at the study sites ranged from 4.2% to 82.4%. The lowest prevalence was at Phoenix, and the highest prevalence was at Fort Lewis (Table 1). The distribution of prevalence at all study sites was normal (Wilks-Shapiro test, $W = 0.97$, $p = 0.67$). Normal distribution of all datasets in this study assured the validity of the following analyses.

BARTONELLA PREVALENCE AND GENUS/SPECIES RICHNESS

Using data from all 24 sites, correlation analysis demonstrated that *Bartonella* prevalence was significantly and negatively correlated with both the genus richness of the community ($r = -0.49$; $p = 0.0141$) and the species richness of the community ($r = -0.50$, $p = 0.0132$). The correlation became more apparent after two sites, Phoenix and Yuma, were removed from the analysis because of biases discussed below ($r = -0.58$, $p = 0.0049$ at the genus level; $r = -0.67$, $p = 0.0006$ at the species level) (Figures 3a, b).

BARTONELLA PREVALENCE AND SHANNON DIVERSITY INDEX

Using data from all 24 study sites, correlation analysis indicated that *Bartonella* prevalence was not significantly associated with the Shannon index at either the genus level ($r = -0.36$, $p = 0.0821$) or the species level ($r = -0.40$, $p = 0.0552$), although a trend was observed. However, a significant correlation was observed between the two variables after data from the Phoenix and Yuma sites were removed from the analysis ($r = -0.52$, $p = 0.0121$ at the genus level; $r = -0.61$, $p = 0.0026$ at the species level) (Figures 4a, b).

DISCUSSION

Transmission of infectious disease agents is an inherently ecological process involving interactions among hosts, pathogens and, when they are involved, vectors. Not surprisingly, the species diversity of ecological communities can potentially affect disease transmission and dynamics, either increasing or decreasing the risk (Ostfeld *et al*. 2000a; Ezenwa *et al*. 2006; Keesing *et al*. 2006). The present study showed that *Bartonella* prevalence varied in rodent communities that differed in diversity and compositions. Our results showed a negative correlation between *Bartonella* prevalence and community diversity: within high diversity rodent communities, *Bartonella* prevalence was lower than in low diversity communities.

As relatively newly discovered organisms, we still know little about bacteria in the genus *Bartonella.* This genus includes many species (multiple infectious agents) that can infect a variety of rodent species (multiple hosts), and likely are transmitted by fleas and other vectors (multiple vectors). These and other factors make the system of *Bartonella* infection very complex. Community diversity may affect the rate of transmission of infectious organisms through a variety of ways within such a system. Following, we consider three possible mechanisms proposed by Keesing *et al.* (2006) to explain our observations of rodent community diversity on *Bartonella* prevalence.

WITHIN-GENUS/SPECIES TRANSMISSION MODEL (ENCOUNTER REDUCTION)

One observation of *Bartonella* occurrence that has been previously documented is that the same *Bartonella* strain rarely infects rodents of species that are taxonomically distant, referred to co-speciation. For example, the *Bartonella* strain harbored by Grasshopper Mice (*Onychomys leucogaster*) does not infect rodents of other species (Bai *et al.* 2007); *B. washoensis* was found only in Ground Squirrels (*Spermophilus* spp.) but not in other rodent species (Kosoy *et al.* 2003). Co-speciation of *Bartonella* with the natural hosts suggests that the transmission of *Bartonella* should be more common within species than between species. In a multi-host system, if transmission rates are higher within species than it is between species, then host diversity should decrease infection rates, and *vice versa* (Holt *et al*. 1985; Bowers *et al*. 1991; Begon *et al*. 1992; Begon *et al*. 1994; Dobson 2004; Rudolf *et al*. 2005). This is because adding non-host species to the community would reduce the probability of encounters between hosts, or limit or regulate host numbers through resource competition. As a result, increasing rodent community diversity may decrease *Bartonella* prevalence. Our observations that *Bartonella* prevalence is lower in a more diverse community supported such a mechanism.

FREQUENCY-DEPENDENT MODEL (TRANSMISSION REDUCTION)

A key factor in defining the effects of community diversity on risk of infection in a multi-host system appears to be whether transmission of an organism is a function of the absolute density of infected hosts (density-dependent) or whether it is a function of the proportion of the total population that is infected with the pathogen (frequency-dependent) (Rudolf *et al*. 2005; Keesing *et al*. 2006). Vector-borne infections are considered to conform broadly to frequency-dependent models of transmission (Thrall *et al*. 1993). If prevalence is frequency-dependent, adding hosts of other species will decrease infection risk whether or not the added hosts reduce the abundance of the principal hosts. This is because added hosts decrease the proportion of infected individuals in the community, resulting in a reduction of contact between susceptible and infected individuals, and leading to a lower rate of transmission, again assuming that transmission between individuals of different species is lower than transmission within individuals of a single species. Since *Bartonella* infections are generally considered as vector-transmitted, the prevalence of the infection could be explained by the frequency-dependent model, why it is lower in a community with higher diversity of rodent hosts. However, we do not have real data supporting the frequency involvement in this setting.

DOMINANT SPECIES EFFECT (SUSCEPTIBLE HOST REGULATION)

Bartonella prevalence in rodents at the Phoenix site was surprisingly low compared with that in other rodent

communities with a similar or higher diversity. A similar situation also was observed at the Yuma site. Although *Bartonella*e are distributed throughout a wide range of rodent hosts with overall high prevalence (Birtles *et al*. 1994; Kosoy *et al*. 1997; Ying *et al*. 2002; Holmberg *et al*. 2003), they rarely infect rodents of certain genera or species, such as *Chaetodipus* mice, *Reithrodontomys* mice, and Cactus Mice. Rodents that are not susceptible to *Bartonella*e or that are of low susceptibility to them could play a crucial role in determining the infection rates of arthropod vectors. If the dominant species in a community is not susceptible to infections of any *Bartonella*, then the overall prevalence of *Bartonella* in the community will be low because the dominant species could constrain the abundance of susceptible hosts through competition for limited resources, a process called *susceptible host regulation* (Keesing *et al*. 2006). The Rock Pocket Mouse and Cactus Mouse were the dominant species in Phoenix (51.2%) and Yuma (83.6%), respectively. However, the Rock Pocket Mouse has never been found culture-positive for *Bartonella* (Kosoy, *personal communication*), and the level of infectivity of *Bartonella* among Cactus Mice was evidently lower compared to other species of *Peromyscus* mice in the current study, which may suggest that Cactus Mice are less susceptible to *Bartonella* infection than are other *Peromyscus* mice. The dominance of these rodents in both communities may explain the overall low prevalence of *Bartonella* at these two sites regardless of the community diversity. Although the Cactus Mouse also was the dominant species at Rio Rancho, the prevalence of *Bartonella* was higher there than in Yuma, though the diversity was higher at Rio Rancho. This higher prevalence may be explained by the lower proportion of Cactus Mice at Rio Rancho compared to at Yuma (40.5% versus 83.6%), which may have fewer competition effects on rodents of other host species. The dominant rodents at the other 21 sites, including Pinyon Mice, Deer Mice, Brush Mice, White-footed Mice, Desert Woodrats, White-throated Woodrats, Mexican Woodrats, Northern Grasshopper Mice, and Southern Grasshopper Mice are all susceptible to *Bartonella*e based on this and other studies (Kosoy *et al*. 1997; Bai *et al*. 2007; Morway, *personal communication*). As noted above, the correlation of *Bartonella* prevalence with community diversity became more apparent after exclusion of the Phoenix and Yuma sites from the analyses, suggesting that dilution effects were more evident when susceptible reservoirs were dominants in rodent communities (Schmidt *et al*. 2001).

POTENTIAL BIAS IN THE CURRENT ANALYSIS

At the Comanche site, *Bartonella* was not found in any of the 30 Deer Mice tested; this result was not related to community diversity. At least two hypotheses might explain this observation: (1) the current Deer Mouse population was newly established after a recent crash of the population and this population remained free of *Bartonella* because there was not sufficient time to re-establish *Bartonella* infection in the population; and (2) the newly established population of Deer Mice at Comanche represented only a small fraction of the genetic variation in Deer Mice (founder effect) and mice of the new population were not susceptible to *Bartonella* infection.

OTHER PERSPECTIVE

Although it is not directly related to our study, we would like to mention that, most likely, vector regulation does not substantially influence the effect of biodiversity dilution in *Bartonella* system. Observations of the dilution effect based on the Lyme disease model require vectors to be generalists and to acquire pathogens orally (as opposed to exclusively transovarial transmission). A vertebrate community with high species diversity can deflect vector meals away from the most competent reservoirs, thereby reducing infection prevalence. However, the above-mentioned requirements may not completely apply to *Bartonella* infections. First, mechanisms of transmission of *Bartonella* between rodents

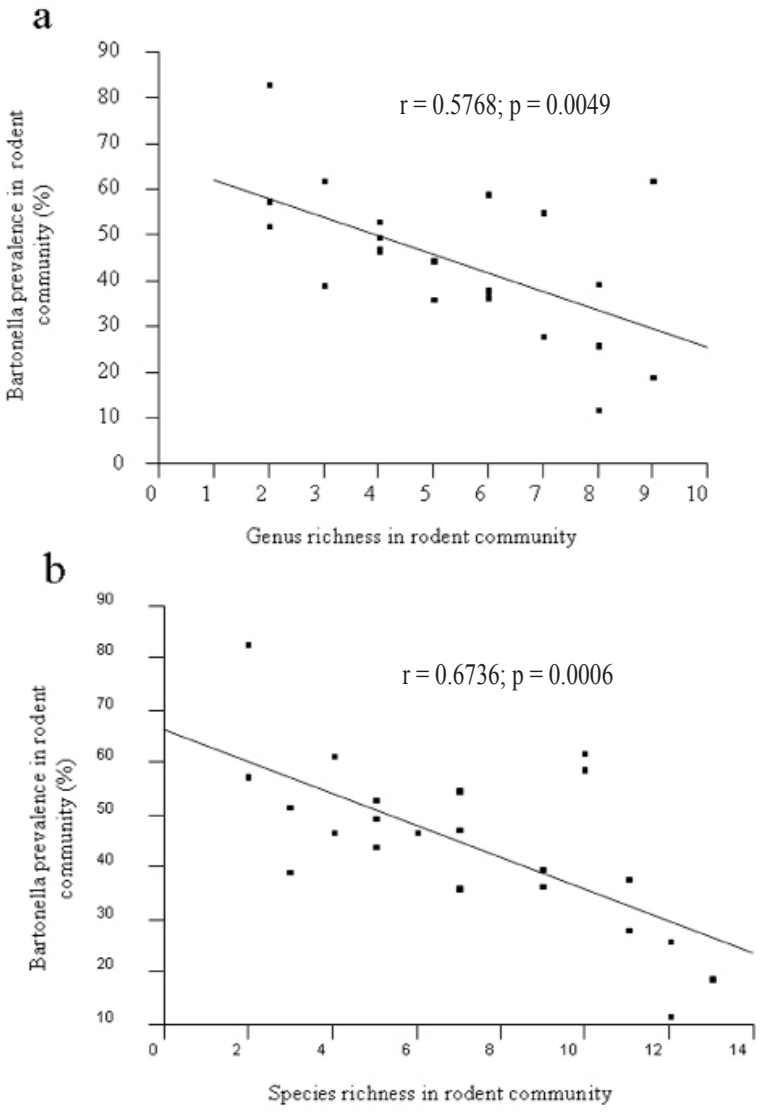

Figure 3. Associations of *Bartonella* prevalence with genus richness (a) and with species richness (b) in rodent communities. *Bartonella* prevalence was measured as the percentage of culture-positive rodents of all tested individuals in the rodent community. Genus richness represents the number of genera of rodents in a community. Species richness represents the number of species of rodents in a community. Simple linear regression was performed after removal of two sites (Phoenix and Yuma), where members of the dominant species showed low or no susceptibility to *Bartonella*.

remain unclear. Although the role of fleas and other potential vectors is generally accepted as a mechanism for *Bartonella* transmission (Bown *et al*. 2004; Breitschwerdt *et al*. 2000; Stevenson *et al*. 2003), there is evidence that vertical transmission of *Bartonella* occurs in rodents (Kosoy *et al*. 1998), indicating arthropods are not the only means by which *Bartonella*e are transmitted. More importantly, rodent fleas are usually specialists feeding on specific rodent hosts, at least at the rodent genus level, although some rodent fleas, such as *Aetheca wagneri, Orchopeas leucopus*, and *Pleochaetis exilis*, can share hosts of different rodent genera, such as Grasshopper Mice and Deer Mice (Thomas 1988). However, as specialists, most rodent fleas will not be drawn away from their hosts by adding non-host species to the community. Therefore, the density of the flea population will not increase in response to increased richness, but will remain at the same or a similar level. The investigation of *Bartonella* infection within vole populations in Ireland by Telfer *et al*. (2005) demonstrated that flea prevalence did not increase with overall rodent density increases, suggesting that vector involvement did not increase. Thus, the requirements for a dilution effect model may be more general than previously considered. In infection systems with specialist vectors (*Bartonella* infection) or non-vector systems (hantavirus) (Mills 2006), dilution effects may also occur within a diverse community.

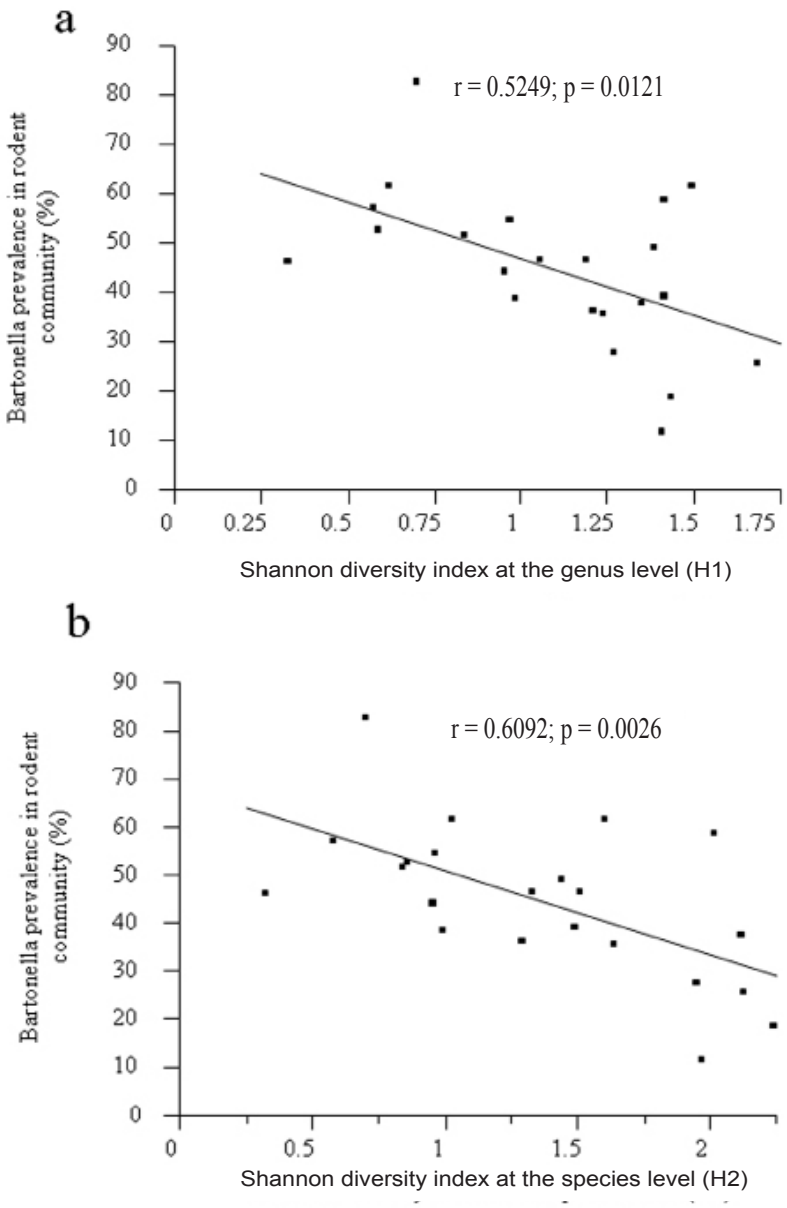

Figure 4. Associations of *Bartonella* prevalence with Shannon diversity index at the genus level (H1) (a) and at the species level (H2) (b) in rodent communities. *Bartonella* prevalence was measured as the percentage of culture-positive rodents of all tested individuals in a rodent community. The Shannon diversity index was calculated from proportional abundances of each genus and /or species of rodent in a rodent community. Simple linear regression was performed after removal of two sites (Phoenix and Yuma), where members of the dominant species showed low or no susceptibility to *Bartonella*.

The potential effects of diversity on risk of infection with *Bartonella*e have drawn the attention of ecologists, conservation planners, and physicians, who work together to understand the factors responsible for disease risk and for maintenance of biological diversity. The present study highlights the critical role of rodent community ecology in risk of *Bartonella* infection. Our results provide empirical support for theoretical models applicable in ecological epidemiology. In addition, they demonstrate that the presence of a diverse assemblage of rodents can bring about a reduction in *Bartonella* prevalence in a rodent community through differing mechanisms. The occurrence and peculiarities of *Bartonella* infection surely are far more complex than we recognize. For example, multiple *Bartonella* species can coexist in the same individual or different individuals of the same host species, individuals of different host species can share the same *Bartonella*, and multiple vectors may transmit the organisms among hosts of multiple species (Birtles *et al*. 1994). Complex interactions of pathogens, hosts, and vectors, as well as other factors make the net effects of rodent community diversity on *Bartonella* prevalence unpredictable. Additional studies are needed so that we may more fully understand the relationship between rodent community structure and *Bartonella* prevalence.

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