



Spatial variation in keystone effects: small mammal diversity associated with black-tailed prairie dog colonies

Jack F. Cully, Jr, Sharon K. Collinge, Ron E. Vannimwegen, Chris Ray, Whitney C. Johnson, Bala Thiagarajan, David B. Conlin and Brian Holmes

J. F. Cully, Jr (bcully@ksu.edu), United States Geological Survey, Kansas Cooperative Fish and Wildlife Research Unit, and Div. of Biology, 204 Leasure Hall, Kansas State Univ., Manhattan, KS 66506, USA. – S. K. Collinge, Dept of Ecology and Evolutionary Biology, and Environmental Studies Program, Univ. of Colorado-Boulder, 334 UCB, Boulder, CO 80309-0334, USA. – R. E. Vannimwegen and B. Thiagarajan, Div. of Biology, 204 Leasure Hall, Kansas State Univ., Manhattan, KS 66506, USA. – C. Ray, W. C. Johnson and D. B. Conlin, Dept of Ecology and Evolutionary Biology, Univ. of Colorado-Boulder, 334 UCB, Boulder, CO 80309-0334, USA. – B. Holmes, BLM White River Field Office, 220 E Market Street, Meeker, CO 81641, USA.

Species with extensive geographic ranges may interact with different species assemblages at distant locations, with the result that the nature of the interactions may vary spatially. Black-tailed prairie dogs *Cynomys ludovicianus* occur from Canada to Mexico in grasslands of the western Great Plains of North America. Black-tailed prairie dogs alter vegetation and dig extensive burrow systems that alter grassland habitats for plants and other animal species. These alterations of habitat justify the descriptor “ecological engineer,” and the resulting changes in species composition have earned them status as a keystone species. We examined the impact of black-tailed prairie dogs on small mammal assemblages by trapping at on- and off-colony locations at eight study areas across the species’ geographic range. We posed 2 nested hypotheses: 1) prairie dogs function as a keystone species for other rodent species; and 2) the keystone role varies spatially. Assuming that it does, we asked what are the sources of the variation?

Black-tailed prairie dogs consistently functioned as a keystone species in that there were strong statistically significant differences in community composition on versus off prairie dog colonies across the species range in prairie grassland. Small mammal species composition varied along both latitudinal and longitudinal gradients, and species richness varied from 4 to 11. Assemblages closer together were more similar; such correlations approximately doubled when including only on- or off-colony grids. Black-tailed prairie dogs had a significant effect on associated rodent assemblages that varied regionally, dependent upon the composition of the local rodent species pool. Over the range of the black-tailed prairie dog, on-colony rodent richness and evenness were less variable, and species composition was more consistent than off-colony assemblages.

Keystone species (Paine 1969) are defined as those that have a disproportionate effect on the distribution and abundance of other species relative to their own abundance (Power et al. 1996), and that perform a role unlike any other species or process in the same ecosystem (Kotliar 2000). Prairie dogs (*Cynomys* spp.) often are considered ecosystem engineers (Jones et al. 1994, 1997, Ceballos et al. 1999, Wright et al. 2002, Bangert and Slobodchikoff 2006, Davidson and Lightfoot 2006, 2008, Davidson et al. 2008, VanNimwegen et al. 2008) as well as keystone species of short- and mixed-grass prairies (Miller et al. 1994, Kotliar et al. 1999) and desert grasslands (Ceballos et al. 1999, Davidson and Lightfoot 2006, 2008, Davidson et al. 2008). When prairie dogs colonize a new area, they create a significant and unique disturbance by reducing both dead and live vegetation cover and changing plant species composition (Coppock et al. 1983, Agnew et al. 1986, Whicker and Detling 1988, Winter et al. 2002). In

addition, they excavate extensive underground burrow systems which provide refugia for other species (Hoogland 2006). Reduced vegetation height creates suitable habitat for species that prefer relatively open habitats, and burrows create shelter for insects (Kretzer 1999, Bangert and Slobodchikoff 2006, Davidson and Lightfoot 2007), reptiles and amphibians (Kretzer and Cully 2001, Shipley and Reading 2006, Davidson et al. 2008), and other animals (Kotliar et al. 1999) such as black-footed ferrets *Mustela nigripes* (Hillman and Clark 1980) and burrowing owls *Athene cunicularia* (Desmond et al. 2000, Winter and Cully 2007). Because black-tailed prairie dogs *C. ludovicianus* have a large geographic range (Fig. 1), they interact with different suites of species in different portions of their range. This variance in species associations may lead to spatial heterogeneity in their keystone role (Stapp 1998).

The direction and magnitude of the effects of prairie dogs on ecological communities may depend on a number

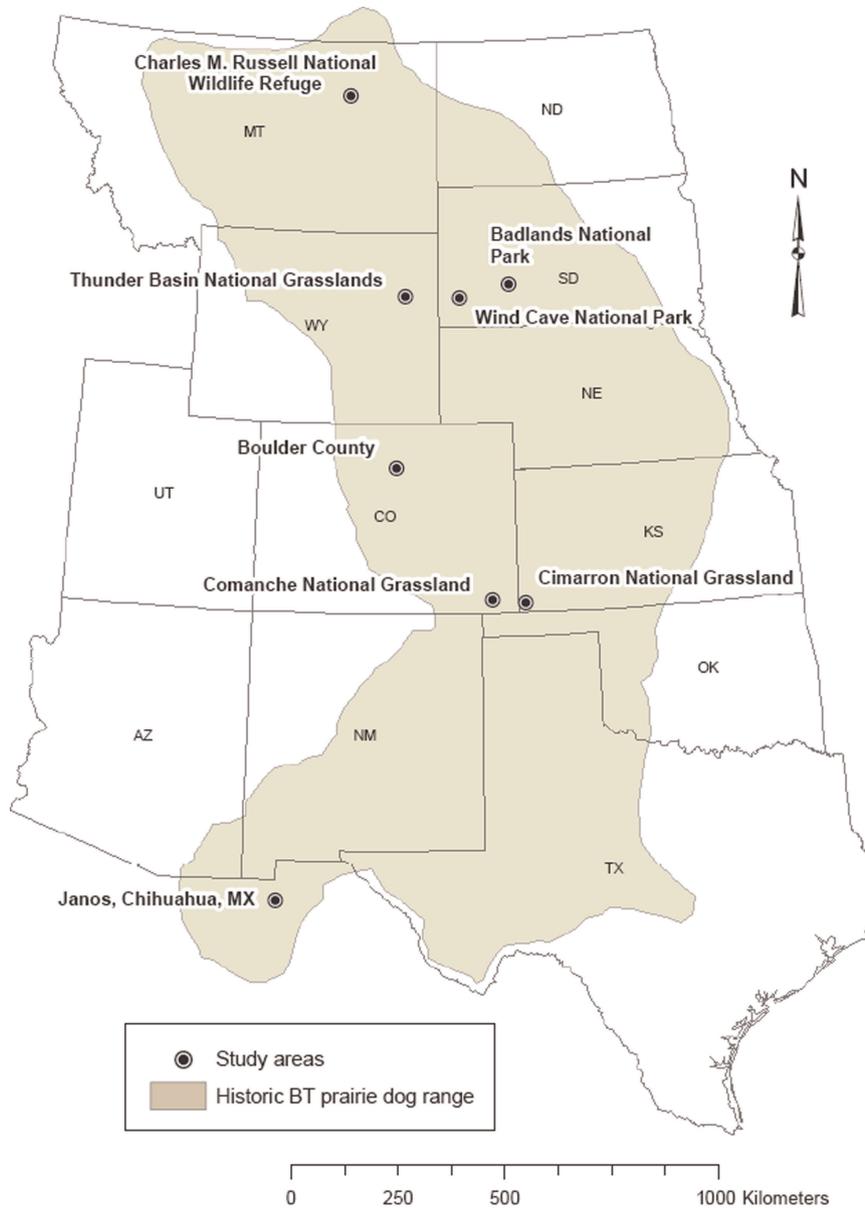


Figure 1. The geographic range of black-tailed prairie dogs in western North America showing the locations of our study areas.

of ecological factors and may vary significantly in space and time (Menge et al. 1994, Navarrete and Menge 1996). If the ecological effects of a keystone species vary significantly across its geographic range, then community structure and species interactions may also differ both qualitatively and quantitatively across the range (Stapp 1998). Further, if a species has strong effects on community structure at a particular place and time, then the decline or local extinction of that species due to disease or other mortality factors may cause large shifts in community structure (Collinge et al. 2008).

Although the black-tailed prairie dog is often identified as a keystone species, this status has been questioned on the basis that too little was known about the functional relationship between prairie dogs and associated species (Stapp 1998), and in a recent literature review only 9 of 208 species previously claimed to be dependent on prairie dogs were found to be strongly associated with grasslands with

prairie dog colonies (Kotliar et al. 1999). Nevertheless, as discussed above, prairie dogs have large effects on short- and mixed-grass prairie and desert grassland vascular plant, insect, bird, reptile, and amphibian community structure, and they perform roles unlike any other grassland species (Kotliar et al. 1999, Kotliar 2000, Davidson and Lightfoot 2006). At the scale of the geographic range of the species, significant changes in the number and distribution of black-tailed prairie dogs likely would result in significant changes in the nature of prairie ecosystems.

The effect of prairie dog colonies on small mammal diversity and composition has been studied separately in Mexico, Arizona, Oklahoma, Colorado, and South Dakota, with variable results (Table 1). Ceballos et al. (1999) reported that small mammal diversity was variable, but on average higher, on grasslands with black-tailed prairie dogs in northeastern Mexico. Density of small mammals also was significantly higher in grasslands with prairie dogs. However,

Table 1. Previous studies of prairie dog effects on small mammal assemblages.

Prairie dog species	Location	Sample size, on-colony/off-colony	Reference
Mexican	San Luis Potosí, MX	1/1/0*	Mellink and Madrigal 1993
Black-tailed	Oklahoma, USA	6/6	O'Melia et al. 1982
Black-tailed	South Dakota, USA	3/3	Agnew et al. 1986
Black-tailed	Chihuahua, MX	2/1	Ceballos et al. 1999
Black-tailed	Oklahoma, USA	36/36	Lomolino and Smith 2003
Black-tailed	Colorado, USA	3/2	Shipley and Reading 2006
Black-Tailed	Colorado, USA	18/6/7*	Stapp 2007
Gunnison's	Arizona, USA	15/15/15*	Bartz et al. 2007

*Active on-colony/inactive on-colony/off-colony.

their sample sizes were small (Table 1); the 2 sites with prairie dogs had variable species richness (4 and 10 species), whereas the site without prairie dogs had 6 species (Ceballos et al. 1999). Mellink and Madrigal (1993) reported that higher small mammal species richness and abundance occurred on an inactive Mexican prairie dog colony than on an active colony, but it was presumably prior habitat changes – caused by prairie dogs – that affected the small mammals. In Oklahoma, O'Melia et al. (1982) documented higher abundance but lower species richness of small mammals on 6 black-tailed prairie dog colonies relative to 6 off-colony grassland plots. In a more recent analysis of grassland vertebrates in the Oklahoma Panhandle, mammal diversity, including rodents and other species, was similar between prairie dog colonies and paired off-colony sites in summer, and lower on colonies during fall (Lomolino and Smith 2003). Shipley and Reading (2006) reported higher numbers of captures and higher species diversity of small mammals incidentally caught in their reptile and amphibian traps on than off prairie dog colonies in Colorado. Stapp (2007) did not observe differences in abundance, richness, or evenness among active colonies, inactive colonies or off-colony sites on the Pawnee National Grassland in northeastern Colorado, although there were differences in abundance of individual species. Likewise, for Gunnison's prairie dogs, Bartz et al. (2007) found that rodent species abundance richness and diversity did not vary among active prairie dog colonies, inactive colonies, and control sites. Agnew et al. (1986) found higher density of small mammals but lower species richness on prairie dog colonies in South Dakota, with northern grasshopper mice *Onychomys leucogaster* and deer-mice *Peromyscus maniculatus* more abundant on 3 prairie dog colonies than on 3 adjacent mixed-grass prairie sites. The above studies indicate that prairie dogs' effects on rodent communities vary spatially.

We sought to test the keystone role of prairie dogs for other rodent species over a large area across the majority of the species range. At 8 study areas in 6 U.S. states and Chihuahua, Mexico, we established live-trapping grids on prairie dog colonies and at nearby off-colony grassland sites to gather contemporaneous data to evaluate relationships between black-tailed prairie dogs and small mammal assemblages. We conducted a multivariate analysis of rodent assemblages on vs. off colonies to see if prairie dogs structure rodent assemblages at the large scale of the black-tailed prairie dog range. Within each study area we evaluated differences in abundance, richness, diversity, and evenness of the small mammal assemblages on and off prairie dog colonies. We posed 2 nested hypotheses. First,

black-tailed prairie dogs perform a keystone role in the grassland rodent assemblage. Second, because species composition changes across the range of the black-tailed prairie dog, characteristics of the keystone role vary spatially. Assuming the keystone role would vary spatially, we sought to identify possible sources for that variation, which might include a west-to-east moisture gradient, or a north-to-south gradient in seasonal temperature differences, day-lengths, and insolation. Both east-west and north-south gradients are associated with the geographic ranges of different species, which may interact differently with prairie dogs.

Methods

Keystone species

“As used by Paine and other ecologists, there are two hallmarks of keystone species. First, their presence is crucial in maintaining the organization and diversity of their ecological communities. Second, it is implicit that these species are exceptional, relative to the rest of the community, in their importance” (Mills et al. 1993, p. 219). We refer to Paine's definition to evaluate the role of prairie dogs structuring rodent assemblages across the black-tailed prairie dog species range as a multivariate phenomenon (see below). Power et al. (1996) identified 2 quantitative criteria to evaluate the keystone effects of a species: Overall Importance $OI_i = (t_N - t_D)/(t_N)$, where t_N is the community trait and t_D is the trait in the absence of species i , and Community Importance $CI_i = (OI_i)/(1/p_i)$, where p_i is the proportional abundance of species i . Power et al. (1996) considered the adjustment of Overall Importance by the abundance of the putative keystone species to be important to demonstrate that the effects are not simply a numerical response to the abundance of that species. Kotliar (2000) noted that for black-tailed prairie dogs, the assumption that community importance is a linear function of prairie dog density is inappropriate. Because our study design considered only the presence or absence of prairie dogs, we were unable to calculate Community Importance. However, we assume that it is not prairie dogs per se, but rather structural habitat changes (ecological engineering) brought about by prairie dogs (vegetation clipping, burrow excavation, etc.) that is important. We did not measure habitat variables, but we would not expect, nor did we observe, large differences among colonies in the structural impacts caused by prairie dogs; thus, we did not expect substantial variation among sites in prairie dog effects. When p_i does not vary, OI and

CI are equivalent. Here we measure OI as the differences in abundance, species richness, diversity, and evenness of rodent assemblages at trap grids on and off colonies at individual study areas. We assume that significant effects of prairie dogs on the abundance of other species, on species richness, or on species evenness, imply a keystone effect (see below). Kotliar (2000) added a third criterion for keystone status: that the species perform unique and non-redundant functions in the ecosystem. For prairie dogs, those unique functions (clipping vegetation, excavation of extensive underground burrow systems) are well established (Whicker and Detling 1988, Ceballos et al. 1999, Davidson and Lightfoot 2006, VanNimwegen et al. 2008).

Study sites and trapping

We collected small mammal data from 8 study areas within the geographic range of the black-tailed prairie dog during 2003 (Fig. 1). The 8 study areas were: 1) Badlands National Park, South Dakota; 2) Wind Cave National Park, South Dakota; 3) Thunder Basin National Grasslands, Wyoming; 4) Comanche National Grasslands, Colorado; 5) Cimarron National Grasslands, Kansas; 6) Janos, Chihuahua, Mexico; 7) Boulder County, Colorado; and 8) Charles M. Russell National Wildlife Refuge and Southern Phillips County, Montana (Russell). We recorded the UTM coordinates for each study area (WGS84 projection; Zone 13N). Within the United States, colony sizes generally varied between 10 and 100 ha, although some may have been smaller or larger. We did not measure colony sites at all areas during 2003. The Mexican sites were more variable, with the largest colony reportedly >10 000 ha. No colony had more than one trap grid (see below).

At each study area except Russell, which was an independent but methodologically similar study (Holmes 2003), we established several sampling sites, each consisting of one “on-colony” and one “off-colony” trapping grid, with off-colony grids located 500 m to 2 km from the edge of the colony in similar habitat and topography. Sampling grids at each study area were associated with different colonies so that the grids were independent of each other. Because our goal was to assess the impacts of prairie dog activities rather than general habitat differences on rodent species composition, we attempted to find off-colony grid locations that were similar in plant community type, soil type, and slope to the nearby on-colony grid. At Russell, we trapped similar numbers of grids on-colony ($n = 29$) and off-colony ($n = 28$), but off-colony grids were not associated with individual on-colony grids. At study areas 1–7, our sample sizes consisted of 4, 4, 6, 6, 6, 6 and 20 pairs of grids, respectively, where each square grid consisted of 7×7 ($n = 49$) large Sherman live-traps ($7.6 \times 8.9 \times 22.9$ cm) spaced 20 m apart. At Russell, each square grid consisted of 10×10 ($n = 100$) traps spaced 10 m apart. We trapped each site twice (early and late in the season) except at Russell and Janos, which were trapped once. Data from early and late sessions were combined for community analyses. Janos was trapped during the early session, and because of the large number of grids, Russell was trapped throughout the summer. The “early” trapping session was completed between 12 May and 29 June 2003 and the “late” trapping

session was completed between 8 July and 12 September 2003. We trapped a total of 36 688 on-colony and 36 288 off-colony trap-nights.

During each session, we opened traps for three successive nights (four at Boulder and Russell). We set and baited traps with oatmeal in late afternoon, and usually checked and closed them within 2 h of sunrise the following morning. We identified animals to species, sex, and age (juvenile or adult), weighed them, and took standard measurements (total length, tail length, ear pinna, hind foot, and mass). At sites 1–6 we uniquely marked individuals with numbered aluminum ear-tags. At sites 7 and 8, we marked individuals uniformly by shaving a portion of the hindquarters. All animals were released at their point of capture. If a marked animal was captured in both early and late trapping sessions, it was counted once for each session (twice for the year). Few animals were captured in both sessions. All animal trapping and handling procedures were approved by the Institutional Animal Care and Use Committees at Kansas State Univ., the Univ. of Colorado, and the Univ. of Montana, and conformed to the Guidelines of the American Association of Mammalogists (Gannon et al. 2007).

In the following analyses, we use univariate and multivariate statistical frameworks to characterize spatial variation in the effects of prairie dog colonies on small mammal communities. We compared species abundance, species richness, diversity, and evenness at on- and off-colony grids within each study area. We used the number of unique individuals trapped (excluding recaptures) as an index of abundance in the analyses. For comparisons among sites, abundances were adjusted to standardize for trapping effort by dividing the number of animals caught by the number of trap-nights. Species richness (S) was the observed (not estimated) number of species on each grid. We used Shannon’s diversity index (H') and calculated evenness as $H'/\log_e S$ (Magurran 1988). To compare the relative role of prairie dog colonies and study area, we modeled each metric (abundance, richness, diversity, and evenness) as a function of colony status (on/off), study area, and their interaction using generalized linear models (GLMs). We did not use geographic coordinates as predictors for these models, having discovered a non-monotonic response to latitude in all 4 metrics. For abundance and richness, we used function glm with a quasipoisson error distribution and a log link function in R for Statistical Computing (R Development Core Team 2008). This model specification accommodated the positively-skewed integer distribution of these metrics and allowed for their overdispersion. For diversity and evenness, we used function glm with a Gaussian error and identity link. Evenness was almost normally distributed after a log transformation and diversity was bimodal; however, the residual plots from both Gaussian models lacked a clear pattern and subsequent results were corroborated graphically by interaction plots (Results), so we allowed moderate departure from our assumption of normally distributed errors for these linear models of diversity and evenness. Significant interactions were interpreted as regional variability in the effect of prairie dogs, whereas insignificant interactions were dropped from the model, which was then re-analyzed for main effects of colony status and study area.

We analyzed the multivariate response of small mammal species composition using several functions in the *vegan* package (Oksanen et al. 2008) for R. Function *adonis* implemented a non-parametric multivariate analysis of variance (np-MANOVA) as described by Anderson (2001). This method implements analyses similar to ANOSIM (Clarke 1993) but is more flexible in that distance matrices can be modeled with multiple continuous and categorical effects, including interactions. We used np-MANOVA to model community dissimilarity as a function of colony status, latitude, and longitude of study areas, and their interactions (colony \times latitude and colony \times longitude). Interactions were treated and interpreted in the same way as described for univariate analyses above; that is, a significant interaction would mean that prairie dog colonies affected small mammal composition differently in different regions of the prairie dog range.

To visualize the effect of colony status and geography on small mammal species composition, we coupled unconstrained ordination with environmental interpretation. Specifically, we used function *metaMDS* to perform non-metric multidimensional scaling (NMDS, Kruskal 1964) on our study areas in 3 dimensions. Upon that ordination, we plotted the variables of colony status, latitude, and longitude using function *envfit*. Three dimensions provided a reasonable NMDS goodness-of-fit diagnostic or “stress” of 5.4% (following Borg and Patrick 2005), and we used the Bray-Curtis distance metric based on its performance in simulations (Faith et al. 1987). Abundance was standardized to trapping effort (number of trap-nights) for each study area, then square root-transformed to downweight inordinately high counts. The ordination mapped our study areas in similarity space; i.e. areas near each other in the ordination had relatively similar species compositions, whereas those farther apart had relatively dissimilar compositions. Finally, we tested for decay-by-distance in community similarity by calculating a Mantel correlation between community distance (Bray-Curtis) and geographical distance (Euclidean). Function *mantel* in the *vegan* package computed this correlation and its permutation-based significance following the implementation of Legendre and Legendre (1998).

Results

We captured 25 species of small mammals at the 8 study areas, including 24 rodent species and 1 lagomorph, the desert cottontail *Sylvilagus audubonii*. Because rabbits, except young juveniles, were too large for our traps, and they were rarely caught, we excluded them from analyses. Species caught differed among areas (Table 2). Observed species richness varied from 4 species at Wind Cave to 11 species at Boulder. Janos, the southernmost study area, had the most “unique” species (4 species not captured at other areas), followed by Russell, the northernmost site with 3 unique species. The deer mouse was the only species caught at all study areas, and the prairie vole *Microtus ochrogaster* was captured at all but Janos. The northern grasshopper mouse was absent from Boulder and Wind Cave, and the thirteen-lined ground squirrel *Spermophilus tridecemlineatus* was absent from Janos and Russell. The most abundant species was the deer mouse with 2127 captures. Next was the

northern grasshopper mouse (277 captures), which was consistently more abundant on colonies, followed by hispid pocket mice (113 captures) and thirteen-lined ground squirrels (77 captures), which were both most abundant off colonies. Other species were more local, caught in lower numbers, and most frequently caught at off-colony grids.

Small mammal abundance, richness, and diversity all varied significantly with colony status, study area, and colony-area interactions (Table 3) indicating an inconsistent effect of prairie dog colonies across study areas. Evenness varied with colony status and study area, but not with their interaction, indicating a consistent effect of prairie dogs, that being a higher evenness on average, across all study areas (Fig. 2).

Interaction terms from GLMs were study area-specific, and were further examined using interaction plots (Fig. 2). For both levels of colony status, the 4 responses were plotted against study areas, which were arranged by latitude to aid in geographical interpretation. Due to its apparent lack of colony effect (Fig. 2) and its extreme northern location, Russell was used as the reference level for GLM model terms containing study area. Compared to Russell, abundance was higher on prairie dog colonies, compared to off-colony grids, at the mid-latitude study areas, Boulder, Wind Cave, and Badlands, but not at Thunder Basin or further south at Janos, Cimarron, or Comanche. Richness and diversity showed an opposite response. Compared to Russell, these metrics were lower on prairie dog colonies at the mid-latitude study areas Boulder and Wind Cave, but did not differ from on colony grids at Thunder Basin, Badlands, or further south at Janos, Cimarron, and Comanche. Evenness responded somewhat consistently across all study areas: on average, evenness was higher on prairie dog colonies compared to Russell, with a marginally significant reversal of that effect at Badlands, and an insignificant reversal at Cimarron (Fig. 2).

Species composition varied with colony status, latitude, and longitude, and also with the colony \times latitude interaction (Table 3, bottom). The colony \times study area interaction was also significant, congruent with colony \times latitude. The nature of the interaction can be visualized in the ordinations: the colony effect was present throughout ordination space, in that the confidence ellipses never intersect, and the strength of this effect increased in northern latitudes. The latitude and longitude arrows on the NMDS plots form a plane perpendicular to the direction of separation between on and off levels of colony status (Fig. 3A). These graphical observations indicate that all 3 variables suggest independent gradients (except for the colony \times latitude interaction, congruent with results from np-MANOVA (Table 3).

Community dissimilarity increased with geographic distance among study areas (Mantel $r = 0.426$, $p = 0.004$). The correlation between dissimilarity and distance was stronger when only considering on-colony grids (Mantel $r = 0.671$, $p = 0.012$) or off-colony grids (Mantel $r = 0.765$, $p = 0.002$) in each study area.

Discussion

Black-tailed prairie dogs functioned as a keystone species across their range in that they consistently altered rodent

Table 2. Numbers of individual small mammals captured at each area at on-colony grids and (off colony grids). Bad = Badlands, Cim = Cimarron, Com = Comanche, TB = Thunder Basin, WC = Wind Cave.

Species	Common name	Bad	Boulder	Cim	Com	Janos	TB	WC	Russell	Total
<i>Chaetodipus hispidus</i>	Hispid pocket mouse	2(2)	24(58)	4(12)	4(6)	5(48)		0(1)		34(79)
<i>Dipodomys merriami</i>	Merriam's kangaroo rat									5(48)
<i>Dipodomys ordii</i>	Ord's kangaroo rat			6(15)	3(0)	18(25)	8(0)			17(15)
<i>Dipodomys spectabilis</i>	Banner-tailed kangaroo rat						0(2)			18(25)
<i>Lemmyscus curtatus</i>	Sagebrush vole									0(2)
<i>Microtus montanus</i>	Montane vole		0(3)							0(3)
<i>Microtus ochrogaster</i>	Prairie vole	3(7)	0(3)	0(1)	0(1)		0(1)	0(6)	1(23)	3(42)
<i>Microtus pennsylvanicus</i>	Meadow vole		0(3)							0(3)
<i>Mus musculus</i>	House mouse		0(4)		1(0)					1(4)
<i>Neotoma albigula</i>	White-throated woodrat					0(1)				0(1)
<i>Neotoma cinerea</i>	Bushy-tailed woodrat								0(2)	0(2)
<i>Neotoma mexicana</i>	Mexican woodrat		0(5)						0(2)	0(5)
<i>Neotoma micropus</i>	Southern plains woodrat			3(0)						3(0)
<i>Onychomys leucogaster</i>	Northern grasshopper mouse	24(3)		95(33)	49(9)	0(2)	31(10)		17(4)	216(61)
<i>Onychomys torridus</i>	Southern grasshopper mouse					0(4)				0(4)
<i>Perognathus fasciatus</i>	Olive-backed pocket mouse									0(9)
<i>Perognathus flavus</i>	Silky pocket mouse		0(1)	2(2)		2(18)				4(21)
<i>Peromyscus leucopus</i>	White-footed deer mouse			2(5)						2(5)
<i>Peromyscus maniculatus</i>	North American deer mouse			21(30)	1(36)	0(3)	113(159)	165(36)	325(378)	1317(810)
<i>Reithrodontomys megalotis</i>	Western harvest mouse	88(25)	604(143)		0(1)				0(11)	0(48)
<i>Reithrodontomys montanus</i>	Plains harvest mouse		0(36)	3(2)	1(0)					4(2)
<i>Spermophilus spilosoma</i>	Spotted ground squirrel				8(13)	1(0)	9(18)	0(4)		1(0)
<i>Spermophilus tridecemlineatus</i>	Thirteen-lined ground squirrel	2(8)	2(0)	6(7)						27(50)
<i>Tamias minimus</i>	Least chipmunk								0(4)	0(4)
Total individuals		119(42)	630(256)	142(107)	67(66)	25(101)	161(190)	165(47)	343(431)	1652(1243)
Total species		5/5(5)	3/9(11)	9/9(10)	7/6(9)	4/7(8)	4/5(6)	1/4(4)	3/7(7)	14/22(24)

Table 3. Significance of univariate and multivariate responses from generalized linear models and non-parametric multivariate analysis of variance (np-MANOVA). Top: the generalized linear models test whether the 4 metrics (abundance, richness, diversity, and evenness) have main effect differences between on- and off-colony status (Colony), among study areas, or an interaction (colony effect differs among study areas). Bottom: the np-MANOVA tests whether the mammal communities, as multivariate phenomena, differ between on- and off-colony, by latitude, or by longitude. The interactions test whether the on-off-colony relationships change with latitude, longitude, or among study areas. Whole-term interactions (as opposed to area-specific interactions from GLMs) were obtained by analysis of deviance (function anova in R). Colony = colony status (on- vs off-colony). See text for study areas. Latitude and longitude = WGS84 Zone 13N UTM Easting and Northing coordinates for each study area.

Generalized linear models	Colony	Study area	Colony × study area
Abundance	0.006	<0.001	<0.001
Richness	<0.001	<0.001	0.003
Diversity	<0.001	<0.001	<0.001
Evenness	0.023	<0.001	0.426

np-MANOVA			
- Main effects	Colony <0.001	Latitude <0.001	Longitude <0.001
- Interactions	Colony × latitude 0.015	Colony × longitude 0.955	Colony × study area 0.001

species composition on colonies compared to off-colony grassland sites. The impacts on abundance, richness, and diversity varied spatially (Fig. 2, Table 3). Rodent assemblage dissimilarity between study areas was correlated with distance; that is, areas close together were more similar than areas far apart. Species composition varied by prairie dog status (on- vs off-colony), latitude, and longitude.

Our use of the term keystone species follows the definitions offered in the Introduction, but differs in important ways from common usage in the conservation biology literature where a keystone species is expected to increase species richness or diversity. That expectation is not met in most instances between prairie dogs and other rodents. Prairie dogs do, however, show strong and

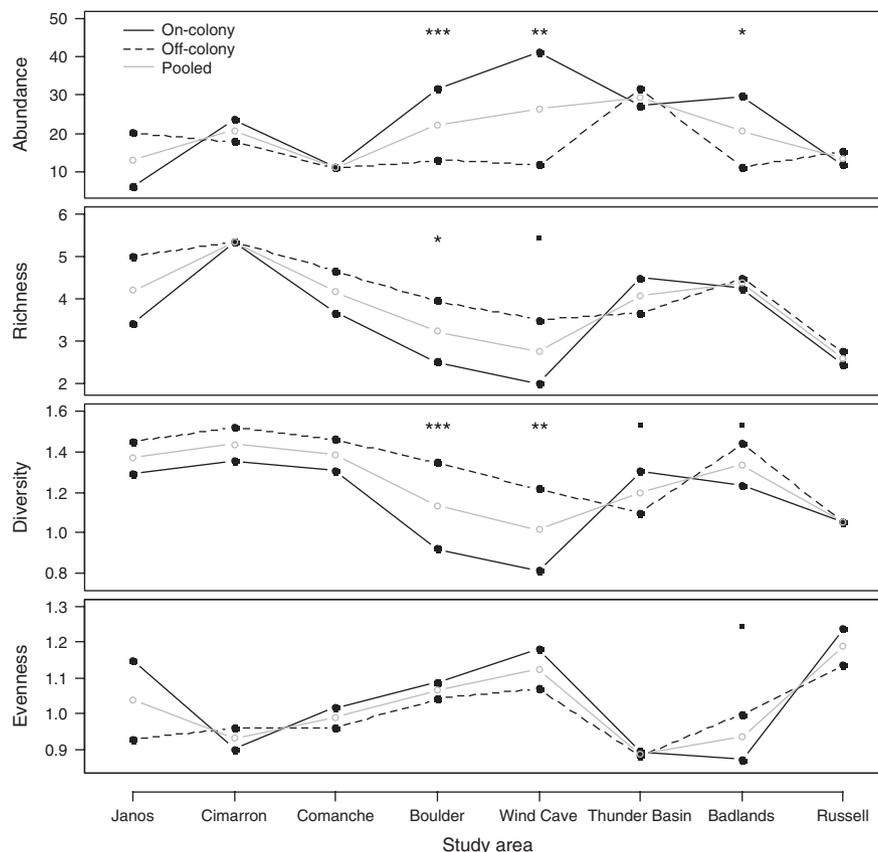


Figure 2. Interaction plots of 4 community response metrics: abundance, richness, Shannon’s diversity (H'), and evenness. Interactions are between colony status (on/off) and study area, using the Russell study area as a reference level. Interaction significance codes: <0.001 (***), <0.010 (**), <0.050 (*), <0.100 (■). The gray line indicates pooled response metrics for each study area had we ignored colony status.

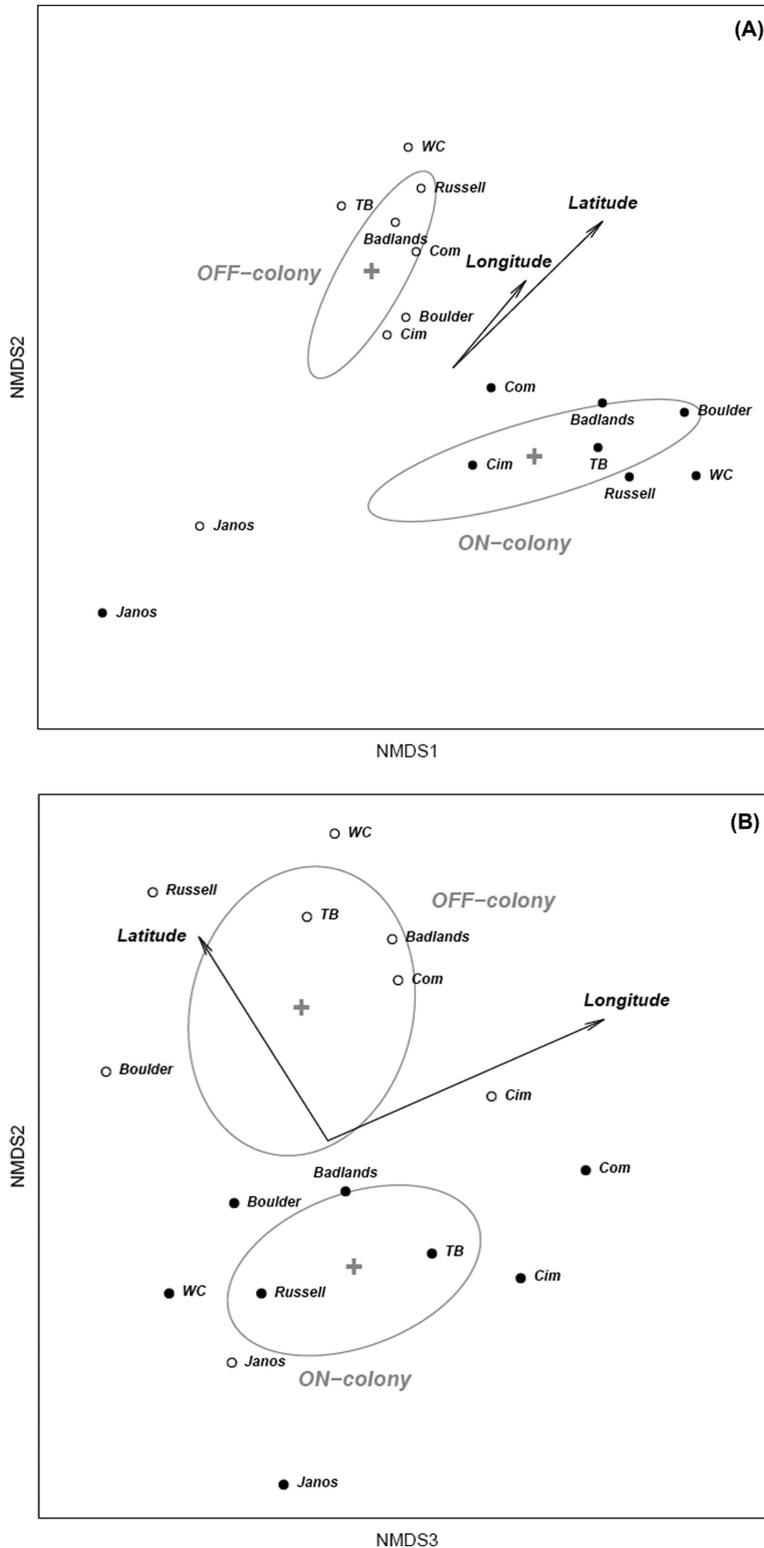


Figure 3. (A) NMDS ordination of study areas in dimensions 1 and 2, with regressed variables Latitude, Longitude (vectors), and Colony status (on/off centroids and ellipses). 95% confidence ellipses are for visualization only; reported significance values were obtained from np-MANOVA analysis (see text). Axis units are omitted due to their lack of information in distance-based ordinations: relative position of site scores provide more interpretive value. Open symbols are the off-colony grids at study areas (Badlands, BO = Boulder County, CIM = Cimarron, Russell = Charles M. Russell and southern Phillips County, COM = Comanche, Janos, TB = Thunder Basin, and WC = Wind Cave) and closed symbols on-colony grids. (B) NMDS ordination of study areas in dimensions 2 and 3 with regressed variables Latitude, Longitude (vectors), and Colony status (on/off centroids and ellipses). View is along the first dimension (previous plot turned 90 degrees clockwise looking down on its vertical axis). Confidence ellipses are for visualization only; reported significance values were obtained from np-MANOVA analysis (see text). Again, axis units are omitted due to their lack of information in distance-based ordinations: relative position of site scores are of more interpretive value.

consistent impacts on the rodent assemblages we encountered; in that context they meet our definition of a keystone species. Their effect is to enhance habitat for two of the dominant rodent species in short- and mixed-grass prairie – deermice and northern grasshopper mice. The reduction in usage for hispid pocket mice, thirteen-lined ground squirrels, and other, less common, grassland rodents that were rarely or never encountered on prairie dog colonies does not diminish prairie dogs' importance as ecosystem engineers and keystone species. The keystone role we observed was to produce consistent habitat patches in varied prairie settings. Such patches characteristically supported a limited, but important group of common species within a larger species pool that varied across the black-tailed prairie dog's range.

Prairie dog colonies affected small mammals at different spatial scales and at different population and community levels. At the Boulder study area, located near the geographic center of our region, abundance was markedly increased on prairie dog colonies, due to large populations of deermice, whereas species richness and Shannon's diversity were decreased. The same trend was noted to a lesser degree at Wind Cave and lesser still at Badlands (but without the effect on richness). Thus, this trio of study areas located at middle to northern latitudes showed a departure from the northern reference area (Russell) that disappeared farther south. Thunder Basin did not show a similar pattern despite its relative proximity to Wind Cave and Badlands, underscoring the geographic variability in the effect of prairie dogs on these metrics. Patterns at Thunder Basin in 2003 may have been influenced by a recent widespread epizootic of plague. In desert grasslands, keystone effects of Gunnison's and black-tailed prairie dogs varied spatially in that they interacted with complementary keystone effects of banner-tailed kangaroo rats *D. spectabilis*. Each species affected changes in lizard and arthropod assemblages, and the effects were synergistic where the 2 keystones occurred together (Davidson and Lightfoot 2006, 2007, Davidson et al. 2008).

Small mammal species composition also was affected by prairie dog colonies, but this effect did not vary across the geographic range of our study. Even though the regional species pool (represented by off-colony grids) changed along latitudinal and longitudinal gradients, the presence of prairie dog colonies always changed that composition at the local level. This multi-scale effect was also apparent in the correlations between community and geographic distances, which were nearly twice as high within a single treatment (on- vs off-colony) as when grids were pooled over both treatments.

Black-tailed prairie dogs had strong and significant effects on rodent species composition across their geographic range. The species composition at off-colony grids in the US varied both latitudinally and longitudinally, and was characterized by regionally abundant species. At the Cimarron National Grassland, the structure of the on-colony rodent assemblage was most likely a result of the presence of prairie dog burrows or other soil disturbance rather than effects of altered vegetation (VanNimwegen et al. 2008), and such may be the case in other study areas as well.

The multivariate analyses demonstrated a strong keystone effect. We suggest that prairie dogs demonstrated two important effects on small mammal assemblages. First, the observed spatial variation in the effects of prairie dogs on small mammals suggests that the effects were variable across the large scale of the western Great Plains of North America. Contrary to our expectations, there were qualitative differences in the effects of prairie dogs on small mammal assemblages across the range of the black-tailed prairie dog that were correlated with latitude and longitude. The variation in the keystone effect with latitude and longitude reflects the changes in composition of the species assemblages across the range of the black-tailed prairie dog. Abundance, evenness, and richness, where significant, were lower at on-colony grids at the three southern sites, as were richness and evenness at Boulder. Abundance was higher at on-colony grids at Boulder, Badlands, and Wind Cave, and unlike at the other sites, evenness was higher at on-colony grids at Thunder Basin (again, perhaps due to plague). Spatial variation in a keystone effect has rarely been studied. A notable exception involves large-scale studies of marine intertidal communities in Oregon and Washington, USA, which show that the effects of seastars on invertebrate communities vary both spatially and temporally (Menge et al. 1994, Navarrete and Menge 1996). For example, the intensity of seastar predation depends on wave exposure and rates of prey production at particular sites. These studies demonstrate that the impact of a keystone species may be high under some conditions but relatively modest under others. The same may be true of the effects (keystone or otherwise) of prairie dogs on small mammal assemblages, and spatial variation in the strength of these effects may have significant implications for disease dynamics or other ecological processes in this system. The abundance and species composition of small mammals within prairie dog colonies may critically affect the likelihood of prairie dogs contracting plague if the main route of transmission is through contact with these other rodent hosts.

Second, prairie dog colonies produced a consistent effect that differentiated small mammal species composition between on- and off-colony grids across the species range. The most important species in this regard were northern grasshopper mice and deermice. Where present, northern grasshopper mice were always more common on prairie dog colonies. Deermice were most common on prairie dog colonies where grasshopper mice were absent or occurred in low numbers. The other species either showed inconsistent patterns, or were more abundant at off-colony grids (Table 2).

Prairie dog alteration of grassland habitats is well documented. Numerous species of plants and both vertebrate and invertebrate animals benefit from the changes produced by foraging, vegetation clipping, and burrow digging activities of black-tailed prairie dogs (Whicker and Detling 1988, Ceballos et al. 1999, Kotliar et al. 1999, VanNimwegen et al. 2008). In addition, prairie dogs themselves are the most important occupants of colonies, and they were not considered in these analyses. We found clear and consistent differences in the rodent assemblages (composition) across the species range. However, the abundance, species richness, and diversity of small mammals were variable on prairie dog colonies compared to

nearby off-colony sites; some species responded positively and some negatively to prairie dog-altered habitats.

Prairie dogs clearly altered habitat suitability for some rodent species, and their extensive manipulation of grassland structure and burrowing provided unique functions that were not provided by other species. The term keystone species is usually applied when the species effect is to increase diversity. The dichotomy of keystone versus non-keystone is oversimplified and the so-called keystone role is really a gradient of effect (Kotliar 2000, Soulé et al. 2003). Whether or not black-tailed prairie dogs are considered keystones depends on the definition chosen, what is clear is that they are an important, highly interactive species on the grasslands of the U.S. western Great Plains with regionally varying effects on rodents depending on the local species pool.

Acknowledgements – We gratefully acknowledge the National Park Service for supporting work at Badlands and Wind Cave National Parks, with special thanks to B. Kenner, D. Roddy, D. Foster, and B. Muenchau, Cimarron, Comanche, and Thunder Basin National Grasslands, with special thanks to D. Augustine, A. Chappell, T. Byer, and C. Lockman, and R. List and J. Pacheco who kindly allowed us to use the research station at the Janos Prairie site. We especially thank John and Theresa Harris for their kindness in showing us around and introducing us to land owners in the Janos area. We gratefully acknowledge the City of Boulder Open Space and Mountain Parks, and Boulder County Parks and Open Space Dept, particularly Mark Brennan, Bryan Pritchett and Cary Richardson for facilitating our research in Boulder County, Colorado, and our 2003 field crews for enthusiastic field assistance. We thank Ken Gage for insightful discussions regarding rodent assemblages and disease ecology. We thank W. Davis, C. Pope, T. Johnson, B. Erie, M. Campbell, and D. Stetson for their long hours in the field. This research was supported by grants from the National Center for Environmental Research STAR program of the US-EPA (R-82909101-0), the NSF/NIH joint program in Ecology of Infectious Diseases (DEB-0224328), and the USGS Species At Risk program.

References

- Agnew, W. et al. 1986. Flora and fauna associated with prairie dog colonies and adjacent ungrazed mixed-grass prairie in western South Dakota. – *J. Range Manage.* 39: 135–139.
- Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. – *Austral Ecol.* 26: 32–46.
- Bangert, R. K. and Slobodkinoff, C. N. 2006. Conservation of prairie dog ecosystem engineering may support arthropod beta and gamma diversity. – *J. Arid Environ.* 67: 100–115.
- Bartz et al. 2007. **Please provide full details for this reference.**
- Borg, I. and Patrick, J.F. 2005. Modern multidimensional scaling: theory and applications, 2nd ed. – Springer.
- Ceballos, G. et al. 1999. Influence of prairie dogs (*Cynomys ludovicianus*) on habitat heterogeneity and mammalian diversity in Mexico. – *J. Arid Environ.* 41: 161–172.
- Clarke, K. R. 1993. Non-parametric analyses of changes in community structure. – *Aust. J. Ecol.* 18: 117–143.
- Collinge, S. K. et al. 2008. Effects of disease on keystone species, dominant species, and their communities. – In: Ostfeld, R. S. et al. (eds.), *Effects of ecosystems on disease and of diseases on ecosystems*. Princeton Univ. Press, pp. 129–144.
- Coppock, D. L. et al. 1983. Plant-herbivore interactions in a North American mixed-grass prairie. I. Effects of black-tailed prairie dogs on intraseasonal aboveground plant biomass and nutrient dynamics and plant species diversity. – *Oecologia* 56: 1–9.
- Davidson, A. D. and Lightfoot, D. C. 2006. Keystone rodent interactions: prairie dogs and kangaroo rats structure the biotic composition of a desertified grassland. – *Ecography* 29: 755–765.
- Davidson, A. D. and Lightfoot, D. C. 2007. Interactive effects of keystone rodents on the structure of desert grassland communities. – *Ecography* 30: 515–525.
- Davidson, A. D. and Lightfoot, D. C. 2008. Burrowing rodents increase landscape heterogeneity in a desert grassland. – *J. Arid Environ.* 72: 1133–1145.
- Davidson, A. D. et al. 2008. Engineering rodents create key habitat for lizards. – *J. Arid Environ.* 72: 2142–2149.
- Desmond, M. J. et al. 2000. Correlations between burrowing owl and black-tailed prairie dog declines: a 7-year analysis. – *J. Wildl. Manage.* 64: 1067–1075.
- Faith, D. P. et al. 1987. Compositional dissimilarity as a robust measure of ecological distance. – *Vegetatio* 69: 57–68.
- Gannon, W. L. et al. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. – *J. Mammal.* 88: 809–823.
- Hillman, C. N. and Clark, T. W. 1980. *Mustela nigripes*. – *Mamm. Species* 126: 1–3.
- Holmes, B. E. 2003. Ecology and persistence of sylvatic plague in Phillips County, Montana. – MS thesis, Univ. of Montana, Missoula, Montana.
- Hoogland, J. L. 2006. Conservation of the black-tailed prairie dog. – Island Press.
- Jones, C. G. et al. 1994. Organisms as ecosystem engineers. – *Oikos* 69: 373–386.
- Jones, C. G. et al. 1997. Positive and negative effects of organisms as ecosystem engineers. – *Ecology* 78: 1946–1957.
- Kotliar, N. B. 2000. Application of the new keystone species concept to prairie dogs: how well does it work? – *Conserv. Biol.* 14: 1715–1721.
- Kotliar, N. B. et al. 1999. A critical review of assumptions about the prairie dog as a keystone species. – *Environ. Manage.* 24: 177–192.
- Kretzer, J. E. 1999. Herpetological and Coleopteran communities of black-tailed prairie dog colonies and non-colonized areas in southwest Kansas. – Unpubl. Masters thesis, Division of Biology, Kansas State Univ.
- Kretzer, J. E. and Cully, J. F. Jr 2001. Effects of black-tailed prairie dogs on reptiles and amphibians in Kansas shortgrass prairie. – *Southwest. Nat.* 46: 171–177.
- Kruskal, J. B. 1964. Nonmetric multidimensional scaling: a numerical method. – *Psychometrika* 29: 115–129.
- Legendre, P. and Legendre, L. 1998. *Numerical ecology*, 2nd English ed. – Elsevier.
- Lomolino, M. V. and Smith, G. A. 2003. Terrestrial vertebrate communities at black-tailed prairie dog (*Cynomys ludovicianus*) towns. – *Biol. Conserv.* 115: 89–100.
- Magurran, A. E. 1988. *Ecological diversity and its measurement*. – Princeton Univ. Press.
- Mellink, E. and Madrigal, H. 1993. Ecology of Mexican prairie dog, *Cynomys mexicanus*, in El Manantial, northeastern Mexico. – *J. Mammal.* 74: 631–635.
- Menge, B. A. et al. 1994. The keystone species concept: variation in interaction strength in a rocky intertidal habitat. – *Ecol. Monogr.* 64: 249–286.
- Miller, B. et al. 1994. The prairie dog and biotic diversity. – *Conserv. Biol.* 8: 667–681.
- Mills, L. S. et al. 1993. The keystone species concept in ecology and conservation. – *Bioscience* 43: 219–224.
- Navarrete, S. A. and Menge, B. A. 1996. Keystone predation and interaction strength: interactive effects of predators on their main prey. – *Ecol. Monogr.* 66: 409–429.

- Oksanen, J. et al. 2008. vegan: community ecology package. – R package ver. 1.11-0, <<http://cran.r-project.org/>>, <<http://vegan.r-forge.r-project.org/>>.
- O'Meila, M. E. et al. 1982. Some consequences of competition between prairie dogs and beef cattle. – *J. Range Manage.* 35: 580–585.
- Paine, R. T. 1969. A note on trophic complexity and community stability. – *Am. Nat.* 103: 91–93.
- Power, M. E. et al. 1996. Challenges in the quest for keystones. – *BioScience* 46: 609–620.
- R Development Core Team 2008. R: a language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna, Austria, <www.R-project.org>.
- ShIPLEY, B. K. and Reading, R. P. 2006. A comparison of herpetofauna and small mammal diversity on black-tailed prairie dog (*Cynomys ludovicianus*) colonies and non-colonized grasslands in Colorado. – *J. Arid Environ.* 66: 27–41.
- Soulé, M. E. et al. 2003. Ecological effectiveness: conservation goals for interactive species. – *Conserv. Biol.* 17: 1238–1250.
- Stapp, P. 1998. A reevaluation of the role of prairie dogs in Great Plains grasslands. – *Conserv. Biol.* 12: 1253–1259.
- Stapp, P. 2007. Rodent communities in active and inactive colonies of black-tailed prairie dogs in short-grass steppe. – *J. Mammal.* 88: 241–249.
- VanNimwegen, R. E. et al. 2008. Ecosystem engineering by a colonial mammal: how prairie dogs structure rodent communities. – *Ecology* 89: 3298–3305.
- Whicker, A. D. and Detling, J. K. 1988. Ecological consequences of prairie dog disturbances: prairie dogs alter grassland patch structure, nutrient cycling, and feeding-site selection by other herbivores. – *BioScience* 38: 778–785.
- Winter, S. L. and Cully, J. F. Jr 2007. Burrowing owl associations with black-tailed prairie dog colonies in southwestern Kansas and southeastern Colorado. – *Prairie Nat.* 39: 69–75.
- Winter, S. L. et al. 2002. Vegetation of prairie dog colonies and non-colonized short-grass prairie. – *J. Range Manage.* 55: 502–508.
- Wright, J. P. et al. 2002. An ecosystem engineer, the beaver, increases species richness at the landscape scale. – *Oecologia* 132: 96–101.