

RESEARCH ARTICLE

Reintroducing a keystone burrowing rodent to restore an arid North American grassland: challenges and successes

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Prairie dogs (*Cynomys* spp.) are important ecosystem engineers in North America's central grasslands, and are a key prey base for numerous predators. Prairie dogs have declined dramatically across their former range, prompting reintroduction efforts to restore their populations and ecosystem functions, but the success of these reintroductions is rarely monitored rigorously. Here, we reintroduced 2,400 Gunnison's prairie dogs (*C. gunnisoni*) over a period of 6 years to the Sevilleta National Wildlife Refuge, in central New Mexico, U.S.A., a semi-arid grassland ecosystem at the southern edge of their range. We evaluated the population dynamics of prairie dogs following their reintroduction, and their consequent effects on grassland vertebrates. We found postrelease survival of prairie dogs stabilized at levels typical for the species (ca. 50%) after approximately 1 month, while average annual recruitment was ca. 0.35 juveniles per female, well below what was required for a self-sustaining, stable population. Extreme drought conditions during much of the study period may have contributed to low recruitment. However, recruitment increased steadily over time, indicating that the reintroduced colony may simply need more time to establish in this arid system. We also found well-known associates of prairie dog colonies, such as American badgers (*Taxidea taxus*) and burrowing owls (*Athene cunicularia*), were significantly more common on the colonies than off. After 7 years, we have yet to meet our goal of establishing a self-sustaining population of Gunnison's prairie dogs in this semi-arid grassland. But despite the uncertainty and challenges, our work shows that reestablishing keystone species can promote ecosystem restoration.

Key words: burrowing mammals, capture-mark-recapture, *Cynomys gunnisoni*, ecosystem engineer, tag loss, wildlife translocation

Implications for Practice

- Offspring recruitment was the primary factor limiting population growth. Management efforts to promote offspring recruitment may help reintroduced prairie dogs become established in arid regions.
- Population growth slowly increased with time, as the extreme drought receded, suggesting that this reintroduced prairie dog population eventually may become self-sustaining.
- Ear-tag loss rate was high, indicating the importance of double-tagging whenever possible.
- Reintroduction of prairie dogs can have positive effects on numerous grassland vertebrates, thereby promoting ecosystem restoration.
- To assess reintroduction success, it is critical to establish quantifiable success criteria (e.g. positive population growth), conduct long-term monitoring, and define how much time can pass before the objectives are achieved (or not).

Introduction

Reintroductions of wildlife are important for recolonizing historically occupied regions, reducing species-level extinction risk, and restoring ecosystem functions (Seddon et al. 2007,

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2014). However, reintroductions often require considerable resources and effort (Seddon et al. 2007, 2014; IUCN/SSC 2013) and, despite this, most reintroductions end in failure or incomplete success (Seddon et al. 2014). This has led to much discussion in the literature on the challenges of reintroductions and strategies to improve reintroduction protocols, including decision support tools and the recent International Union for the Conservation of Nature (IUCN) guidelines for wildlife translocations (Pérez et al. 2012; Batson et al. 2015). Here, we report on the challenges and successes of our efforts to restore Gunnison's prairie dog (*Cynomys gunnisoni*) populations and their ecological role to a semi-arid grassland ecosystem at the southern and xeric edge of their range.

Prairie dogs (Cynomys spp.), like other social, burrowing, and grassland herbivores around the world, play an important role in shaping the grassland ecosystems they inhabit (Whicker & Detling 1998; Kotliar et al. 2006; Davidson et al. 2012). By grazing and clipping vegetation, prairie dogs create a low mat of dense forbs and grazing-tolerant grasses within their colonies, and their burrows provide refuge for many animals (Whicker & Detling 1998; Davidson et al. 2012). Prairie dog colonies attract numerous species that prefer the open grassland habitat they create, such as burrowing owls (Athene cunicularia) and mountain plovers (Charadrius montanus), and they attract large herbivores, such as bison (Bison bison) and cattle (Bos taurus), that prefer the higher quality forage found on their colonies (Kotliar et al. 2006; Bayless & Beier 2011). In addition, many predators rely on prairie dogs as a primary food source, such as coyotes (Canis latrans), American badgers (Taxidea taxus), raptors, and the endangered black-footed ferret (Mustela nigripes) (Goodrich & Buskirk 1998; Kotliar et al. 2006; Davidson et al. 2012; Eads et al. 2015, 2016; Grassel et al. 2015).

Prairie dogs have been persecuted as pests and face a suite of anthropogenic threats, in part due to their conspicuous activities as herbivores and ecological engineers (e.g. grazing, burrowing, and clipping; Miller et al. 2007; Delibes-Mateos et al. 2011; Davidson et al. 2012). Consequently, prairie dogs now occupy an estimated 2% of the area they occupied a century ago (Hoogland 2006). Prairie dog occupied habitat and prairie dog numbers continue to be lost due to deliberate persecution (e.g. poisoning and shooting) (Miller et al. 2007; Bergstrom et al. 2014), in addition to introduced exotic disease (sylvatic plague; (Cully et al. 2006; Eads & Biggins 2015), habitat loss (Ceballos et al. 2010), and, increasingly, climate change (Ceballos et al. 2010; Davidson et al. 2014; Eads & Biggins 2017; Eads & Hoogland 2017).

Functional grassland ecosystems depend on the existence of prairie dogs in large enough numbers to support associated wildlife (Davidson et al. 2012). The documented consequences of prairie dog loss demonstrate their critical role in maintaining grassland ecosystems (Ceballos et al. 2010; Ponce-Guevara et al. 2016). For example, the dramatic decline in prairie dog distribution and abundance has resulted in declines in associated species (Kotliar et al. 2006; Davidson et al. 2012; Eads & Biggins 2015). Additionally, declines of black-tailed prairie dogs (C. ludovicianus) have contributed to the desertification and

shrub invasion of North American semi-arid grasslands (Ceballos et al. 2010; Ponce-Guevara et al. 2016).

Because of their ecological importance, prairie dog population restoration is likely to have cascading effects on associated species and the grassland ecosystem (Fig. 1), similar to the rippling effects that other keystone species have had when reintroduced, such as beavers (Castor Canadensis), sea otters (Enhydra lutris), and wolves (Canis lupus) (Hastings et al. 2007; Gibson & Olden 2014; Ripple et al. 2014). Therefore, understanding how to successfully restore and maintain prairie dog populations across their range, and if/how reintroduction efforts help to restore their functional role, is important for grassland conservation and management (Davidson et al. 2014). Reintroductions of prairie dogs often entail translocating them from areas where they are in conflict with humans to wildlife refuges (e.g. Truett et al. 2001; Bly-Honness et al. 2004; Dullum et al. 2005; Shier 2006; Davidson et al. 2014). Considerable research has been conducted to determine successful methodologies for reestablishing prairie dogs (especially C. ludovicianus), which involves releasing them into pre-existing burrows or constructed artificial burrow systems (Long et al. 2006). Despite numerous reintroduction efforts occurring across their ranges over the last two decades, the long-term (decadal) success of reintroductions and how success varies across different parts of their geographic range remains limited (Facka et al. 2010; Davidson et al. 2014). Such information is needed in the context of climate change, especially in the southern portion of their ranges that is projected to become increasingly warmer and drier (Seager et al. 2007; Gutzler & Robbins 2010). Additionally, few studies have reported on the response of grassland ecosystems to prairie dog reintroductions (Davidson et al. 1999).

The purpose of our research was to evaluate the population status and dynamics of three newly established (since 2010) Gunnison's prairie dog colonies at the Sevilleta National Wildlife Refuge in central New Mexico, and the impact of their reintroduction on grassland wildlife. To understand whether our large-scale reintroduction effort was successful in terms of multi-year population viability and ecosystem restoration, we addressed the following questions: (1) Was the population self-sustaining (positive population growth rate) after 6 years following several years of reintroductions? (2) Which (if any) environmental variables were important drivers of demographic success during this time? (3) Were grassland vertebrate species influenced by prairie dog reintroduction (e.g. increased activity on versus off colonies) 6 years following project initiation?

Methods

Study Area

The Sevilleta National Wildlife Refuge (SNWR), located in Socorro County, New Mexico (Fig. 2), is a unit of the National Wildlife Refuge system. The SNWR is available specifically for research and education purposes, and, because there are no cattle grazing on the refuge, we were able to avoid potential conflicts between prairie dog reintroduction efforts and livestock producers. The 92,000 ha refuge previously supported colonies

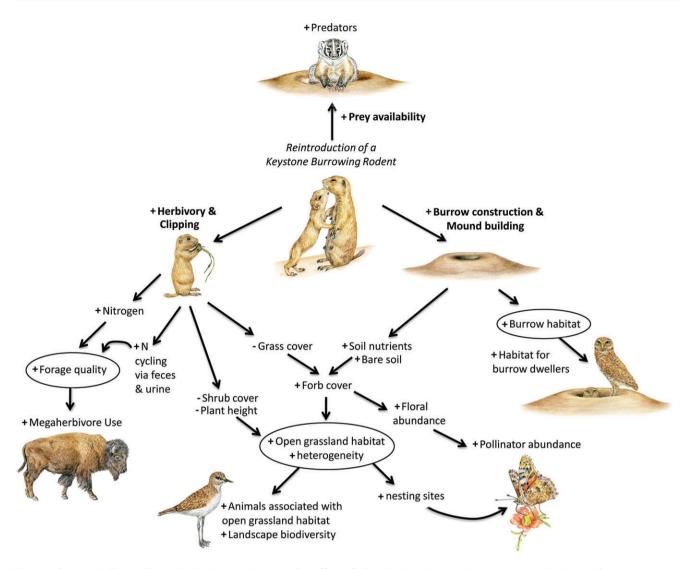


Figure 1. Conceptual diagram illustrating the hypothesized, cascading effects of reintroducing a keystone burrowing mammal, using prairie dogs (*Cynomys* spp.) in North America's central grasslands as an example. Reintroduction of prairie dogs should result in the addition of their trophic (herbivory, prey) and ecosystem engineering (clipping, burrow construction, and mound building) effects on the grassland, with consequent increases in predators (e.g. black-footed ferrets [*Mustela nigripes*], raptors, swift and kit foxes [*Vulpes velox*, *V. macrotis*], coyotes [*Canis latrans*], badgers [*Taxidea taxus*]), large herbivores (e.g. Bison [*Bison bison*]), invertebrate pollinators, and species that associate with the open habitats and burrows that they create (e.g. burrowing owls [*Athene cunicularia*], mountain plovers [*Charadrius montanus*], pronghorn [*Antilocapra americana*], swift and kit foxes, cottontail rabbits [*Sylvilagus* spp.], rodents, and many species of herpetofauna and invertebrates). Black arrows depict the effects of prairie dogs. Plus signs indicate an increase in an ecosystem property as a result of the addition of prairie dogs; minus signs indicate a decrease. Drawings are by Sharyn N. Davidson.

of Gunnison's prairie dogs prior to extermination in the 1960s (J. Ford 1999, Wildlife Services, US Department of Agriculture, personal communication; Fig. 2). The site with the most extensive southern shortgrass steppe habitat at SNWR that Gunnison's prairie dogs once occupied, was chosen as the reintroduction site. The reintroduction site, located in the western foothills of the Los Piños Mountains (NAD 83, UTM Zone 13 N, Northing 3,800,855 Easting 349,376, elevation 1,650 m; Fig. 2), was dominated by short-stature blue grama (*Bouteloua gracilis*), a mixture of black grama (*B. eriopoda*), and James' galleta (*Pleuraphis jamesii*) grasses, various annual and perennial forbs, and some small shrubs. The SNWR is on the southern edge of the geographic range of *C. gunnisoni*. The majority of

the precipitation at the SNWR comes during the summer rainy season (June–September) (Moore 2017). Precipitation during the winter–spring period (October–May) is highly variable from year to year in this system, with droughts (< 50 mm) during this period being frequent (Moore 2017).

Field Methods

Prairie Dog Reintroductions. We established four Gunnison's prairie dog colonies in 2010, and installed four 9-ha plots on each colony and four 9-ha off-colony control plots with no prairie dogs added (Fig. S1). We randomly selected the treatment and control plots. Because there were no pre-existing

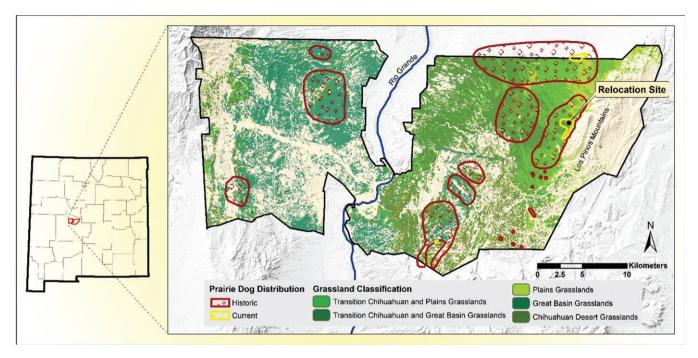


Figure 2. Historical and current distribution of Gunnison's prairie dogs (*Cynomys gunnisoni*) on the Sevilleta National Wildlife Refuge, New Mexico, U.S.A. Supervised vegetation classification created from Thematic Mapper satellite imagery (Muldavin et al. 1998).

burrows at our release site, we reintroduced prairie dogs into artificial burrows on the colony plots in 2010–2015 (releases on plots B and C began in 2010, plot F in 2012, and plot G in 2014; Fig. S1). We installed 70 artificial burrows across each 9-ha colony plot. Detailed methods on artificial burrow construction are provided by Davidson et al. (2014) and in Appendix S2.

We captured prairie dogs to be released on the study plots from nearby urban and rural areas in and around Albuquerque, New Mexico (approximately 105 km away), Belen, New Mexico (approximately 37 km away), and a small number from Taos, New Mexico (approximately 300 km away). Methods used in this project were approved by the University of New Mexico's Institutional Animal Care and Use Committee (Protocol 15-200326-MC). We strived to ensure that all reintroduced animals were from the prairie population of Gunnison's prairie dogs and were of the putative C. g. zuniensis subspecies that occurs in the lower elevation grasslands, and which are the native residents of SNWR (Sackett et al. 2014). Capture efforts occurred in spring (March-April) and summer (mid-June through August) in 2010–2016 (Fig. 3). We captured prairie dogs using water flushing and trapping methods, and followed similar protocols to those discussed by Long et al. (2006) and Davidson et al. (2014). We kept individuals in a holding facility with their apparent family groups (hereafter, clans) and fed them daily for a period of at least 1 week to optimize health for release. We dusted all animals with flea powder (DeltaDust, Bayer CropScience LP, Research Triangle Park, NC, U.S.A.) to prevent transporting potentially plague-infected fleas to the release site (Long et al. 2006). For all captured individuals, we obtained data on weight and sex and applied numbered ear tags or Passively Induced Transponder (PIT) tags (Appendix S1).

We released prairie dogs into artificial burrows during the first time releases occurred on each study plot (plots B and D: 2010; plot F: 2012; plot G: 2014). We released animals from the same clan into the same or adjacent burrows. Once released into the burrows, we covered the artificial burrow with an acclimation cage for 2-5 days following release (Appendices S1 and S2; Table S1). The cages allowed individuals to explore the surface visually, communicate with nearby captive individuals (or previously released free-ranging prairie dogs), eat, and acclimate before cage removal. We fed newly released animals daily while cages were in place. After the cages were removed, the animals at release sites were fed as needed (i.e. if drought conditions were present) throughout the summer (Appendix S2). Once released, prairie dogs dug their own natural burrow systems, often continuing to use the artificial burrows as well. Releases into artificial burrows only occurred during the first releases on each plot; subsequent releases were into abandoned natural burrows or augured burrows, in unoccupied areas of the plots.

Prairie Dog Population Monitoring Following Reintroduction. Population assessment during the course of the study consisted of live capture-recapture trapping (Figs. S1 and S2A). We used 100 welded wire mesh treadle-type live traps $(19 \times 19 \times 48 \text{ cm})$ across each 9-ha plot, with two traps placed at each numbered, flagged, and mapped trapping station near active burrows. We wired traps open and prebaited them for 2 days prior to trapping events, set and baited them at dawn each day of a trapping event, and left them open during the morning

until ambient temperature reached 25°C. Trapping occurred during the spring (March/April, 2012–2014, 2016) and summer (June/July 2011–2016) before new animals were released (see Fig. 3). Each trapping period consisted of three consecutive trap days (Appendix S1).

Vertebrate Diversity and Activity. To investigate whether vertebrate species were more commonly found on prairie dog colonies compared to off-colony grasslands, we conducted camera trap surveys for one summer activity season at the end of this study, from April 2015 to October 2015. We deployed 40 infrared-triggered camera traps (Bushnell Trophy Cam 11-9636c, Bushnell Outdoor Products, Overland Park, KS) across our study site to detect the presence of vertebrate species, with each camera operating 24 h/day. For each of the eight 9-ha study plots (on-colony and off-colony), we installed one camera trap at each of the four plot corners and one at the center (Fig. S1). Each camera was attached to a T-post at ca. 1 m above the ground and faced the center of the plot (Fig. S2B). We processed all photos using the Picture Information Extractor (PIE) for georeferencing and cataloging. We identified all detected vertebrates to species where possible, and recorded the number of individuals of each species captured in each photo.

Environmental Variables. We obtained monthly precipitation and soil moisture data from a Sevilleta Long-Term Ecological Research Program (LTER) meteorological station (Blue Grama Core Site) located in the center of our study site, which was fitted with a tipping bucket rain gauge (Moore 2017). We used Sevilleta LTER data on seasonal plant canopy cover obtained from the Blue Grama Core Site, as an indicator of net primary productivity (NPP) (Sevilleta LTER Program 2017). Plant canopy cover was measured once in May (representing spring growth from February to May) and once in September (representing summer growth from May to September), from 2010 to 2016.

Data Analysis

Annual survival rates were estimated from capture–recapture data using a modified Cormack–Jolly–Seber model (inclusion of individuals in the likelihood function was conditioned on initial capture; Royle & Dorazio 2008). For estimating vital rates, we considered our reestablished colonies (one colony per study plot; a total of four colonies) to be a part of a single study population. We assumed population closure between consecutive-day trap visits, or "secondary" capture occasions (standard "robust-design" assumption; Kendall et al. 1997), with populations open to mortality among seasonal visits, or "primary" capture occasions (total of 12 visits separated by an average of 6.5 months). We implemented this model in a Bayesian framework (see Appendix S1 for details on implementation in WinBUGS and R).

Capture Probability and Abundance. We modeled expected probability of capture (p) as constant per secondary sampling period (i.e. each day within a 3-day trapping session) because the trapping protocol at each sampling site was standardized

(9-ha trap area). We included a logit-normal random effect to account for differential capture probability among individuals. We estimated total prairie dog abundance within each sampled colony using a Horvitz-Thompson estimator (McDonald & Amstrup 2001).

Survival. Survival rates between seasonal trapping bouts were modeled as a logit-linear function of environmental variables and intrinsic characteristics (e.g. sex and age). The apparent survival rate between survey periods for resident individuals (≥1 month postrelease) was modeled with the following equation:

$$logit\left\{ \left(\varphi_{t}\right)^{\frac{1}{per}}\right\} = \beta_{0} + \beta_{1}.precip_{t} + \beta_{2}.soilH20_{t} + \beta_{3}.isJuv_{i} + \beta_{4}.isMale_{i}$$

$$(1)$$

where φ_t represents survival rate between consecutive trapping periods, per represents the interval between sampling periods in units of years, β_0 represents mean annual survival rate on the logit scale, β_1 represents the logit-linear effect of seasonal precipitation (precip_t, see below for details) on survival, β_2 represents the logit-linear effect of mean seasonal soil moisture $(soil H20_t)$, and β_3 and β_4 represent the expected logit-scale difference in expected annual survival for juvenile (isJuv_i) and male (isMale;) individuals, respectively. All quantitative covariates (e.g. seasonal precipitation) were standardized prior to analysis. Seasonal precipitation and soil moisture were defined as averages for either winter (November-April) or summer (May-October) periods. We modeled the influence of environmental conditions (precipitation and soil moisture) with no time lags; e.g. the probability of surviving from September 2011 to April 2012 (the period of time between two successive capture periods) was modeled as a function of precipitation and soil moisture in November 2011-April 2012. We also tested for a 1-year time lag in the influence of environmental conditions on survival rates as well as the effects of spring and summer NPP (average plant canopy cover) on survival (in models without precipitation effects, as NPP and precipitation are correlated), but we failed to detect any lag effects or effects of NPP, so these effects were not included in the final model.

The survival rate for newly-released individuals (≤ 1 month postrelease) was modeled as a function of variables describing the context of release from captivity (e.g. released in spring and released as a juvenile), as follows:

$$logit \left\{ \left(\varphi_{rel_{t}} \right)^{12} \right\} = \beta_{0} + Rel.Eff + \beta_{6}.Released.as.Juv_{i}.$$

$$+ \beta_{7}.Released.in.Spring_{i} \tag{2}$$

where $\varphi_{\rm rel}$ represents survival in the first month postrelease, Rel.Eff represents the change in annualized survival rate for newly released individuals on the logit scale (relative to "resident" individuals), and β_6 and β_7 are terms representing the difference in expected survival for individuals released as juveniles (vs. adult) or during the spring (vs. summer) season. Although

the survival (φ) terms technically represent apparent survival (compound parameter indicating death or permanent emigration), we interpret these terms as true survival because, given the isolation of the release sites from other existing colonies, permanent emigration from these colonies most likely is functionally equivalent to mortality.

Tag Loss. Loss of ear tags was observed frequently in the marked population at SNWR, such that failing to account for tag loss as a factor eliminating individuals from the marked population would substantially underestimate survival rates (permanency of markings is a key assumption of capture-recapture analysis; Silvy et al. 2005). Since most individuals in the population were double-tagged (left and right ear tags), we were able to estimate tag loss rates explicitly and thereby relax the no-tag-loss assumption (e.g. Rivalan et al. 2005; Oosthuizen et al. 2010). We built the tag loss process into survival model, so that survival, tag loss, and detection were modeled simultaneously (Appendix S1).

Estimation of Recruitment and Finite Population Growth Rate. We used Approximate Bayesian Computation (ABC; Beaumont 2010) to estimate the set of recruitment rates that could plausibly yield the observed fraction of native-born prairie dogs (those juveniles and adults recorded as having no signs of ever having ear tags) observed in 2012-2015 (2012 was the first year native-born individuals were observed; Fig. 4). To do this, we simulated population dynamics of female prairie dogs using the known history of releases (year and season of release, number released, and age at release), posterior distributions of survival and capture rates, and estimated effects of release status, age, and environmental covariates on survival. Annual recruitment rate (juveniles produced per female per year, representing the product of pregnancy probability, litter size, and underground pup survival rates) was assigned a uniform prior between 0.01 and 2.5 juveniles per female per year. We ran 10,000 simulations, retaining only those parameter sets (including recruitment rates) that yielded a number of native-born individuals within 5% of the observed value for each year 2012-2015 (recruitment rates were estimated separately for all 4 years). Drawing 4,000 times from the ABC joint posterior distribution for recruitment and adult and juvenile survival rates, we then calculated an ABC posterior distribution for the discrete population growth rate (lambda).

Vertebrate Diversity and Activity. We conducted occupancy analyses for photos of vertebrate species on and off the prairie dog colonies using the formulation of Royle and Nichols (2003), as implemented in the "unmarked" package in R (Fiske & Chandler 2011). This model assumes that capture probability is (logit-) linearly related to local abundance, such that denser populations correspond to higher capture probabilities. However, camera traps often have the limitation of spatial and/or temporal autocorrelation whereby the same individual may be detected by multiple camera traps; this can be especially relevant for species with large home ranges relative to between-camera

distances. Because we lacked sufficient data to model spatial autocorrelation explicitly, we conservatively treated our regression coefficients (effect of being on-colony vs. off-colony) as representative of differences in relative activity between treatments. To reduce serial autocorrelation, we summarized detections at each camera trap station at 1-week intervals (whereby "presence" represents one or more observations in a given week) from April 2015 through October 2015. Habitat structure at our study site was open within the field of view of the cameras, such that detection probabilities of larger vertebrates were similar across the two treatment types. We only conducted analysis on those species for which we had a sufficient number of detections (algorithm nonconvergence signified insufficient sample size) and confidence in photo identifications.

Results

Environmental Variables

An extreme drought occurred across the region and most of the Southwest during much of our study period. The study area was not in drought in 2010, but then went into extreme drought in 2011, severe drought in 2012 and 2013, abnormally dry in 2014, and no drought in 2015–2016 (The National Drought Mitigation Center, University of Nebraska-Lincoln U.S. Drought Monitor 2017; Fig. S3). Soil moisture was not directly correlated with precipitation, likely due to lags in the amount of time required for the soil to recover from drought. NPP (as measured by plant canopy cover) was correlated with precipitation, with low productivity occurring during the drought and especially high productivity following large rainfall totals in the summer of 2013 at the end of the severe drought (Fig. S3).

Population Dynamics

Overall, 2,412 individual prairie dogs were released at SNWR between the years 2010 and 2015 (Fig. 3). Of the 2,412 animals released, 286 were recaptured at least once during subsequent surveys.

Capture Probability and Abundance. Daily capture probability for reintroduced prairie dog colonies at SNWR was 0.3 (95% credible interval [CI]: 0.27 to 0.34), and among-individual variation in detectability was estimated at 0.94 (95% CI: 0.81–0.99) (Table 1). From these detection probability estimates, we estimated that total resident abundance increased from approximately 15 individuals in summer of 2011 to a high of approximately 217 in summer of 2013, after which resident abundance exhibited a sharp decline (Fig. 3). The recently estimated increase in abundance from spring 2016 to summer 2016 was the first observation of population increase that was not directly attributable to supplementation of additional individuals, and occurred after the drought period broke in 2014 (Fig. 3).

Survival. Estimated mean annual survival for resident females at SNWR was 0.52 (95% CI: 0.29–0.77). Adult males generally experienced lower survival rates than females, averaging

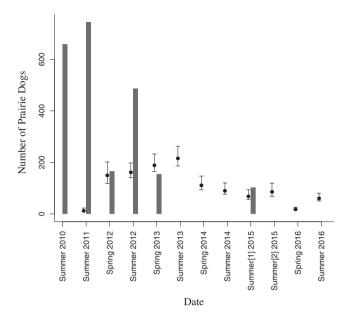


Figure 3. Estimated prairie dog abundance across the 9-ha reintroduction plots over time (2010–2016) at the Sevilleta National Wildlife Refuge, Socorro County, New Mexico. Columns represent the number of prairie dogs released at the start of each time period. Points (and 95% credible intervals) represent estimates of population size before releases were conducted (e.g. the point estimate in "Summer 2011" represents the estimated number of prairie dogs that survived from "Summer 2010" until the next set of releases in "Summer 2011"; this is why there is no point estimate for "Summer 2010" as there were no prairie dogs to survive from a previous time period).

0.31 (95% CI: 0.14–0.57). Survival of resident juveniles was comparable with females, averaging 0.53 (95% CI: 0.26–0.81). Precipitation and soil moisture (with or without a temporal lag effect) were not major factors influencing survival during the study period (Table 1).

Survival rates in the first month postrelease were much lower than for equivalent residents, regardless of year or season; estimated 1-month survival rates for newly released individuals were 0.22 for spring-released females (95% CI: 0.18–0.27; males: 0.17–0.25 [95% CI]) and 0.17 for summer-released females (95% CI: 0.13 to 0.22; males: 0.12–0.21 [95% CI]). No

significant differences in survival were detected between spring and summer releases or between releases of adults and juveniles.

Tag Loss. Retention of individual ear tags (left or right ear) in this study averaged 0.56 per year (Table 1). At this rate, many individuals released at the beginning of the study, during which most individuals were double-ear-tagged (years 2011 to 2012; i.e. tagged in both left and right ears), would be expected to have lost both tags by the final year of monitoring (2016); e.g. a double-tagged individual released in 2012 would have an approximately 81% chance of losing both ear tags by 2016.

Recruitment and Population Growth Rate. We estimated mean recruitment for the years 2012–2015 to be 0.32 juveniles/female/year (95% ABC credible interval: 0.05–0.94). Accounting for the full range of uncertainty in recruitment and survival rates, estimated mean population growth rate (combining recruitment estimates with survival rates) was 0.75 (95% ABC credible interval: 0.44–1.16). Estimated recruitment rates exhibited a steady increase over time, from a mean of 0.15 in 2012 to 0.48 in 2015 (Fig. 4A). Similarly, mean lambda estimates increased from 0.66 in 2012 to 0.83 in 2015 (Fig. 4B).

Vertebrate Diversity and Activity

Our cameras captured over 6,000 photos of vertebrate animals in 2015. Species detected at camera traps included American badgers, banner-tailed kangaroo rats (Dipodomys spectabilis), black-tailed jackrabbits (Lepus californicus), coyotes, desert cottontails (Sylvilagus audubonii), gray foxes (Urocyon cinereoargenteus), kit foxes (Vulpes macrotis), mule deer (Odocoileus hemionus), pronghorn (Antilocapra americana), spotted ground squirrels (Xerospermophilus spilosoma), Chihuanuan ravens (Corvus cryptoleucus), common ravens (Corvus corax), curve-billed thrashers (Toxostoma curvirostre), greater roadrunners (Geococcyx californianus), horned larks (Eremophila alpestris), northern mockingbirds (Mimus polyglottos), raptors, sparrows (e.g. lark sparrow [Chondestes grammacus], rufus-crowned sparrow [Aimophila ruficeps], Cassin's sparrow [Aimophila cassinii], and Vesper sparrow [Pooecetes gramineus]), turkey vultures (Cathartes aura), white-winged doves (Zenaida asiatica), and coachwhip snakes

Table 1. Prior probabilities and parameter estimates for a Bayesian model built to infer key demographic rates for a reintroduced population of prairie dogs at Sevilleta National Wildlife Refuge, New Mexico, U.S.A. on the basis of capture-mark-recapture data. *Annualized effect size, on logit scale.

Parameter	Prior	Posterior mean (95% credible interval)	
Φ (Base annual survival rate)	Uniform (0.01, 0.99)	0.52 (0.42 to 0.65)	
Release effect*	Uniform $(-28, 0.5)$	-21.37 (-24.89 to -17.95)	
Spring release effect*	Uniform $(-8, 8)$	3.23 (-0.49 to 6.85)	
Juvenile effect*	Uniform $(-2, 2)$	0.04 (-0.59 to 0.73)	
Male effect*	Uniform $(-2, 2)$	-0.94 (-1.33 to -0.55)	
Precipitation effect*	Uniform $(-2, 2)$	-0.25 (-0.68 to 0.10)	
Soil moisture effect*	Uniform $(-2, 2)$	-0.24 (-0.74 to 0.28)	
p (Capture probability per day)	Uniform (0.01, 0.75)	0.30 (0.27 to 0.34)	
Among-individual variability in capture probability (StDev)	Uniform (0.1, 1)	0.94 (0.81 to 0.99)	
r (annual tag retention rate)	Uniform (0.4, 0.99)	0.56 (0.48 to 0.63)	

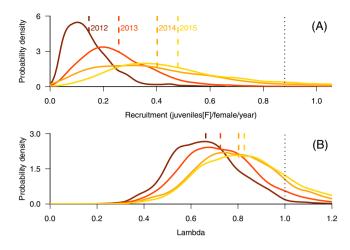


Figure 4. Offspring recruitment and population growth rates for reintroduced prairie dogs at the Sevilleta National Wildlife Refuge, Socorro County, New Mexico. Posterior distributions for (A) per capita recruitment rates and (B) lambda for each year, 2012–2015, derived from an ABC algorithm. Dashed vertical lines are means for each year, indicating an increase in both recruitment and lambda over time. Dotted vertical lines indicate (A) the recruitment rate required to achieve a self-sustaining population (lambda = 1) when survival is held at the estimated mean, and (B) population growth rate required for a self-sustaining population (lambda = 1).

(Masticophis flagellum). We documented positive associations between the presence of reintroduced prairie dogs and the presence of a wide diversity of vertebrate species (Figs. 5 and 6), including species known to associate with prairie dog colonies (Fig. 5). Burrowing owls, black-tailed jackrabbits, desert cottontails, and American badgers generally exhibited the strongest associations with prairie dog colonies (p < 0.05; Fig. 5). Mule deer (p < 0.05; Fig. 5) and white-winged doves (failed to converge, but all of the nearly 100 observations occurred off-colony; Fig. S4) were the only species that were not associated with reintroduced prairie dog colonies.

Discussion

After 7 years of intensive monitoring of reintroduced prairie dog colonies at the SNWR, the colonies are not yet self-sustaining. The colonies were characterized by unsustainably low recruitment rates and severe declines in the absence of augmenting the population through additional reintroductions. These results echo those by Davidson et al. (2014), Facka et al. (2010), and Hale et al. (2013), who found reestablishment of self-sustaining prairie dog colonies in the southern and drier portions of their range to be challenged by the variable and arid climate. However, in our study, the colonies showed slow, steady increases in population growth and recruitment rates with time, and attracted species known to strongly associate with prairie dog colonies. Our results indicate that if population trends continue, the colonies may become self-sustaining with more time and management investment. We also show that even under extreme drought and these modest recovery conditions, reestablishing

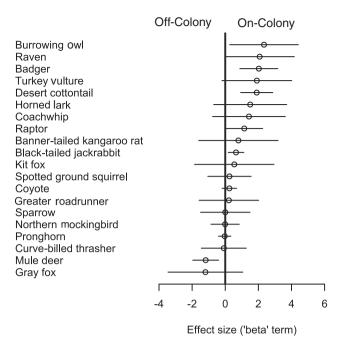


Figure 5. Influence of prairie dog presence/absence on the population densities of vertebrate species at a prairie dog reintroduction site on the Sevilleta National Wildlife Refuge, New Mexico, U.S.A., estimated on the basis of data from automated camera-traps. Using a Royle-Nichols occupancy model, we found the presence of prairie dogs and their colonies had a positive influence on the log-transformed densities of many other wildlife species. Point estimates for effect sizes are represented by open circles, and 95% confidence intervals are indicated by solid lines. Species positively associated with reintroduced prairie dog colonies included burrowing owls (Athene cunicularia), Chihuahuan ravens (Corvus cryptoleucus), American badgers (Taxidea taxus), black-tailed jackrabbits (Lepus californicus), and desert cottontails (Sylvilagus audubonii). Mule deer (Odocoileus hemionus) had lower estimated densities on reintroduced prairie dog colonies. Note that the statistical algorithm did not converge for several species due to insufficient data (not shown). Effect sizes represent the increase in log-transformed densities on a prairie dog colony versus off a prairie dog colony. See text for all species names in figure.

prairie dogs can have positive effects on grassland vertebrate communities.

Offspring recruitment, rather than adult survival, appears to be the primary factor limiting reintroduction success in this semi-arid system. After the initial 1-month acclimation period, average survival estimated over the course of this study (females 52% and males 31%) was higher than in our previous reintroduction of Gunnison's prairie dogs (17% across both females and males over an 8-year period, and 26% after the initial establishment period, Davidson et al. 2014). The average survival over the course of our current study matches approximately with rates for naturally occurring Gunnison's prairie dog colonies (ca. 30-50%; Hoogland 2001). This suggests that resident adult survival may have reached sustainable rates soon after the initiation of the reintroduction program at SNWR in 2012. In contrast, our estimates of offspring recruitment are substantially lower than similar estimates in the literature that report average recruitment rates of at least

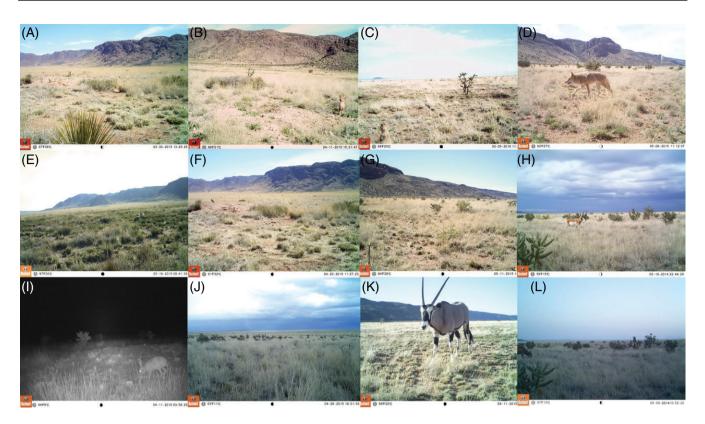


Figure 6. Photos from camera traps at our prairie dog reintroduction site on the Sevilleta National Wildlife Refuge, New Mexico, U.S.A., showing: (A), (B), and (C) Gunnison's prairie dogs (*Cynomys gunnisoni*), (D) a coyote (*Canis latrans*), (E) a burrowing owl (*Athene cunicularia*) pouncing on the back of a badger (*Taxidea taxus*) crossing a colony, (F) a burrowing owl on a mound and a spotted ground squirrel (*Xerospermophilus spilosoma*) standing up next to the mound, (G) a coachwhip snake (*Masticophis flagellum*) looking over grass, (H) a pronghorn, (I) a black-tailed jackrabbit (*Lepus californicus*), (J) pronghorn running (*Antilocapra americana*), (K) an oryx ([*Oryx gazelle*] introduced to New Mexico for hunting), and (L) mule deer (*Odocoileus hemionus*) running in the background, and a cow (*Bos taurus*) in the foreground (although cattle are not allowed on the SNWR).

1 juvenile/female/year (Hoogland 2001; Haynie et al. 2003). Coupled with our survival estimates, estimated recruitment rates at our study site were insufficient to produce positive rates of population growth (lambda ≥ 1). Therefore, efforts to improve reintroduction at this and other arid sites should focus on enhancing rates of offspring recruitment—such as by food and water supplementation and by decreasing predation of pups in their first year of life (Hoogland 2003; Hayes et al. 2016).

We observed no recruitment in the first 2 years of the study (2010 and 2011), and very low recruitment in 2012, corresponding to the years that the site experienced extreme drought (although these years had a very small population size, and it is possible that recruitment would have been low even without drought). Recruitment only began to increase once the drought receded in winter 2013-2014. Drought periods have been found to limit reproductive success in other prairie dog populations (Facka et al. 2010; Davidson et al. 2014; Hayes et al. 2016). Furthermore, several previous studies have suggested that prairie dog recruitment (and to a lesser extent, survival) is most limited by winter-spring precipitation, which triggers spring green-up of vegetation that provides critical forage resources during the energetically-demanding periods of mating, pregnancy, and lactation (Hoogland 2003; Hayes et al. 2016).

Despite our conjectures about the role of drought as a driver of low recruitment in our study, we did not detect a statistical correlation between the relatively constant and linear increase in recruitment and population growth rate and drought-related environmental factors like precipitation, soil moisture, or vegetation canopy cover. Perhaps this is due to our small sample size of annual recruitment estimates (n = 4), but it could also be because supplemental feeding was sustained during drought periods, potentially reducing our ability to detect drought effects on recruitment (and indicating the importance of this practice during droughts, see also Hale et al. 2013). The slow, steady increase in population growth that we observed could be related to the time it takes for prairie dogs to dig deep, complex burrow systems, which are important in protecting prairie dogs from predators and may take several years to construct (Long et al. 2006). Whatever the underlying drivers of the slow population increase in our study, assuming this rate of population growth continues, it may take more years of intensive management and further releases to achieve a self-sustaining population of prairie dogs at the SNWR, and by implication, other semi-arid grassland areas.

Although our relocation methods followed similar protocols as others (Long et al. 2006), prairie dogs in our study had a ca. 20% chance of surviving the first month following release

(averaged across all release events), which is lower than reported in other studies of translocated black-tailed prairie dogs during the first few months postrelease (40–80%; Bly-Honness et al. 2004; Dullum et al. 2005; Long et al. 2006). Low 1-month postrelease survival is likely due in part to movement off-site and predation (Long et al. 2006; Shier 2006; Davidson et al. 2014). For example, we radio-collared 11 reintroduced prairie dogs (unpublished data) and found that several individuals moved far from our release sites (with one confirmed mortality via predation), so some of the losses during the first month were due to animals leaving the relocation site, and probably resulted in mortality. First-month survival may be improved by encouraging site fidelity for longer periods with supplemental feed and deploying acclimation cages for longer periods of time, and would probably be higher if existing burrows were available.

Ear-tag retention probability in our study was remarkably low, averaging just 0.56 per year. Although tag loss is common in the literature (Rivalan et al. 2005; Oosthuizen et al. 2010), these rates are very low. Because being able to track individuals over time is critical for determining population vital rates, our findings on tag loss underscore the importance of proper training of ear tag attachment. We encourage the use of PIT tags for permanent marking whenever possible, which have a negligible loss rate when properly injected subcutaneously (Gibbons & Andrews 2004). At the very least, double-tagging is required to estimate and correct for tag loss rates, and should be instituted for any study system for which tag retention is uncertain; the present study would not have been possible (or would have yielded severely biased results) if most animals had not been tagged in both ears.

Results from our camera traps suggest that the reintroductions of prairie dogs at the SNWR have helped restore their functional role to the grassland ecosystem, even despite the minimal growth of the prairie dog population. Vertebrate species were more commonly found on the colonies overall, compared to the off-colony grasslands, and the colonies attracted species that are associated with prairie dog colonies. Burrowing owls, American badgers, and desert cottontails were among the species that responded most strongly to our reintroductions, and are species well-known to associate with prairie dog (Cynomys spp.) colonies (Clark et al. 1982; Goodrich & Buskirk 1998; Kotliar et al. 2006; Bayless & Beier 2011; Davidson et al. 2012; Eads et al. 2015, 2016; Grassel et al. 2015). Cottontails and burrowing owls use prairie dog burrows for homes, nesting, and shelter, and American badgers and raptors are important predators of prairie dogs (Goodrich & Buskirk 1998; Cully 2011; Grassel et al. 2015; Eads et al. 2016). Black-tailed jack rabbits may have been attracted to the more nutritious forage that is often found on prairie dog colonies compared to surrounding grasslands (Whicker & Detling 1998). Some of the species that were more common on the colonies (but most not significantly so), such as coyotes, kit foxes, and horned larks are also known to associate with prairie dog ecosystems and open grassland landscapes (Clark et al. 1982; Kotliar et al. 2006; Ceballos et al. 2010; Davidson et al. 2012; Eads et al. 2015). We suspect ravens were more common on colonies than off because they were attracted to supplemental feed provided during relocation efforts (which is unlikely to be the case for other colony-associated species because of their known associations with burrows and/or prey, for example). Overall, our camera-trapping efforts support the concept that restoration of keystone species (e.g. wolves, bison, beavers, sea otters, and prairie dogs) can play a fundamentally important role in ecosystem restoration (U.S. Fish and Wildlife Service 2013).

We used standard techniques to perform prairie dog translocations and to prepare reintroduction sites, but our efforts have been only partially successful to date, and the long-term success of this project remains uncertain. Our study illustrates the importance of monitoring postrelease population demographics and ecosystem responses to inform management decisions. By doing so, and testing quantifiable success criteria, we found that substantial and costly management efforts (e.g. supplemental feeding) in addition to continual population replenishment may be required for years following the initiation of reintroduction efforts. Previous efforts to establish prairie dog colonies on the SNWR had limited success despite multiple release efforts, and the chances for success in this system may become increasingly difficult with the climate becoming more arid and drought-prone (Davidson et al. 2014). While restoration goals may be more easily obtained in the mesic parts of their range, those populations are often exposed to more frequent sylvatic epizootics, and so require intensive management efforts via regular dusting with insecticide or distribution of the sylvatic plague vaccine to facilitate their persistence (Biggins et al. 2010; Rocke et al. 2017; Tripp et al. 2017). With such uncertainty regarding reintroduction success for prairie dogs, and for wildlife reintroductions in general, we underscore the importance of setting well-defined, quantifiable success criteria with associated time frames for assessment, prior to implementing a reintroduction project and embarking upon long-term monitoring (Seddon et al. 2007; IUCN/SSC 2013).

Unsurprisingly, restoring animal populations to complex and changing ecological systems is not trivial (Seddon et al. 2007, 2014). Because of the suite of challenges reintroductions can face, IUCN guidelines encourage implementing adaptive approaches to augment postrelease success and population persistence, aimed at mitigating mortality losses, limiting barriers to dispersal, and enhancing offspring production (IUCN/SSC 2013; Batson et al. 2015). Such adaptive approaches are needed to help bolster prairie dog populations reintroduced in the southern and more arid parts of their ranges. Given the challenges associated with reintroducing them into these more arid regions, we concur with Davidson et al. (2014) that the primary goal of managers should be to focus on maintaining and growing already-established prairie dog colonies. Reintroductions can be a valuable tool to restore their functional role in natural areas where they have been extirpated, but should only occur when sufficient resources are available to support and monitor these efforts. Translocations should not be used simply as a mitigation tool, because recreating prairie dog colonies is not a trivial endeavor and is not likely to result in equivalent ecological outcomes without significant resource commitment and proactive management. Whether management efforts are directed at maintaining, growing, or restoring populations, our

work provides novel insights into the fundamentally important role prairie dogs play in restoring grassland communities (U.S. Fish and Wildlife Service 2013).

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Supporting Information

The following information may be found in the online version of this article:

 $\label{eq:Appendix S1.} \textbf{Supplemental methods}.$

Table S1. The number of Gunnison's prairie dogs released to each plot in each year. **Figure S1.** Gunnison's prairie dog (*Cynomys gunnisoni*) reintroduction project experimental design.

Figure S2. Photo of (A) Gunnison's prairie dog (*Cynomys gunnisoni*) live-trapping effort and (B) camera trap installation.

Figure S3. Mean annual winter/spring and summer/fall precipitation from 2005 to 2016.

Appendix S2. Gunnison's prairie dog release protocol.

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Supplementary Materials for: Reintroducing a keystone burrowing rodent to restore an arid North American grassland: challenges and successes

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APPENDIX S1.

SUPPLEMENTAL METHODS

Artificial burrows:

Before releases in 2010, we installed 70 artificial burrows across each 9-ha colony plot, with 13 burrows clustered at the center and 57 in a circular arrangement around the central cluster. Artificial burrows consisted of 40 L nursery pots turned upside down, fitted with wire mesh floors, hay and two 1.5 m corrugated plastic tubing tunnels extending out of holes cut on opposite sides of each pot (see Davidson et al. 2014 for images). We placed this apparatus approximately 1 m deep in a 2-m long \times 0.5-m wide trench dug with a backhoe (Davidson et al. 2014). The plastic tunnels extended laterally in the trench and angled gently toward the surface to provide 2 entrances at each burrow (Davidson et al. 2014). After the first releases at each site, augured burrows were occasionally used when abandoned natural burrows were not available. Augured burrows were dug according to recommended protocols (Utah Prairie Dog Recovery Team 2006). These burrows were constructed with double entrance burrows. Paired augured holes were dug to approximately 1.5-2m deep/long and intersected at a 45-65 degree angle. At the intersection, the area was dug out by hand tools to create a larger space (Utah Prairie Dog Recovery Team 2006). We also note that the SNWR staff burned 1 plot and mowed another in 2014 to improve habitat for releases, but because these treatments were not replicated, we did not test the effects of those management treatments here.

Capture and transport:

We captured prairie dogs using water flushing and trapping methods, and followed similar protocols to those discussed in Long et al. (2006) and Davidson et al. (2014). We housed captured individuals in a humane setting certified by the Prairie Dog Coalition, a part of The Humane Society of the United States. We kept individuals in holding with their apparent family groups (hereafter, clans) and fed them daily for a period of at least 1 week to optimize health for release (Bly-Honness et al. 2004; Long et al. 2006; Shier 2006; Curtis & Conover 2012). We dusted all animals with flea powder (© DeltaDust, Bayer CropScience LP, Research Triangle Park, NC) to prevent transporting potentially plague-infected fleas to the release site (Long et al. 2006). We transported prairie dogs to the release site in pet carriers and an air-conditioned van, and followed release procedures similar to those described by Long et al. (2006) and in Davidson (2014). All releases began early in the morning to minimize heat stress while animals awaited release into artificial burrows. We transported pet carriers filled with hay to the burrows by hand.

Acclimation:

One burrow entrance was fitted with an aboveground acclimation cage consisting of 1-cm wire mesh $(0.5 \times 0.5 \times 0.25 \text{ m})$. Each acclimation cage had a hole in the floor center to fit over the artificial burrow entrance. We supplied each cage with hay, black-oil sunflower seeds, and

carrot slices before fitting it over each burrow entrance with rebar stakes and wire to keep the cage door propped open (see Davidson et al. 2014 for images). Depending on size and sex of the prairie dogs, we released up to 8 individuals from the same clan into the other burrow entrance before we covered it with the same cage configuration. If a clan was larger than 8 individuals, we placed one half of the group in one artificial burrow and the other in an adjacent artificial burrow. We removed animals from the pet carrier by hand and carefully transferred them to the burrow entrances. The ratios of age classes varied depending on the season we captured them (i.e., spring vs. summer). Often we placed adult males in separate artificial burrows, adjacent to clan-member females and pups to avoid potential aggressive male behavior towards pups.

Trapping:

We used 100 welded wire mesh treadle-type live traps $(19 \times 19 \times 48 \text{ cm})$ with double doors across each 9-ha plot, with 2 traps placed at each numbered, flagged, and mapped trapping station near active burrows. We wired traps open and pre-baited them for 2 days prior to trapping events, set and baited them at dawn each day of a trapping event, and left them open during the morning until ambient temperature reached 25 C. We labeled captured individuals with the trap station number and carried them to a shaded processing location off-plot. We transferred individuals from the trap to a canvas cone where they could be processed (Hoogland 1995), and recorded their trap station and ear-tag numbers.

Tag loss:

To be available for observation at time t, an individual must survive from time t-l to time t, and must remain tagged in at least one ear (or PIT-tagged; see below). Individuals alive and tagged at time t remained tagged (available for capture) according to the following process:

$$\begin{aligned} n. \, tag \, s_{i,t} &= Bernoulli \big(tag L_{i,t-1} * r^{per} + retagged L_{i,t} \big) \\ &\quad + Bernoulli \big(tag \, R_{i,t-1} * r^{per} + retagged R_{i,t} \big) + PIT. \, tag_i \\ Tagged_{i,t} &= \begin{cases} 1, & n. \, tags \geq 0 \\ 0, & n. \, tags = 0 \end{cases} \end{aligned} \tag{Eq. 3}$$

Where $n.tags_{i,t}$ represents the (latent or observed) number of unique tags on each individual (maximum of three; left ear, right ear, and PIT tag), $tagL_{i,t-1}$ and $tagR_{i,t-1}$ are (latent or observed) binary indicators of whether an individual had ear tags of their left and right ears (respectively) at time t-1, r represents the tag retention rate (free parameter, estimated on the basis of data - e.g., known tag loss instances whereby double-tagged individuals transitioned to single-tagged individuals), $retaggedL_{i,t}$ and $retaggedR_{i,t}$ are binary indicators representing whether or not an individual was re-tagged on the left or right ear, respectively, and $PIT.tag_i$ is a binary indicator of whether an individual i was marked with a PIT (passive integrated transponder) tag. PIT tags were assumed to be permanent (no tag loss rate). An individual i was available for capture at time t ($Tagged_{i,t}$) if it retained one or more tags.

Finally, we used the estimated tag retention rate to compute the probability of a double-tagged individual losing both tags (and therefore becoming lost from the marked population) after *t* years using the following equation:

$$Prob.lost = (1 - p^t)^2$$
 (Eq. 4)

Bayesian implementation of survival model in WinBUGS

We estimated survival, capture, and tag loss parameters with Markov-Chain Monte Carlo (MCMC) using WinBUGS 1.4 (Lunn et al. 2000), which was called from the R environment via the R2WinBUGS package (Sturtz & Gelman 2005; R Development Core Team 2016). We assigned uninformative uniform prior probability distributions to all free parameters (Table S1 in Supp. Mat.). We ran three independent Markov chains, discarding the first 25,000 MCMC samples as a burn-in and storing every fifth sample of the remaining 25,000 MCMC iterations for further analysis. We tested for convergence of the Markov chains to the stationary posterior distribution with the Gelman-Rubin diagnostic (Bolker 2008). We summarized posterior distributions for all parameters with the mean of all MCMC samples as a point estimate and the 2.5 and 97.5 percentiles of the MCMC samples as a 95% credible interval (Bolker 2008). Scripts for running this analysis (R and WinBUGS) are posted on GitHub (https://github.com/kevintshoemaker/Sevilleta_pdogs).

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SUPPLEMENTAL TABLES

Table S1. The number of Gunnison's prairie dogs released to each plot in each year. Plot G was not included in analyses.

	Year						
Plot	2010	2011	2012	2013	2014	2015	
В	272	368	169			35	
D	387	377	143			33	
F			341	155		35	
G						95	

SUPPLEMENTAL FIGURES

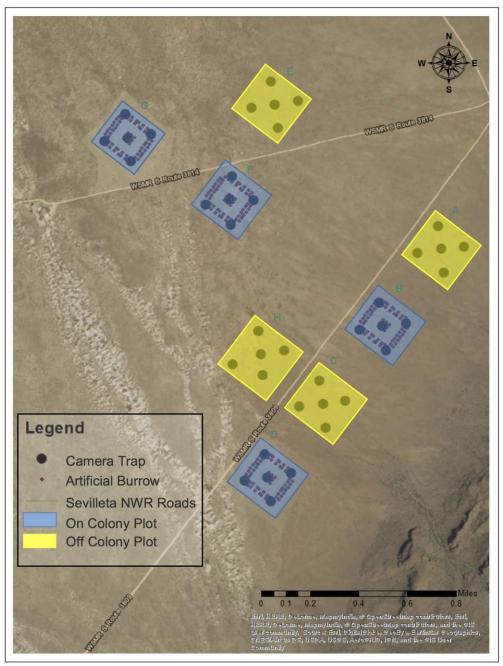


Figure S1. Gunnison's prairie dog (*Cynomys gunnisoni*) reintroduction project experimental design at the Sevilleta National Wildlife Refuge (NWR), Socorro County, New Mexico, USA. Blue squares represent 9 ha on-colony plots where prairie dogs were released into artificial burrows (70 artificial burrows on each plot, with 13 burrows clustered at the center and 57 in a circular arrangement around the central cluster); yellow squares represent 9 ha off-colony control plots where no prairie dogs were released. Prairie dog population monitoring via mark-recapture live trapping occurred on plots B, D, and F. Camera traps were established at the four corners and center of each on-colony and off-colony plot (A, B, C, D, E, F, G, H).

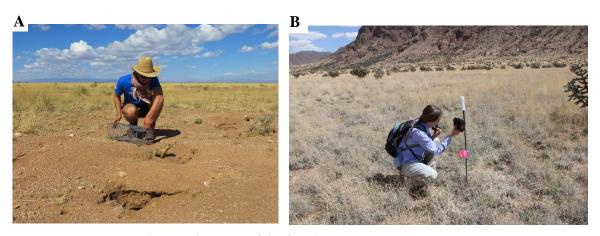


Figure S2. Photo of (A) Gunnison's prairie dog (*Cynomys gunnisoni*) live-trapping effort and (B) camera trap installation at the prairie dog reintroduction study site, Sevilleta NWR, Socorro County, NM, USA.

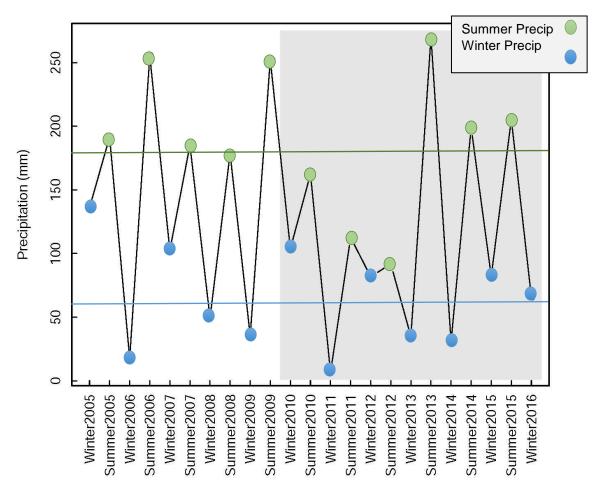


Figure S3. Mean annual winter/spring (November – April) and summer/fall (May – October) precipitation from 2005 to 2016 at the Gunnison's prairie dog reintroduction study site, Sevilleta NWR, Socorro County, NM, USA. Blue and green lines represent the mean precipitation from 2005 through 2016 during the winter/spring and summer/fall periods, respectively.

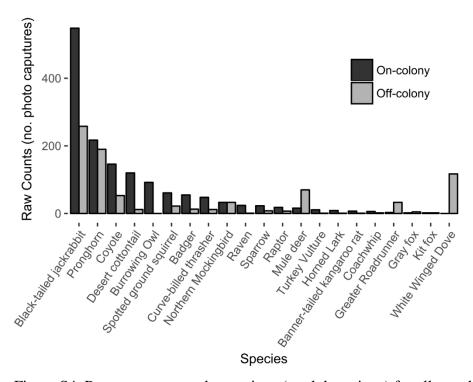
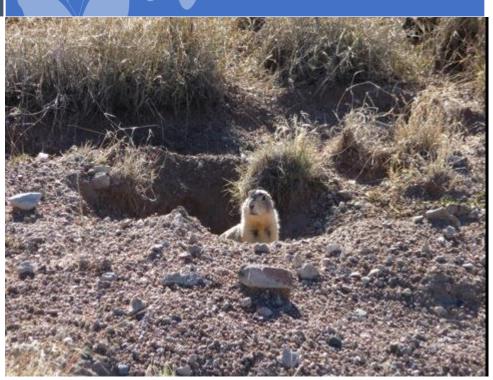


Figure S4. Raw camera trap observations (total detections) for all vertebrate species observed (excluding prairie dogs) on the on-colony and off-colony plots at the prairie dog reintroduction study site, Sevilleta NWR, Socorro County, NM, USA. Raw counts are ranked in order of on-colony detections (most to least commonly detected species). Note that the occupancy analysis algorithm we used did not converge for some species, so more species are listed in this figure than are listed in Fig. 5.

APPENDIX S2.

Gunnison's Prairie Dog Release Protocol (following pages):



June 2012



Contents

- Background
- Site Preparation
- Processing Gunnison's Prairie Dog
- Gunnison's Prairie Dog Release: Burrow Release
- Daily Feeding
- Gunnison's Prairie Dog Release
- Plot Monitoring

Gunnison's Prairie Dog Release Protocol

Background

Prairie Dogs are considered ecosystem engineers as well as a keystone grassland species. Prior to becoming a National Wildlife Refuge in 1973 the Sevilleta was heavily ranched. Ranchers eradicated the prairie dogs from land where they were historically abundant. Gunnison's Prairie Dogs are now being released on the Sevilleta National Wildlife Refuge, which they historically inhabited in order to promote biodiversity as well as grassland ecosystem health. The following is a list of materials and procedures for the release of Gunnison's Prairie Dogs (*Cynomys gunnisoni*) on the Sevilleta National Wildlife Refuge. The methodology has been developed over several releases and the following has been shown to be the most effective.





Site Preparation

McKensie Flats on the East side of the Sevilleta National Wildlife Refuge is excellent Prairie Dog habitat and its location within refuge boundaries will allow for the expansion of colonies upon establishment.

The reintroduction is done at prepared sites using a combination of manmade burrows and natural burrows. The site must be scouted to ensure suitability for the release. Artificial and natural burrows are checked to see if they are occupied or blocked by using a burrow cam, stick or tubing.

Scouting The Site

- Remove damaged pots or tubing
- Check all artificial burrows to ensure they are unoccupied
- If more burrows are needed check natural burrows for availability
- Flag and number burrows for next release



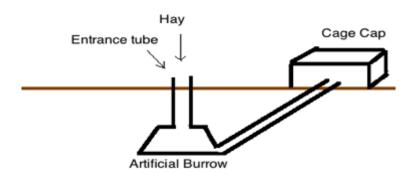
Supply List

- Cage Cap
- Bailing Wire
- Rebar or stakes (4 per cage cap)
- Wire snips
- 2 lbs. Sledge Hammer
- Hay
- Pole/ Broom Handle
- Canvas Bag (for carrying tools and hay)
- Hand Cart (for hauling supplies/ flags)

Final Preparations

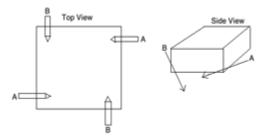
- Cage cap:
 - Close top hatch with bailing wire (at least 6" each) on corners of top lid.
- Entrance tube:
 - Put half flake of hay down tube with pole/broom handle
 - Be sure hay is loose and separated and is not stuck in tube
 - Place vented plastic cap on entrance tube
- Label burrows with flagging

Burrow Design and Preparation



Each burrow has two entrance tubes. Locate the tube with the shallower angle using a pole or broom handle. This is where the cage cap should be placed to allow prairie dogs to move between the artificial burrow and the cage cap during the transition period.

- Be sure the tube fits snugly into the hole at the bottom of the cage cap. The cage cap should sit flush with the ground, if needed use a piece of rebar to push through the top of the cage and push down the bottom
 - Tube should not stick out about 6 inches above the opening, use knife to trim if needed



Staking the Cage

- Use 4 pieces of rebar or landscape stakes
- Follow Above Diagram
- Insert "A" stakes one column from the edge at 45° angle
- Insert "B" stakes at 80° angle

Prairie Dog Processing

The processing of Gunnison's Prairie Dogs takes place at the Prairie Dog Pals headquarters in Albuquerque, New Mexico. The animals are captured mostly from the Albuquerque metropolis area in locations where they have been labeled a nuisance, hazard, or are in danger from human activities. WARNING! These are wild animals that are capable of biting. Use caution when handling!

Procedure

- Approach Prairie Dog from behind. Remove prairie dog from the bin quickly and carefully. Use one hand to grasp behind head and other to hold around rump.
- Always use a firm but not crushing grip to prevent the animal from reaching around to bite.



- Be sure the canvas cone is zipped and closed off
- Place animal in cone head first
- Use scale to obtain mass in grams (Record on data sheet)
- Unzip small end of cone just enough to allow animal's head out
- Using one hand close the rear end of the cone to encourage prairie dog to tunnel into the small opening



Supplies

- Canvas cones with zipper (pillow case may substitute)
- Scale zeroed for mass of canvas cone
- Ear tags & tagging pliers
- Forceps
- Clip board & data sheet
- Gloves
- Pen



Work in Teams of Two!

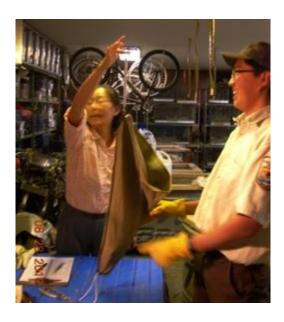
• Handler/Holder

- o Catch animal
- Hold for tagging
- Release into processed bin

• Recorder/Tagger

- Record weight, tag number, gender & source location
- o Tag Ear

Processing Procedure (Continued)



- The head should emerge from the narrow end
- Use zipper to carefully unzip and expose as much of the ear as necessary
- Use one hand to grasp Prairie dog around the shoulders and pin it to the table (be firm but not excessive)
- Support Prairie dog's rear with other hand

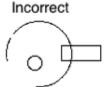
Alternate Method

If canvas cone fails or pillow case is used the holder can hold the animals with hands as instructed above and continue holding the animal gently against the table



Ear Tagging

- Tagger should first record tag number
- Then tag the ear
 - o Place tag in tool
 - Use forceps to grasp the left ear of the animal
 - Place tag as deep as possible, so the inside of the tag is against the edge of the ear (otherwise the tag could possibly be ripped off)
 - o Repeat for the right ear
- Place animal in already processed bin



To much room between tag and ear



RELOCATION TO REFUGE

After processing, the prairie dogs are divided up by source location and their placement on the plot is determined on "relocation map"

ON-SITE:

- Take kennels to burrows as directed by the "relocation map"
- Remove vented cap from tube
- Capture animal from kennel by hand
- Place prairie dog in tube head first and gently push rear to encourage it into the hole
- Place all designated animals in the burrow (rarely exceeding 5-6)
- Replace vented cap securely
- Place hay and food in cage and secure the hatch

Daily Feeding

Gunnison's Prairie Dogs released need to be fed after being placed in burrows. This is an acclimation period to allow the animals to become accustomed to the climate and atmosphere of their new location. The feedings are intended to ensure a successful release and to ease the animal's transition back to the wild.

During Post Release feedings food can be placed around the burrow holes on the ground. If you drop a piece of food down a hole and no noise is detected take note and inform supervisor.





Supplies Needed

- Ziploc bag
- Food (Gallon size Ziploc of each)
 - o Sunflower seeds
 - Chopped carrots or baby carrots
 - o Chopped apples into eighths
- Canvas shoulder bags
- Wire tools for repairs as needed
 - o Snips
 - o Pliers
 - o Bailing wire
- A set of food (one bag of sweet feed and carrots each) should feed five burrows
 - "Handful" of carrots in tray or 7-8 baby carrots
 - o "Handful" of sweet feed in tray

Gunnison's Prairie Dog Release

The release takes place in two parts. After 2-3 days in the cage caps, a "soft release" takes place. After another 1-2 days, the full release is completed.

Soft Release

- Soft release can be done after feeding
- Remove vented caps
- Leave cage caps in place
 - This is done to allow the prairie dogs the opportunity to leave the artificial burrow, while still providing a food source and safe area
 - This allows the animals to slowly acclimate in hopes of increasing their chances of survival

Final Release

- Remove cage caps, trays and rebar
 - Continue feeding for up to four days
 - o Move cage caps to the next relocation burrows



Supplies

- Bailing wire and tools (extra for repairs if necessary)
- Hay
- Food Trays
- Canvas bags
- Gloves







