Endangered Species Research: Texas Kangaroo Rat
Dipodomys elator

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Executive Summary

The Texas kangaroo rat is a rare and potentially threatened species that today only occurs in the state of Texas. Because of its small endemic geographic distribution this species is currently being considered for listing under the Endangered Species Act (ESA). Before an informed decision of whether to list *D. elator* under the ESA can be made, comprehensive assessment of the ecology, distribution, abundance, and genetic diversity of this species should be conducted. Although qualitative anecdotal accounts of habitat affinities are available in the literature, little is known about actual habitat characteristics that are important to this species. Moreover, important quantitative habitat characteristics can be determined and used to predict presence/absence or abundance of Texas kangaroo rats as well as to inform conservation and restoration efforts. Complicating this, however, are numerous observations of Texas kangaroo rats no longer occurring at sites where they were previously observed as well as novel records of occurrence. This research enhances our understanding of the biology of *D. elator* in the state of Texas and helps to inform the U.S. Fish and Wildlife Service regarding the decision to list this species under the ESA. Here, we report results of research to better understand five facets of the biology of the Texas kangaroo rats: 1) distribution in Texas and patterns of co-occurrence with other rodent species, 2) quantitative habitat characteristics across its geographic range, 3) potential distribution in the state of Texas based on Ecological Niche Models, 4) patterns of population genetics and an estimate of number of population in the state 5) predation pressure by coyotes (*Canis latrans*) and 6) efficacy of mapping burrows to estimate density of Texas kangaroo rats across their geographic range.

Distribution in Texas and patterns of co-occurrence with other rodent species.-- Previous research on the distribution on *D. elator* was carried out via road surveys, with the last published survey taking place over 15 years ago. To improve our understanding of the present-day distribution of *D. elator*, we surveyed over 800 locations along unpaved county roads across the historical range of this species. In addition to identifying sites of presence, we examined patterns of species co-occurrence within the rodent communities of these sites. We determined that *D. elator* was sufficiently abundant to be detected in five counties in Texas within its historical range (i.e., Childress, Cottle, Hardeman, Wichita, and Wilbarger) and was the eighth most abundant species of the 14 that we encountered. Moreover, we found that the majority of pairwise species associations were random (i.e., there was not strong evidence that pairs of species within rodent communities were aggregating or segregating from each other). For *D. elator*, specifically, we found that most associations with other species were random (i.e., there was no clear pattern of co-occurence). There was, however, a significant positive association with *Peromyscus leucopus*, meaning that the two species occurred together more frequently than expected and two negative associations with Ord's kangaroo rat (*Dipodomys ordii*) and cotton rats (*Sigmodon hispidus*). These findings suggest that interspecific interactions do not play a strong role in limiting the distribution of *D. elator*, although future work should examine co-occurrence patterns within the context of the surrounding habitat and across meaningful environmental gradients (e.g., precipitation or soils).

Quantitative habitat characteristics across the geographic range of *Dipodomys elator*.--Characterizing quantitative habitat characteristics of organisms is useful for improved understanding of distribution and abundance of understudied species such as *D. elator*. Little is known about actual habitat characteristics that are important to this species. Identifying important quantitative habitat characteristics can be used to better understand factors influencing distribution and abundance of rare species like *D. elator* and to inform conservation and restoration efforts. Here, we intensively characterized habitat features and distribution and abundance of *D. elator* at 35 sites across the geographic range of this species. We found that *D. elator* is associated with loam soils across its distribution, which supports similar findings provided by studies that were more local in nature. Additionally, variation partitioning analyses indicated that soil and abundances of other rodent species accounted for the most variation in *D. elator* presence and abundance across its distribution. Based on our results, we suggest that more sampling is needed to better understand habitat associations of *D. elator*. Preliminary results indicate that *D. elator* is not colonizing all available habitat given the overlap in habitat characteristics in sites where the species is present versus sites where the species was not encountered. As such, in addition to continued habitat sampling, future work should focus on the degree to which *D. elator* can successfully disperse into and colonize available habitat to determine whether the rarity of this species is attributable more to being distribution-limited or abundance-limited.

Potential distribution of *Dipodomys el*ator in Texas based on Ecological Niche Models.-- Species distribution modeling is used to predict probability of occurrence at unsampled sites based on information about locations of documented presence. Environmental variables such as climate measures, vegetation types and soils at those localities of known presence are used to construct the models. Areas with a high probability of occurrence are typically viewed as areas highly suitable for the organism in question. We used MAXENT (a program which uses geographic coordinates and associated environmental data to predict probability of occurrence in a selected area) to build historical and present-day species distribution models for *D. elator*. We gathered geographic data from museums for our historical models. Georeferenced individuals from our contemporary surveys were used to build present-day models of habitat suitability for

D. elator. Our present-day models suggest that Wichita and Wilbarger counties contain areas of high probability of occurrence for *D. elator*, while regions in Foard and Hardeman counties indicate areas of intermediate occurrence probability.

Patterns of population genetics and an estimate of number of population in Texas.--With underpinnings in evolutionary theory, conservation genetics emphasizes investigation of the ramifications of small population sizes, inbreeding and other factors that characterize threatened, rare or endangered species. Studies in conservation genetics can also help resolve unclear taxonomy, be used to understand gene flow and its barriers, investigate reproductive fitness, predict reintroduction successes, or define management units. Current population genetic information is lacking for *Dipodomys elator*. Between 2015-2017, we collected DNA samples from Texas kangaroo rats in Childress, Cottle, Hardeman, Wichita and Wilbarger counties. DNA was isolated from different tissues such as liver, salvaged tail clips, and whiskers. To detect fine-scale population genetic structure that may not be detected using coarser molecular markers such as microsatellites or mitochondrial sequences, we employed a variation of a next-generation sequencing technique known as restriction-site associated DNA sequencing (RAD-Seq). The RAD-Seq protocol utilized restriction enzymes that cleaved the genome at random locations. These loci contained single nucleotide polymorphisms (SNPs), the most abundant type of polymorphism in a genome. Analysis of these SNPs, metrics such as observed heterozygosity, *F*-statistics, principle components analysis and Mantel tests showed that current populations exhibited no loss in heterozygosity from historical estimates (i.e., genetic diversity did not decrease) over 30 years. Furthermore, distance-based measures suggest that current populations are structured. However, conservative *F*-statistics did not corroborate this result. Nonetheless, population structure, though slight, in *D. elator* may correspond to an environmental gradient, geographic feature or anthropogenic barrier, which demonstrates the need for spatially explicit genetic analysis.

<u>Predation pressure by coyotes (*Canis latrans*) on *Dipodomys elator*.— Based on 590 coyote scats collected over a 15 year period, w</u>e found no evidence that coyotes represent an important predator on Texas kangaroo rats at the Holcomb Ranch in Hardeman County Texas

<u>Efficacy of mapping burrows to estimate density of Texas kangaroo rats across their geographic range. —W</u>e determined the potential for using high-resolution imagery to count *D. elator* burrows across its entire range and discuss the implications for landscape level detection and mapping. Specifically, we surveyed a private property located in Wichita County, TX for *D. elator* burrows and used an Unmanned Aerial System (UAS) to collect very high-resolution RGB imagery and digital elevation models (2.5 mm pixel size) over active and inactive burrows located in mesquite mounds and anthropogenic features (roadsides, fences, etc.). We used 26 identified burrows locations to characterize these based on topography and vegetation density. Circular and linear mounds used by *D. elator* for construction of burrows were characterized by prominent slope and aspect ranges in the digital surface models. Burrow entrances and disturbed soils from trails outside burrow entrances were observed using the RGB imagery. We found that *D. elator* burrows can only be identified with <5 cm pixel resolution data which rules out the possibility of using high-resolution imagery data currently available at the state level. Alternatively, we propose that use of NAIP imagery at 0.5 and 0.6 m pixel resolution in combination with resampled Digital Elevation data can provide effective means for identifying potential TKR burrow locations at the county level. We present three different spatial models at the county, landscape and site scale that combine topographic and vegetation fractional cover information using a weighted overlay approach. These modeling approaches have strong

predictive capabilities and can be integrated with UAS data for visual confirmation of active or inactive burrows. The study concludes that very high resolution imagery and topographic information at pixel resolutions <5 cm collected by airborne systems can effectively help locating active *D. elator* burrows. However, to remain cost effective, upscaling to county level will require reducing the sampling area to the most suitable habitat areas. Modeling approaches, such as the ones proposed in this study, can help locating these sampling areas effectively.

Taken together, our key findings are: 1) the relationship between *D. elator* and quantitative habitat characteristics is weak, perhaps because it may not be colonizing all available habitat and may therefore be abundance limited; 2) *D. elator* was detected in five counties in Texas (Childress, Cottle, Hardeman, Wichita, and Wilbarger) and interspecific interactions do not appear to play a substantive role in their distribution; 3) habitats that are relatively highly suitable for *D. elator* currently occur in six counties in the state and the distribution of this species is expected to shift slightly towards the west in the future, while encountering a decrease in suitable habitat over its distribution and 4) *Dipodomys elator* appears to have two different genetic populations in the state despite the fact that no marked loss of heterozygosity in 30 years, and present-day genetic diversity estimates do not indicate high levels of inbreeding or population subdivision; 5) it will likely be possible to obtain a statewide estimate of abundance based on burrow counts generated from remote sensing technologies once resolution reaches a higher level of precision of 5cm resolution; 6) predation pressure from coyotes appears to be weak, and likely not determining the distribution and abundance of Texas kangaroo rats. Indeed, more work needs to be done to make an informed decision to list *Dipodomys elator* under the ESA.

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CHAPTER I: PRE-EXISTING INFORMATION ON TEXAS KANGAROO RATS

Introduction

The Texas kangaroo rat, *Dipodomys elator* (Heteromyidae), is a large kangaroo rat that was first described as a species by Merriam (1984) over 100 years ago. Historically, *D. elator* has occurred within two counties in Oklahoma and eleven counties in north-central Texas (Carter et al. 1985, Baumgardner 1987, Martin and Matocha 1991, Martin 2002, Schmidly and Bradley 2016). However, surveys performed across the historical distribution of the species within the last few decades suggest that the population is declining (Moss and Mehlhop-Cifelli 1990, Martin 2002), likely due to habitat conversion and the encroachment of grasses and forbs (Diamond and Shaw 1990).

The status of *D. elator* has not been comprehensively updated in almost a quarter of a century (Jones et al. 1988), and little is known regarding population genetics or habitat characteristics that are important to distribution and abundance of the Texas kangaroo rat across its entire distribution. Despite the paucity of information about this species, *D. elator* is currently under review for a twelve-month finding, an initial step for listing under the Endangered Species Act (ESA). Decisions on whether to list a species under the ESA should be made using only the best and most up-to-date scientific information. Nonetheless, present or future threats to the range of *D. elator* and other natural or manmade factors affecting its survival are poorly known. Because our understanding of ecology, distribution, abundance and genetic health of *D. elator* is dated, an update on such information is urgently needed to better inform decisions involving listing this species under the ESA.

Classification and Nomenclature

Scientific Name: *Dipodomys elator* (Merriam 1894)

Original Publication: Merriam, CH. 1894. Preliminary descriptions of eleven new kangaroo rats of the genera *Dipodomys* and *Perodipus*. Proceedings Biological Society of Washington 9: 109-116.

Type Specimen: US National Museum No. 64,802, adult male from Henrietta, Clay County, TX.

Common Names: Texas kangaroo rat; Loring's kangaroo rat.

Family: *Heteromyidae*

Present Legal Status

International: Classified as Vulnerable in the IUCN Red List (Wahle et al. 2018).

Federal: Previously listed as a Category 2 species (Jones et al. 1988, Martin 2002), although insufficient data existed to justify an elevated listing (USFWS 1996). More recently, a 90-day finding determined that a 2010 petition (WildEarth Guardians 2010) presented substantial scientific information indicating that listing the species as threatened or endangered may be warranted (USFWS 2011), although it is not presently protected under federal law.

State: Listed as Threatened within the state of Texas (Martin 2002, Schmidly and Bradley 2016), due to its apparent scarcity and small geographic range (Stangl and Schafer 1992).

Genetics and Systematics

All members of the genus *Dipodomys* have chromosomal diploid numbers between 52 and 74; *Dipodomys elator* has a diploid number of 72 chromosomes with a fundamental number of 82. There are 29 uniautosomal pairs in *D. elator*, and six pairs are biautosomal. The X chromosome is submetacentric, similar to other members of the genus. However, the Y chromosome within the genus varies; in *D. elator* it is acrocentric (Stock 1974).

Johnson and Selander (1971) investigated the protein variation of *Dipodomys*, using 11 enzymes and 6 non-enzymatic proteins. Twenty-three examined specimens from Wichita Falls, Texas had a mean allele frequency of 1.06. Of the mean proportion of loci, 0.00 were polymorphic within the population, and individual heterozygosity was reported at 0.002, the lowest (along with *D. panamintinus*) reported within *Dipodomys*. However, the authors made no significant conclusions regarding these data.

Mazrimas and Hatch (1972) reported principle, intermediate, and satellite DNA components using neutral cesium chloride (CsCl) gradients of 12 species of the genus including *D. elator*. Proportions of medium satellite (MS; 1.707 g ml^{-1}) and heavy satellite (HS; 1.713 g ml^{-1}) HS were reported as 15 and 10, respectively, for a specimen trapped in Wilbarger County, Texas. They conclude that the amount of satellite DNA within the genus provides a unique opportunity for evolutionary study among rodents. Hamilton *et al*. (1987) detected six polymorphic loci [creatine kinase-4 (Ck-4), glutamate oxaloacetate transaminase-1 and -2 (Got-1 and Got-2), peptidase-D (Pep-D), phosphoglucomutase-3 (Pgm-3), and 6-phosphogluconate dehydrogenase (6-Pgd)] in *D. elator* (n=21) by using standard starch-gel electrophoresis. Additionally, 12 other loci exhibited a relative degree of interspecific variation along with 4 other members of the genus (Hamilton *et al*. 1987).

Although there is consensus that *D. elator* is a distinct species, there is systematic uncertainty concerning which species group *D. elator* should be placed in. For example, different studies have placed this species in either the *elator* (Davis 1942), *heermanni* (e.g., Best 1993), *merriami* (e.g., Dalquest and Collier 1964), *phillipsi* (e.g., Jannett 1976), or *spectabilis* (Merriam

1894) species groups, or even a separate lineage deserving recognition at the genus level (Dalquest et al. 1992), based on morphological, allozyme, or chromosomal data. More recently, Mantooth (2000) utilized the 1143 base pair (bp*)* cytochrome-*b* gene to further clarify the systematic placement of *D. elator*. Cytochrome-*b* has been found to be phylogenetically informative when considering sister species. The resulting sequence data placed *D. elator* within the *D. phillipsii* group, echoing a similar phylogenetic placement by alloenzyme data already noted above (Hamilton *et al.* 1987). *Dipodomys phillipsii* is an inhabitant of the Chihuahan Desert in north-central Mexico (Knox Jones, Jr. and Genoways 1975). If *D. elator* shares a common ancestor with *D. phillipsii*, then the current distribution of *D. elator* may represent a relictual population along the Red River Valley.

Physical Description

Morphology

Dipodomys elator is a relatively large kangaroo rat (65 to more than 100g; Carter et al. 1985). Similar to other kangaroo rats, the tail of *D. elator* is longer than its body (i.e., approximately 160%; adult tail length: 161-205mm; total length: 260-345mm; Davis 1942, Carter et al. 1985). Mean cranial measurements of 15 adult specimens in Carter et al. (1985) were: greatest length of skull, 37.2 mm; maxillary breadth, 6.7 mm; mastoid breadth, 23.7 mm; interorbital constriction, 13.5 mm; length of maxillary toothrow, 5.3 mm. Males are significantly larger than females for a number of cranial characteristics as well as body weight (Webster and Jones 1985, Best 1987). The degree of sexual dimorphism documented by Best (1987) varied among populations with greater differences between sexes on the margins of the geographical range of this species than in the middle of its geographical range.

The dental formula of *D. elator* is the same as all heteromyids: I 1/1, C 0/0, P 1/1, M 3/3 for a total of 20 teeth (Carter et al. 1985). However, the cusp pattern of P4 is unique in this species and highly differentiated from other teeth described within *Dipodomys* (Dalquest et al. 1992, Carrasco 2000). The baculum of *D. elator* most closely resembles that of *D. phillipsi* (Best and Schnell 1974, Jannett 1976). Moreover, the dorsal skin gland in *D. elator* is larger and more variable in size in males compared to females, while similar to that of five other species (*D. agilis*, *D. deserti*, *D. merriami*, *D. ordii*, *D. panamintinus*; Quay 1954) and markedly different than that of *D. spectabilis* (Stangl et al. 2006).

Appearance

The Texas kangaroo rat is a four-toed species with a conspicuous white tuft on the tip of its tail (Carter et al. 1985, Schmidly and Bradley 2016). The tail also has a dorsal and ventral stripe, both more pale in color than the buff-colored tail (Carter et al. 1985). The upperparts of *D. elator* are buffy, the underparts are white, and a white stripe runs across the thighs. Molt occurs once a year, beginning on the nose and between the shoulder and proceeding in all directions (Webster and Jones 1985).

Dipodomys elator is one of two kangaroo rat species in Texas, along with *D. spectabilis*, with a white plume at the tip of its tail. However, the geographic ranges of the two species do not overlap. The only other kangaroo rat that occurs within the geographic range of *D. elator* is *D. ordii*, although the latter species lacks the distinctive white plume at the tip of its tail, is significantly smaller in body size, and has five toes on each hind foot as opposed to the four toes characteristic of *D. elator* (Lewis 1970, Schmidly and Bradley 2016).

Distribution

This species has been documented from 11 counties in north-central Texas (i.e., Archer, Baylor, Childress, Clay, Cottle, Foard, Hardeman, Motley, Montague, Wichita, and Wilbarger; Blair 1949, 1954; Dalquest 1968; Packard and Judd 1968; Baccus 1971; Martin and Matocha 1972; Cokendolpher et al. 1979; Dalquest and Horner 1984; Carter et al. 1985; Jones and Bogan 1986; Jones et al. 1987, 1988; Martin 2002; Schmidly and Bradley 2016) and Comanche and Cotton counties in southwestern Oklahoma (Bailey 1905, Baumgardner 1987). However, Schmidly (2002) suggested that most remaining populations may occur only in Hardeman, Wichita, and Wilbarger counties in Texas, whereas the most recent distribution-wide survey for the species did not find *D. elator* in Oklahoma and encountered the species in only five counties in Texas: Archer, Childress, Hardeman, Motley, and Wichita (Martin 2002). The apparent absence of this species from Oklahoma agrees with other researchers who have been unable to locate populations of *D. elator* (Jones et al. 1988, Moss and Mehlhop-Cifelli 1990). Moreover, a follow-up survey by Nelson et al. (2009) did not encounter *D. elator* in any of the sites of presence reported by Martin (2002), perhaps due to those sites no longer providing suitable habitat. This suggests that the population is declining, likely due to habitat conversion (i.e., rangeland to agricultural and urban areas), as well as the encroachment of grasses and forbs due to control of wildfires, mesquite (*Prosopis glandulosa*), and other disturbance-related shrubs (Diamond and Shaw 1990). Although *D. elator* is conspicuously absent from the fossil record (Dalquest and Schultz 1992), Dalquest and Horner (1984) speculated that the species may have evolved in mesquite grasslands of northern Texas and southern Oklahoma. It has been hypothesized that the short, sparse, grassland habitat used by *D. elator* was maintained by buffalo (*Bos bison*) and/or prairie dogs (*Cynomys ludovicianus*) as well as naturally-occurring wildfires (i.e., 5-6 year intervals; Stangl et al. 1992). Because neither fire nor these species have significant impacts in these areas presently, systems of moderate to intense grazing pressure may need to be implemented to mimic these disturbances (Stangl et al. 1992, Nelson et al. 2009).

Only a small amount of land (i.e., 12,386.11 ha; Goetze et al. 2015) within the historical geographic range is managed by the Texas Parks and Wildlife Department (i.e., Copper Breaks State Park, Lake Arrowhead State Park, Matador Wildlife Management Area). Of these, the Texas kangaroo rat has previously been reported from Copper Breaks State Park (Martin and Matocha 1998, Martin 2002). However, poor habitat in Copper Breaks State Park has been noted by a number of researchers (Martin 2002, Best and Wahl 1985, Nelson et al. 2011, Goetze et al. 2015) due to lack of grazing and fire as well as mesquite and juniper invasion. As such, the two most recent surveys for *D. elator* in Copper Breaks State Park did not encounter the species (Nelson et al. 2011, Goetze et al. 2015), although Goetze et al. (2015) did encounter a *D. elator* individual outside of the park along a nearby roadside.

Habitat Characteristics

There is general agreement that *D. elator* requires a sparse, short grassland habitat (Dalquest and Collier 1964, Roberts and Packard 1973, Carter et al. 1985, Stangl et al. 1992, Martin 2002, Goetze et al. 2007), but this type of habitat is becoming less common throughout the present range of the Texas kangaroo rat (Goetze et al. 2007). An association between honey mesquite (*Prosopis glandulosa*) and *D. elator* has been well documented (e.g., Dalquest and Collier 1964, Roberts and Packard 1973, Carter et al. 1985). Importantly, however, a number of more recent investigations have demonstrated that woody vegetation is not essential for *D. elator* burrows, and rather that burrow site selection by this species appears to be based primarily on a disturbance regime and presence of bare ground (Stangl et al. 1992, Martin 2002, Goetze et al.

2007, Stasey 2005, Stasey et al. 2010). It appears that *D. elator* prefers areas disturbed by grazing, fire, or drought, although it has been observed that *D. elator* will readily excavate burrows on elevated, open areas (e.g., fence rows, decaying brush piles, road berms; Goetze et al. 2007, Nelson et al. 2009, Stasey et al. 2010, Nelson et al. 2011a,b). As such, associated disturbances such as road construction and discarded equipment that accumulates soil, are thought to be beneficial for kangaroo rats (Roberts and Packard 1973, Stangl and Schafer 1990, Stangl et al. 1992, Martin 2002, Goetze et al. 2007, Stasey et al. 2010).

Sites where *D. elator* are present are also associated with firm clay-loam soils (Roberts 1969; Lewis 1970; Roberts and Packard 1973, Goetze et al. 2007), which may be due in part to the burrow characteristics of this species. In a study of *D. elator* burrows, burrows in more sandy soils were less complex with tunnels less closely interwoven. Moreover, temperatures in sandy burrows varied more than in those with greater clay structure, although it is important to note that at the time of this study burrow temperatures only varied 2.5°C when surface temperatures varied between 8-36°C. Burrow systems average 2.5 m in length, with tunnels 5 to 12.5 cm in diameter usually about 45 cm in depth below the surface (Roberts and Packard 1973). A single nest chamber is typically located near the bottom of the network of tunnels (Carter et al. 1985). The burrow entrances are often left unplugged (Dalquest and Collier 1964), although plugged burrow openings have been observed (Goetze et al. 2008). Moreover, *D. elator* may occupy more than one burrow per day (Goetze et al. 2008).

Texas kangaroo rats can be very active on unpaved county roads at night (Martin and Matocha 1972, Jones et al. 1988, Martin 2002), suggesting that this may be an important habitat feature for the species. Moreover, Stangl et al. (1992) offered that regular maintenance of dirt roads would help provide suitable burrow sites, as well as dispersal routes between populations or from established populations into suitable but uninhabited areas. However, in Wichita County, Texas, *D. elator* uses pastureland more frequently than adjacent roadsides, likely due to differences in vegetation between the two habitats (e.g., roadside vegetation was taller, included more introduced grass species, and contained less bare ground; Goetze et al. 2016).

Behavior

Much of the existing literature on *D. elator* suggests that this species is most active two to three hours after darkness (Packard and Roberts 1973, Carter et al. 1985), although Goetze et al. (2008) observed activity less than an hour after darkness until early morning hours, with no differences in activity levels or foraging behavior. Similarly, Texas kangaroo rats were seen foraging during new, crescent, half, and full moon phases, despite other studies suggesting that *D. elator* is not active during moonlit periods (Packard and Roberts 1973, Dalquest and Horner 1984, Jones et al. 1988). When foraging, *D. elator* appears to move slowly on all fours and utilize its forelimbs to place food in its cheek pouches, which are unloaded at burrow entrances where the seeds are pushed into the burrow with its forelimbs (Goetze et al. 2008). Texas kangaroo rats have been observed foraging up to 20 m from a burrow (Goetze et al. 2008), although Roberts and Packard (1973) found that some *D. elator* traveled more than 300 m along roads at night. Regardless of distance traveled, individuals appear to have good knowledge of the location of burrow entrances, usually returning to their own burrow system when released from live traps (Roberts and Packard 1973).

The Texas kangaroo rat likely has a number of predators (Dalquest and Horner 1984), although there are no published reports of predation on *D. elator*. However, Bailey (1905) described a specimen taken from the throat of a rattlesnake that had partially swallowed an individual captured in a snap trap. In a study of regurgitated barn owl (*Tyto alba*) pellets from Wichita County, Stangl et al. (2005) provided the first documentation of predation on *D. elator*, although determined that its representation as a prey species of the barn owl was lower than expected. The authors suggest that this may be due to well-developed predator avoidance mechanisms in *D. elator*.

Like other kangaroo rat species, Texas kangaroo rats maintain small, cleared patches of dust near burrow openings that are used for dust bathing (Dalquest and Collier 1964, Packard and Roberts 1973, Carter et al. 1985, Goetze et al. 2008). Dust bathing by this species likely helps remove ectoparasites, including *Geomylichus dipodomius*, *Echinonyssus incomptis*, *Androlaelaps fahrenholzi*, *Euschoengastia decipiens*, *Ixodes* sp., *Fahrenholzia pinnata, Meringis agilis*, *M. arachis*, and its own species of nematode, *Trichuris elatoris* (Hedeen 1953, Lewis 1970, Pfaffenberger and Best 1989, Thomas et al. 1990).

The Texas kangaroo rat is asocial (Dalquest and Collier 1964), with two or more adult *D. elator* never observed together in burrows (Packard and Roberts 1973). Thumping noises, similar to those described for other species of *Dipodomys*, are produced by *D. elator* (Packard and Roberts 1973). Agonistic behavior by *D. elator* has been documented in both confinement and in the field. In a laboratory setting, females were observed harassing males by nipping them on the bulky portion of their body (Packard and Roberts 1973). In the field, Goetze et al. (2008) noted both seed pilfering at a burrow entrance and an encounter between two individuals at a dust bathing site along a trail connecting their burrows, with both scenarios resulting in conflict between the individuals. These observations led Goetze et al. (2008) to suggest that Texas kangaroo rats may forage in distinct, defended territories along their trails, and that agonistic behavior may only occur within well-defined territories such as dust-bathing sites and trails associated with burrows.

Diet

Dipodomys elator feeds on seeds, stems, and leaves of grasses, forbs, and some perennials (Schmidly and Bradley 2016). Dalquest and Collier (1964) noted the presence of goathead (*Tribulus terrestris*), a widespread and non-native plant in the study area, in cheek pouches of most of the specimens they examined. Another study analyzing the contents of live *D. elator* cheek pouches found grass seeds (e.g., *Avena sativa* and *Sorghum halepense*) in about 70% of the pouches (Chapman 1972). The lack of mesquite (*P. glandulosa*; <2% occurrence) in *D. elator* cheek pouches suggests that the plant species serves primarily as a habitat feature for *D. elator* (e.g., burrow placement; see Habitat Characteristics). Similarly, seeds of other perennial shrubs that were common in the area (e.g., *Opuntia* spp.) were underrepresented in the cheek pouches of *D. elator*.

Ontogeny and Reproduction

Presently, little is known about mating or reproduction for this species. There is evidence that *D. elator* may breed year-round, as pregnant females have been collected in February, June, July, and September (Carter et al. 1985). As such, two peaks in reproductive activity may occur, in early spring and again in late summer, with mature females giving birth early in the year and their young becoming reproductively active later in the year (Webster and Jones 1985, Schmidly and Bradley 2016).

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CHAPTER II: HABITAT ASSOCIATIONS INFLUENCING PRESENCE AND ABUNDANCE OF THE TEXAS KANGAROO RAT (*DIPODOMYS ELATOR*) ACROSS ITS DISTRIBUTION

EXECUTIVE SUMMARY

Characterizing quantitative habitat characteristics of organisms is useful for improved understanding of distribution and abundance of understudied species such as *D. elator*. Although qualitative anecdotal accounts of habitat affinities are available in the literature, little is known about actual habitat characteristics that are important to this species. Identifying important quantitative habitat characteristics can be used to better understand factors influencing distribution and abundance of rare species like *D. elator* and to inform conservation and restoration efforts. Here, we characterized habitat features and distribution and abundance of *D. elator* at 35 sites across the geographic range of this species. We found that *D. elator* is associated with loam soils across its distribution, which supports similar findings provided by studies that were more local in nature. Additionally, variation partitioning indicated that soil and abundances of other rodent species accounted for the most variation in *D. elator* presence and abundance across its distribution. Based on our results, we suggest that more sampling is needed to better understand habitat associations of *D. elator*. However, preliminary results indicate that *D. elator* may not be colonizing all available habitat given the overlap in habitat characteristics at sites where the species is present versus sites where the species was not encountered. As such, in addition to continued habitat sampling, future work should focus on the degree to which *D. elator* can successfully disperse into and colonize available habitat to determine whether the rarity of this species is attributable more to being distribution-limited or abundance-limited.

Main takeaway points: 1) soil associations may not be as rigid as previously believed, although there is still question about ability of *D. elator* to persist in soils with higher sand content; 2) our analyses demonstrate that, of the explained variation in *D. elator* distribution and abundance across our survey sites, soil composition and shared variation between climate and vegetation were the most important characteristics 3) given the rarity of this species and the potential that it is distribution- or abundance-limited, additional access to private land and sampling across the entire distribution is important to more comprehensively understand habitat associations of this species.

INTRODUCTION

One of the fundamental goals of ecology is to understand patterns in the distribution and abundance of organisms. Rare taxa, which tend to have sparse or restricted spatial distributions (Kunin and Gaston 1997), are often habitat specialists with a limited number of sites of known occurrence. These species are therefore more likely to be governed by habitat availability than are generalist species that utilize a wider range of habitat types (Brown 1984). Importantly, a common hurdle in developing conservation plans for rare and/or endangered species threatened by habitat loss is lack of basic information on distribution and factors affecting population densities (Price and Endo 1989). As such, characterizing quantitative habitat features for understudied and/or rare species is important for better understanding distribution and abundance and informing management strategies.

Little is known regarding habitat characteristics that are important to the distribution and abundance of *D. elator*. A few studies have identified important, qualitative environmental features. For example, Texas kangaroo rats tend to occur in areas with few short grasses that may or may not include mesquite (*Prosopis glandulosa*; Martin and Matocha 1972), in particular heavily grazed short-grass prairie with earth exposed by concentrated traffic of vehicles or animals (Stangl et al. 1992). Sites are also associated with firm clay-loam soils (Roberts, 1969; Roberts and Packard 1973). Nelson et al. (2009) more recently concluded that *D. elator* prefers areas disturbed by grazing, fire, or drought including many prairie mounds (natural, elevated and relatively bare areas possibly uplifted by clay soils swelling in cracks; Diggs et all 1999) used to make burrows. Importantly, as a result of overgrazing and fire suppression, mesquite (*Prosopis glandulosa*) and other disturbance-related shrubs, grasses, and forbs have increased in abundance across the region. Moreover, habitat modifications (e.g., conversion of pastureland to monoculture) have likely resulted in extensive fragmentation of habitat (Diamond and Shaw 1990), such that habitat loss and degradation is considered a major threat to the continued existence of this species (Hafner 1996).

Although habitat associations of *D. elator* have been documented, we still lack a detailed understanding of how different habitat features affect the distribution and abundance of this species, thereby complicating management effort. Moreover, most of the existing studies have been local in scale (e.g., focused on a small portion of the distribution of *D. elator*). Because the distribution of *D. elator* spans the convergence of two ecoregions in Texas, the Rolling Plains to the west and the Cross Timbers and Prairies to the east, it is possible that some of the inferences that have been made about *D. elator* habitat associations may be biased or incomplete because they fail to incorporate all of the variation in environmental characteristics across the distribution of the species. Here, we sampled across the entire geographic range of *D. elator* to identify habitat characteristics important to the distribution and abundance of this species.

METHODS

We estimated *D. elator* distribution (i.e., presence/absence) and abundance and quantified habitat features at 50 sites on state and private land distributed across the historical range of this species. Our initial surveys took place in 2016 at 35 sites (Fig. 1), followed by an additional 15 sites in 2018. At each site we used paired, 500-m transects that were separated by 100 m to estimate *D. elator* distribution and abundance. We placed Sherman live traps (H.B. Sherman Traps, Inc., Tallahassee, Florida) every 10 m (i.e., 51 traps per transect) and operated traps for two consecutive nights, for a total of 204 trap nights per site. Captured individuals were marked to identify recaptures and released each morning. Such a trapping design has been successful for estimating presence and abundance of rodents in other arid systems (Stevens and Tello 2009, 2011, 2012, Stevens et al. 2012). Voucher specimens are deposited in the Natural Sciences Research Laboratory at the Museum of Texas Tech University. We sampled environmental characteristics at an additional eight transects, each 2 m wide × 25 m long, running perpendicular to each mammal transect (i.e., four per transect located at 0-, 167-, 333-, and 500-m marks). Along there transects we identified all perennial plants to species and measured their height, width, and length to estimate biomass. At three equidistant locations within each vegetation transect we measured soil compaction with a penetrometer; soil compaction was quantified as the depth (up to 24 cm), at which 2 MPa was attained, which is a level of soil compaction that affects plant performance (Bassett et al. 2005), and therefore potentially a direct or indirect effect on *D. elator* distribution and abundance. In the center of each transect within a 2 m × 2 m area we estimated percent cover of annuals and grasses and extracted a 1 dm^3 soil sample. From these soil samples, we quantified soil composition for each site using particle size analysis, which is a measurement of the size distribution of individual particles in a soil sample and can be used to determine the amount of sand, clay, and silt in each sample (Gee and Bauder 1986). Particle size analysis was completed at the Weindorf Lab at Texas Tech University for each of the eight samples collected per site (Fig. 1). Soil composition (i.e., percent sand, clay, silt) of these samples was then averaged to obtain one value per site. Using the composition of the soil, we were also able to describe it qualitatively (e.g., loam, clay loam, sandy loam; Fig. 4).

Statistical analyses

Based on our surveys of the initial 35 sites, we characterized habitat using biomass of 32 perennial plant species (Fig. 3), 3 soil particle size classes (i.e., percent sand, clay, and silt), 19 BioClim variables, which span a number of characteristics related to temperature and precipitation (Hijmans et al. 2005), and percent cover of annuals and grasses. We used principal component analysis (PCA) based on a correlation matrix to reduce redundancy and hence dimensionality of the perennial, soil, and climate data sets. Variables were square-root transformed to normalize the data and, in the case of the perennials data set, reduce influence of species with very high biomass. PCA reduced perennial, soil, and climate data sets to 6, 1, and 3 variables (principal components [PCs]; Table 4), respectively, based on those derived axes that had eigenvalues greater than one (Peres-Neto et al. 2005).

We used multivariate analysis of variance (MANOVA) to compare environmental characteristics at sites where *D. elator* was present versus sites where *D. elator* was not encountered. Soil properties included soil compaction and the soil profile PC. Vegetation properties included percent cover of annual plants and grass and the perennial PCs.

To further examine the relationship between habitat characteristics and *D. elator*, we used redundancy analysis (RDA; Legendre and Legendre 1998) to determine the amount of variation in both presence/absence (i.e., distribution) and abundance of *D. elator*, whereby perennial PCs, soil profile PC, climate PCs, and abundance of other rodent species at each site were independent variables and *D. elator* presence/absence and abundance were dependent variables in two separate analyses. RDA selects a combination of environmental variables that maximally accounts for variation in dependent variables (Jongman et al. 1995). This analysis also provides amount of variation accounted for by dependent variables (i.e., adjusted R^2) plus shared variation among variables, as well as statistical significance of the result based 10,000 permutations of the original data. All analyses were conducted in R (R Core Team 2018).

RESULTS

During our first survey period we sampled 35 sites, resulting in 7,140 trap nights. At these 35 sites we encountered 30 *D. elator* across six sites (Table 2), making it the fourth most abundant and fifth most widespread species in the rodent community (Fig. 2). Qualitatively, based on the average amount of sand, clay, and silt in the soil samples, *D. elator* was associated with loam soils (sand: 34.23 ± 13.31; clay: 23.67 ± 5.31; silt: 42.11 ± 9.89), although there was some variation in soil type among the different sites (Figs. 4 and 5).

Principal component analysis reduced the perennial plant variables to six derived variables that accounted for 65.6% of the variation among sites. Together, these principal components represented axes that encapsulated much of the environmental variation present at these sites (Table 4). A second PCA reduced three soil compositions to 1 derived variable that accounted for 91.2% of variation among sites. This PC represented an axis ranging from sandy soils at low values to soils with more silt and clay at high values. The third PCA reduced the climate variables to 3 derived variables that accounted for 95.7% of variation among sites. The first principal component represented an axis ranging from precipitation range at low values to precipitation extremes at high values. The second principal component represented a contrast between summer precipitation and summer temperature, whereas the third principal component was a contrast between winter temperature and winter precipitation (Table 4).

The MANOVA testing for habitat differences between sites of presence and absence of *D. elator* was not significant ($F_{5,29} = 1.38$, $P = 0.26$, Pillai = 0.18). Because the overall MANOVA was non-significant, we did not test for univariate differences among the variables (Table 3).

Soil and the shared variation between climate and vegetation accounted for the most variation in the abundance (soil $R^2_{\text{adj}} = 0.055$; climate and vegetation $R^2_{\text{adj}} = 0.081$) and distribution (soil $R^2_{\text{adj}} = 0.080$; climate and vegetation $R^2_{\text{adj}} = 0.104$) of *D. elator* (Fig. 6). Neither of the overall models was significant (abundance: $F = 1.08$, $P = 0.56$; distribution: $F = 1.26$, $P = 0.39$). Similarly, none of the individual fractions for either model was significant (all $P > 0.18$).

Our surveys of an additional 15 sites added 3,060 more trap nights of effort, although we did not encounter *D. elator* at any of these additional sites (Table 5), even though soil and vegetation did not deviate from conditions at earlier sites of presence. Rodent abundance was much lower during these surveys, with only 6 total species being encountered. *Chaetodipus hispidus*, the only heteromyid species encountered across the sites, was both the most abundant species (4 individuals) and, along with *Sigmodon hispidus*, the most widely distributed (i.e., present at 13% of the sites; Table 5).

DISCUSSION

A common hurdle in identifying effective management strategies for rare and/or threatened species is paucity of basic information on factors influencing presence and abundance across its distribution. We sampled 35 sites across the distribution of *D. elator* to provide a distribution-wide examination of how habitat characteristics influence abundance as well as presence of this species. We found that *D. elator* occurred at six of the 35 sites and was the fourth most abundant species in our surveys (Fig. 2).

Soil has long been considered to be an important determinant of *D. elator* presence across its geographic range. For example, Roberts (1969) and Roberts and Packard (1973) both concluded that *D. elator* was typically associated with firm clay-loam soils. More recently, however, Martin and Matocha (1991) suggested that *D. elator* may not be restricted to areas with clay-loam or clay soil habitat. Although we found an overall association with loamy soils (Fig. 4), our findings indicate that the species can be found in areas with soils ranging from sandy loam to loam, clay loam, and silty clay loam, adding some support to the idea that the species may be able to persist in a wider range of soil characteristics than previously thought. We documented the species in soils with up to 55% sand, whereas Martin and Matocha (1991) documented the species in Motley County at a location with soil that was 79% sand. Nevertheless, it’s worth noting that to our knowledge the species has not been documented in Motley County since Martin’s (2002) status report. Indeed, in addition to our own surveys of the county, Nelson et al. (2013) revisited 10 locations of occurrence in Motley County from the Martin (2002) report and either did not encounter the species or deemed the location was now poor habitat. One possibility is that these sandier soils still represent suboptimal conditions for this species, such that these records documented sink habitats (i.e., populations temporarily persisting in suboptimal habitat).

For the variation partitioning analyses, both soil and shared variation between climate and vegetation accounted for the most variation in abundance and distribution of Texas kangaroo rats (Fig. 6), although a large amount of variation remained unaccounted for in the analyses.

Similarly, many of our analyses comparing habitat features at sites of presence versus sites of absence indicated a large amount of overlap (Fig. 5; Table 3). Together, these findings suggest that *D. elator* may not be colonizing and saturating all available suitable habitat, such that continued habitat sampling will be necessary to better understand the habitat associations for this species. This idea is further corroborated by patterns of abundance and distribution across the rodent community. Compared to species that occurred at a similar number of sites (e.g., *Baiomys taylori* and *Onychomys leucogaster*; Fig. 2), *D. elator* reached higher average abundance at sites of presence (Fig. 2). This finding agrees with earlier research that suggests that at places where habitat is suitable, *D. elator* can be relatively abundant (e.g., Jones et al. 1988). This suggests that *D. elator* may be distribution-limited (i.e., unable to colonize all available habitat). As such, future work should focus on the degree to which *D. elator* can colonize available habitat in order to determine whether the rarity of this species is due more to being distribution-limited or abundance-limited. For example, little is still known about *D. elator* dispersal (but see Goetze et al. 2008, Stasey et al. 2010) or predation (Stangl et al. 2005).

Ancillary analyses on community composition suggest that lack of any strong patterns explaining the distribution or abundance of *D. elator* is not simply due to inadequate sampling, although we due recommend additional sampling (see below). Importantly, we were able to identify strong relationships between habitat variables and relative abundance of a number of species, both rare and common, in our dataset (Fig. 7). Nevertheless, the results of the community-wide redundancy analysis did not detect any relationship between habitat features and *D. elator* relative abundance. This further indicates a high degree of habitat heterogeneity among sites in which we encountered *D. elator*.

One of the existing questions regarding *D. elator* habitat associations is the importance of honey mesquite (*P. glandulosa*) for *D. elator* occurrence (Martin and Matocha 1972). Although we did not detect a significant effect of perennial biomass on *D. elator* presence or abundance, there was a trend towards less perennial biomass at sites where *D. elator* was present, and the lack of significance is likely due to the high amount of variation in perennial plant biomass across the 29 sites where *D. elator* was not encountered (Table 3). This suggests that continued overgrazing and fire suppression, both of which promote the spread of shrubs and forbs like *P. glandulosa*, *Ambrosia psilostachya*, and *Solanum elaeagnifolium*, could pose an increasing threat to *D. elator*, especially if it is unable to easily move among habitats. Moreover, many of the individuals that we trapped at these sites were in close proximity to a road or fence line, suggesting that any habitat features that provide sufficiently open area and a raised surface for *D. elator* to burrow into and forage near are sufficient, as opposed to the presence of *P. glandulosa* in particular (e.g., Goetze et al. 2007, Stasey et al. 2010).

Finally, it is worth noting the temporal significance of our study. Our surveys took place immediately following a period of heavy rainfall that occurred after several years of drought conditions, as evidenced by the high prevalence of *S. hispidus* (Fig. 2) and overall vegetation biomass and cover (Fig. 3, Table 3). As such, this stands in contrast to many of the recent reports on the status of the species that were based on data collected during drought years (e.g., Martin 2002, Nelson et al. 2013), and may explain some of the differences in habitat patterns that we found. For example, Nelson et al. (2013) documented an average of 65.1 ± 16.3% bare ground around *D. elator* burrows in 2011, a much higher value than the average amount of bare ground that we found at sites where *D. elator* was present (Table 3). Thus, the fact that we did not see any significant difference in amount of bare ground between sites of absence and sites of presence of *D. elator* could be representative of *D. elator* populations currently persisting in areas that were previously suitable but may be more marginal now as heavy precipitation has facilitated vegetative growth across the entire region.

Given the preliminary results that we have found, we ultimately believe that more sampling is needed, and specifically more sites of presence, to gain a firm understanding of habitat associations of Texas kangaroo rat. More specifically, we encourage more sampling across both the eastern and western portions of the geographic range, as variation driven by habitat differences across the region may be masking certain patterns. Regardless, continued sampling will help further identify habitat features that are important to the abundance and presence of *D. elator* so that we can make informed management decisions.

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Figure 1a. Number of habitat and rodent survey sites per county out of the 50 total surveys completed on private and state land in 2016 and 2018.

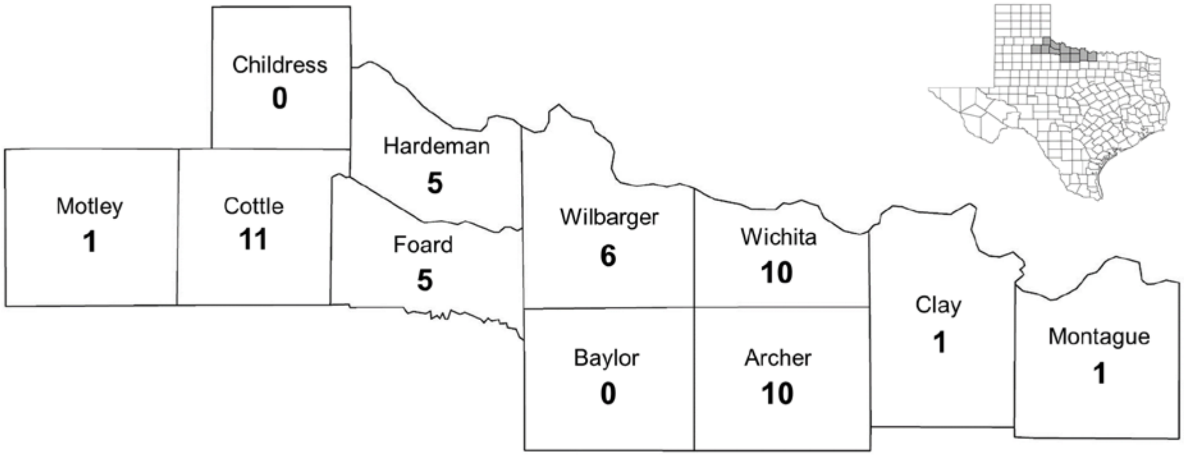

Figure 1b. Diagram representing the arrangement of trapping and vegetation survey transects at each site.

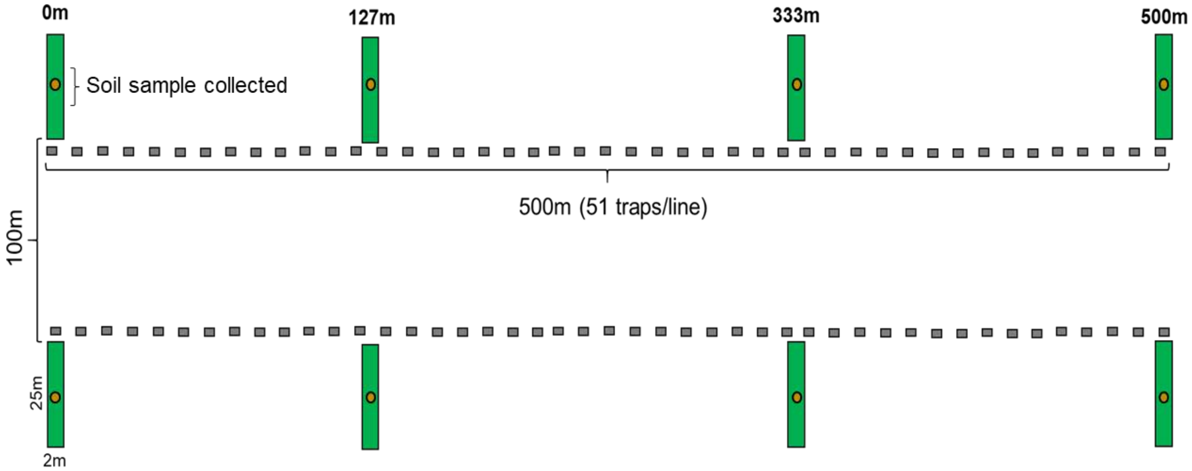

Figure 2. Summary of the distribution and abundance of the 15 rodent species encountered during our habitat surveys. The y-axis on the left corresponds to the line chart, which indicates the proportion of the 35 sites at which a species was present (i.e., the distribution). The y-axis on the right corresponds to the grey bars, which represents the average number of individuals per site, using only sites where the species was present.

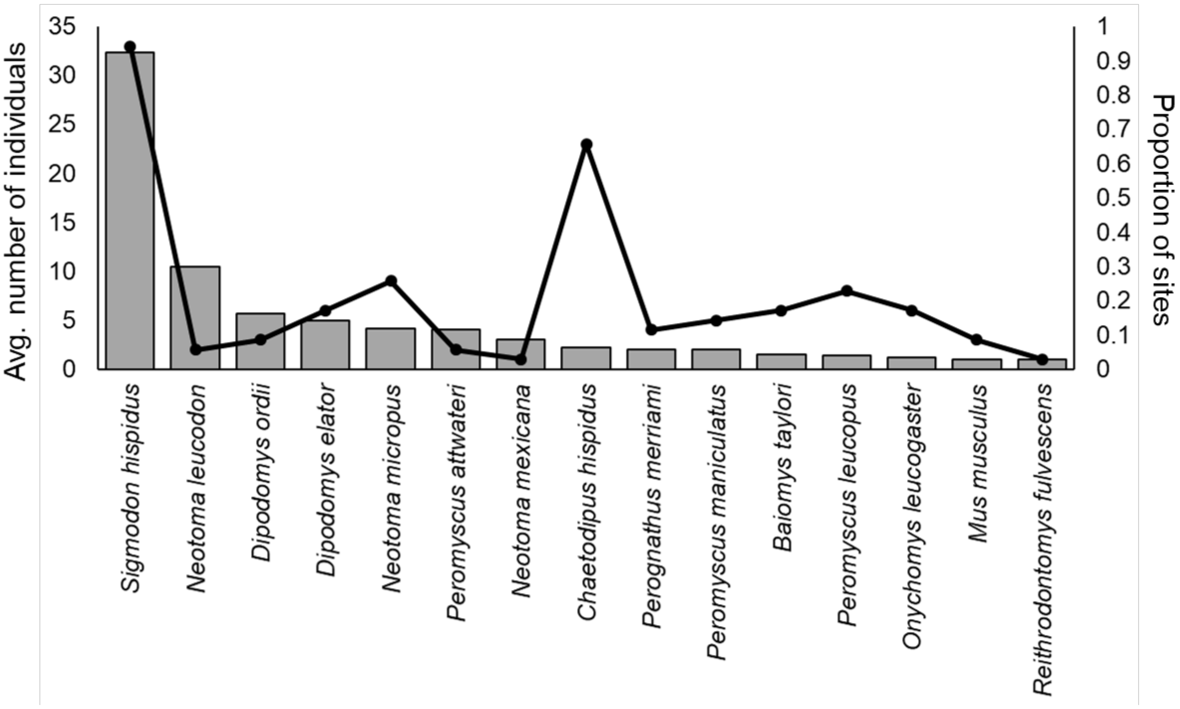

Figure 3. Summary of the distribution and abundance of perennial plant species encountered during our habitat surveys. The y-axis on the left corresponds to the grey bars, which represents the average aboveground biomass of a species per site, using only sites where the species was present. The y-axis on the right corresponds to the line chart, which indicates the proportion of the 35 sites at which a species was present (i.e., the distribution). This data was square root transformed to reflect the data used in the PCA, as well as facilitate visual comparison between species.

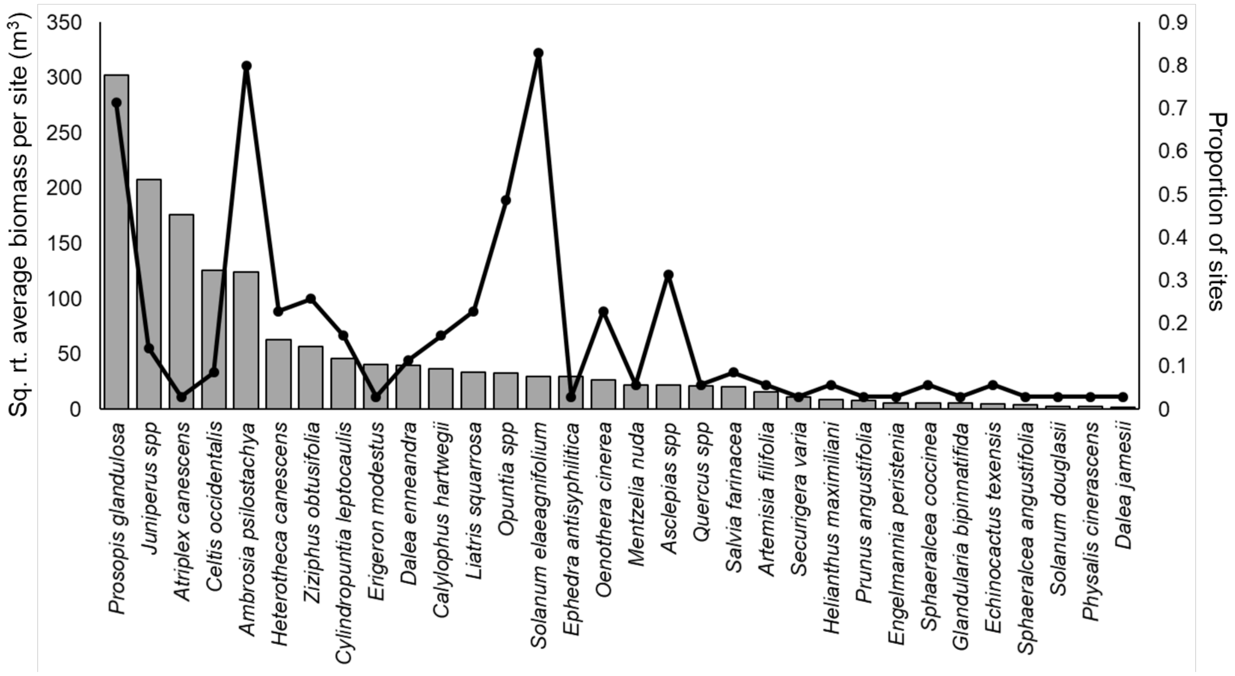

Figure 4. Soil association breadth of *Dipodomys elator*. Each circle represents the average percent of sand, clay, and silt at a site where the species was present, with the corresponding qualitative soil category based on the soil composition (Natural Resources Conservation Service), as determined by particle size analysis.

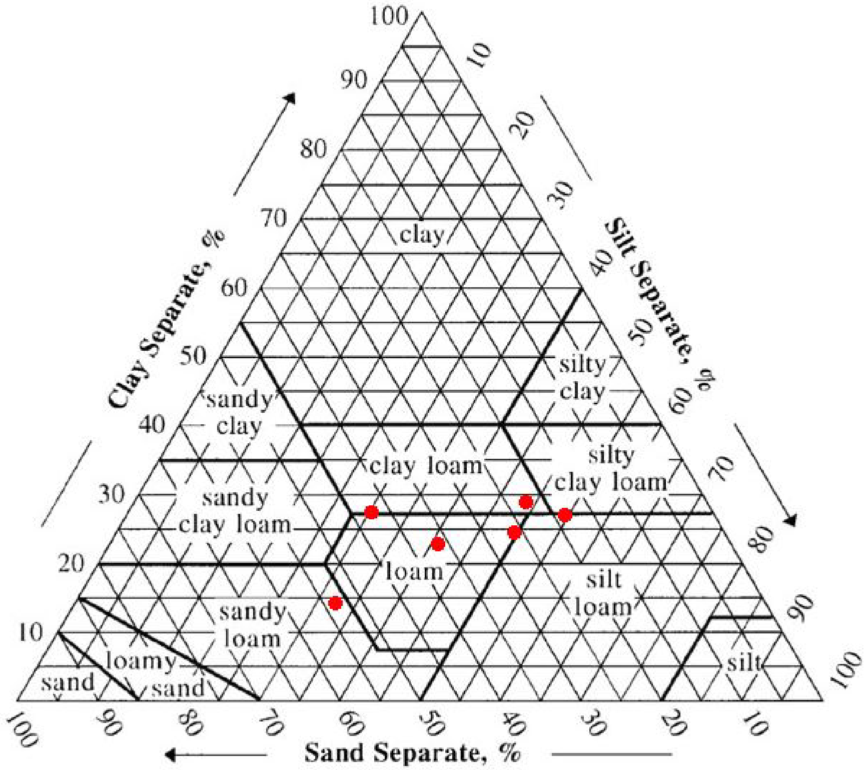

Figure 5. Soil characteristics of sites where *D. elator* was not encountered versus sites where *D. elator* was present. The y-axis represents a contrast between sites with more silt and clay versus sites with more sand and is based on a principal components analysis performed on the average percent sand, clay, and silt at each site obtained by particle size analysis.

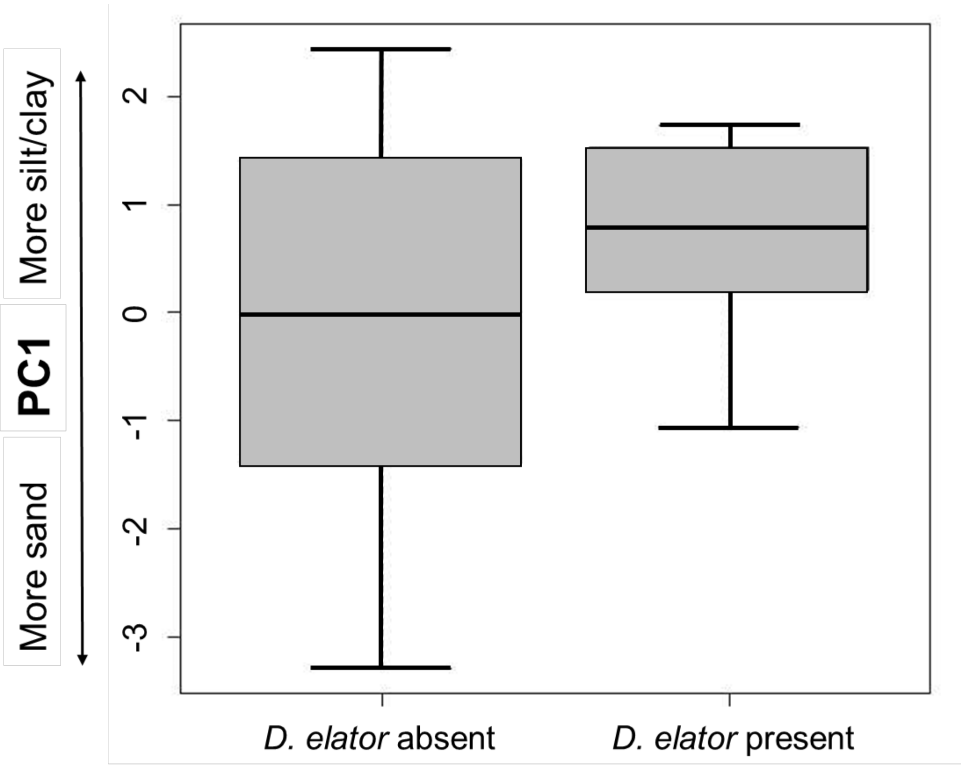

Figure 6. Results of variation partitioning indicating the amount of variation in *D. elator* a) abundance and b) distribution explained by soil, vegetation, climate, rodent abundance, and their shared variation. Values reported within the figure represent the adjusted R^2 for each fraction of variation. Negative values are not shown.

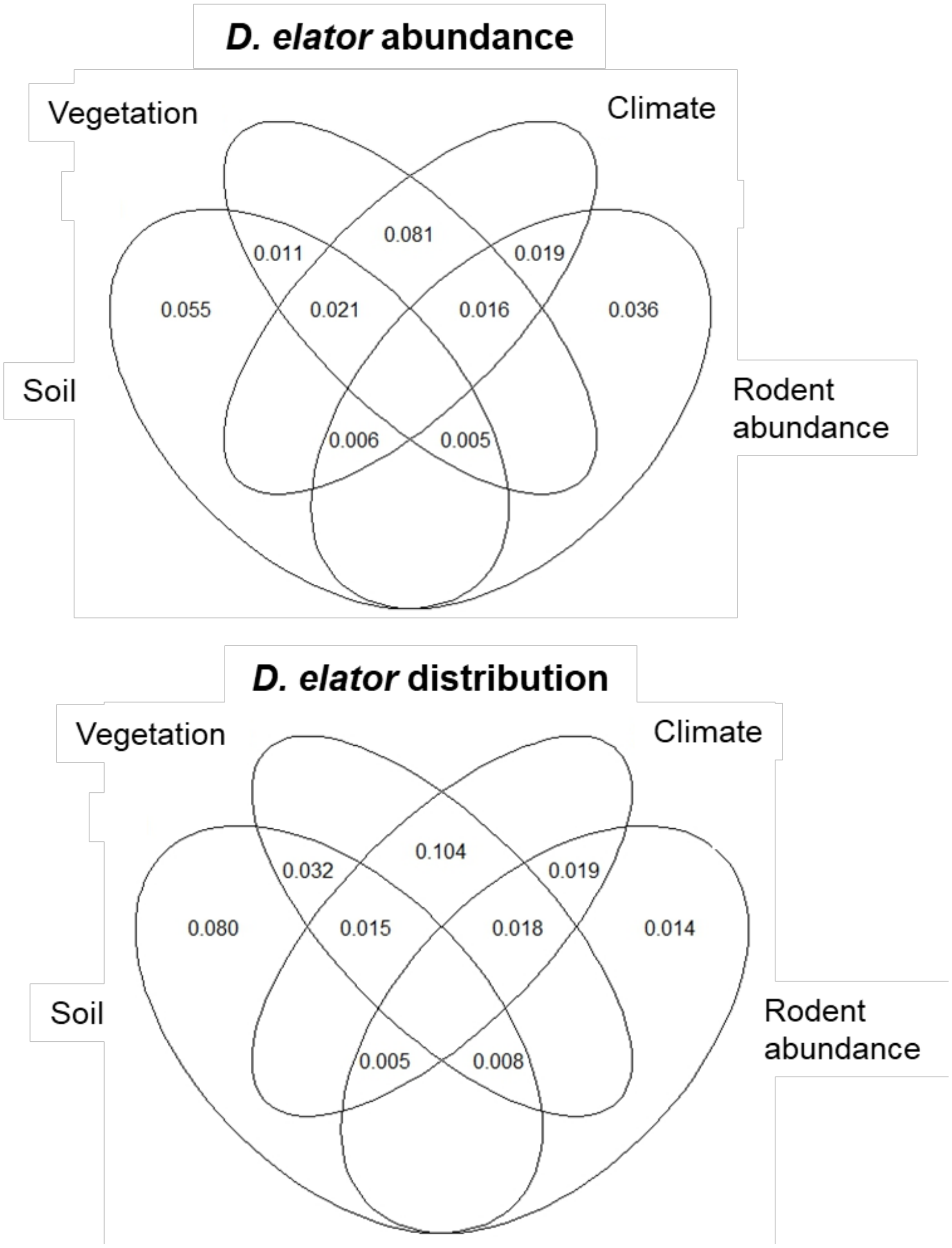

Figure 7. Results from redundancy analysis examining the relationship between rodent species composition and habitat variables. Arrows represent vectors describing the relationship of habitat variables and rodent relative abundance to relationships defined by the redundancy axes. Habitat variables are as follows: Veg1-6, 6 perennial plant PCs; Climate1-3, 3 climate PCs; Soil, soil PC; Cover, annual plant and grass percent cover; Compaction, soil compaction.

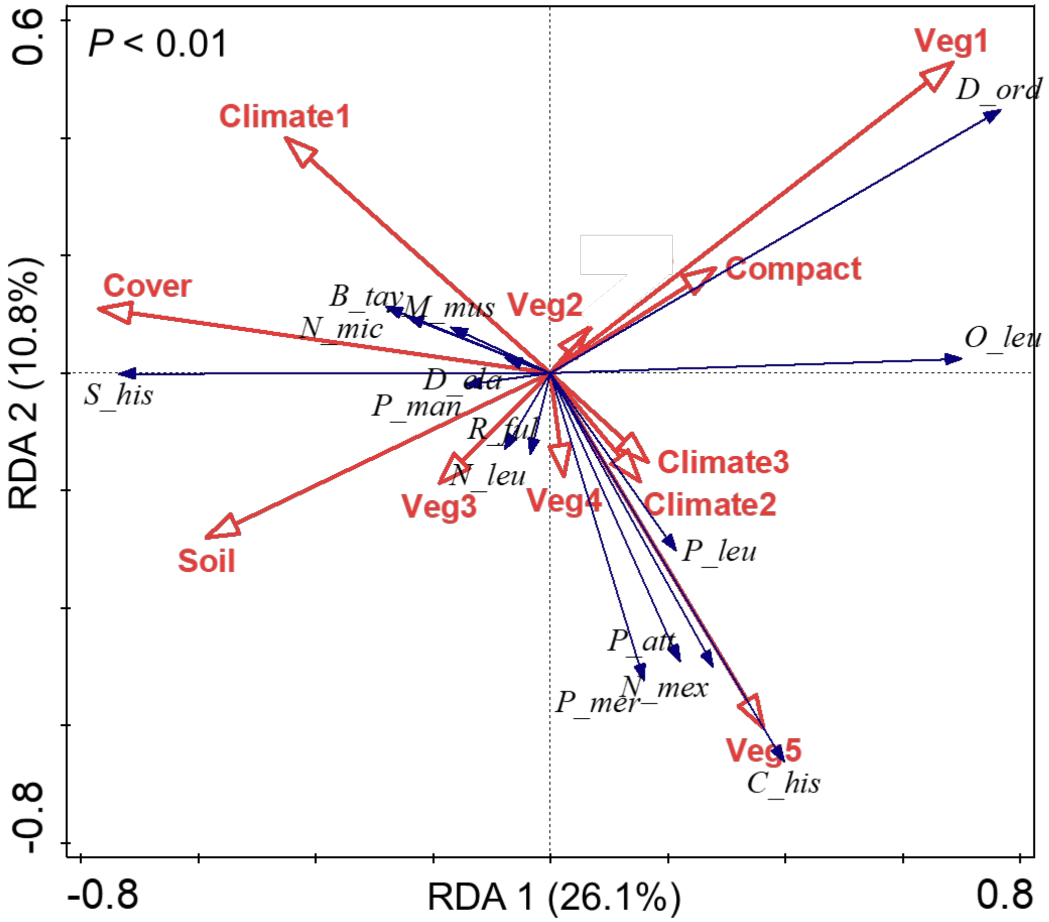

Table 1. Locations, in decimal degrees, of 35 sites. Sites with an asterisk (*) indicate locations where *D. elator* was present during sampling.

Site	Latitude	Longitude
1	34.14988	-100.29970
2	34.14105	-100.31420
3	34.04843	-98.62900
4	34.04918	-98.65710
5	34.12135	-100.36610
6	34.10885	-100.37200
7	34.11311	-99.73580
8	34.11550	-99.76324
9	34.29269	-99.63920
10	34.28359	-99.64170
11	34.13772	-100.35820
12	33.43129	-98.84739
13	33.98012	-99.93700
14	33.98940	-99.95520
15*	34.05604	-98.69980
16*	34.04890	-98.78680
17*	34.05778	-98.81600
18	33.43352	-98.74230
19	33.48521	-98.77480
20*		
21	33.60342	-98.5031
22	33.74569	-98.3949
23		
24		
25	33.78522	-97.9517
26	34.43985	-99.8367
27	34.09242	-100.8893
28	34.15427	-100.4473
29*		
30		
31*		
32	34.07529	-99.34530
33	34.06638	-99.35360
34	34.05402	-99.36697
35	34.13920	-99.44670

Table 2. Site by species matrix of the number of unique individuals (i.e., not recaptures) per species at the 35 sites.

Site	***Baiomys taylori***	***Chaetodipus hispidus***	***Dipodomys elator***	***Dipodomys ordii***	***Mus musculus***	***Neotoma leucodon***	***Neotoma mexicana***	***Neotoma micropus***
1	0	2	0	0	0	0	0	0
2	0	0	0	13	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	1	0	0	1
5	0	2	0	0	0	0	0	0
6	0	2	0	0	0	0	0	0
7	1	1	0	0	0	0	0	0
8	0	8	0	0	0	0	3	0
9	0	0	0	0	0	0	0	7
10	0	1	0	0	0	0	0	11
11	0	1	0	0	0	0	0	0
12	2	0	0	0	1	0	0	0
13	0	6	0	0	0	13	0	0
14	1	1	0	0	0	0	0	3
15	0	1	2	0	0	0	0	0
16	0	1	4	0	0	0	0	0
17	0	0	9	0	0	0	0	0
18	3	0	0	0	0	0	0	5
19	1	1	0	0	0	0	0	3
20	0	1	3	0	0	0	0	0
21	0	0	0	0	0	0	0	1
22	0	0	0	0	0	0	0	0
23	0	2	0	0	1	0	0	0
24	0	2	0	0	0	0	0	1
25	1	0	0	0	0	0	0	0
26	0	8	0	0	0	0	0	5
27	0	1	0	0	0	0	0	0
28	0	0	0	0	0	8	0	0
29	0	2	7	0	0	0	0	0
30	0	1	0	0	0	0	0	0
31	0	0	5	0	0	0	0	0
32	0	2	0	2	0	0	0	0
33	0	2	0	2	0	0	0	0
34	0	1	0	0	0	0	0	0
35	0	2	0	0	0	0	0	0
TOTAL	9	51	30	17	3	21	3	37

Site	*Onychomys leucogaster*	*Perognathus merriami*	*Peromyscus attwateri*	*Peromyscus leucopus*	*Peromyscus maniculatus*	*Reithrodontomys fulvescens*	*Sigmodon hispidus*
1	0	1	0	0	0	0	5
2	1	0	0	0	0	0	0
3	0	0	0	1	0	0	19
4	0	0	0	0	2	0	18
5	0	0	0	0	0	0	19
6	1	0	0	0	0	0	21
7	0	0	0	0	0	0	7
8	0	3	5	2	0	0	5
9	0	2	0	0	0	0	32
10	0	2	0	0	0	0	51
11	0	0	0	0	0	0	29
12	0	0	0	0	4	0	33
13	0	0	3	0	0	0	20
14	0	0	0	0	0	0	82
15	0	0	0	2	0	0	49
16	0	0	0	0	0	0	1
17	0	0	0	0	1	0	31
18	0	0	0	0	0	0	59
19	0	0	0	1	0	0	62
20	0	0	0	0	0	0	64
21	0	0	0	0	0	0	55
22	0	0	0	0	0	0	80
23	0	0	0	1	0	0	61
24	0	0	0	0	0	0	39
25	0	0	0	2	0	0	75
26	0	0	0	0	0	0	53
27	0	0	0	0	0	1	6
28	0	0	0	0	0	0	34
29	1	0	0	1	1	0	2
30	0	0	0	1	0	0	1
31	0	0	0	0	0	0	10
32	1	0	0	0	0	0	0
33	2	0	0	0	0	0	19
34	1	0	0	0	0	0	11
35	0	0	0	0	2	0	15
TOTAL	**7**	**8**	**8**	**11**	**10**	**1**	**1068**

Table 3. Habitat characteristics of sites where *D. elator* was present versus sites where *D. elator* was not encountered. Values represent mean ± SD.

	***D. elator* present**	***D. elator* absent**
Soil		
Soil Compaction (depth to 300 psi)	6.50 ± 8.21	5.15 ± 4.60
Sand (%)	34.23 ± 13.31	46.14 ± 20.22
Clay (%)	23.67 ± 5.31	20.76 ± 7.04
Silt (%)	42.11 ± 9.89	33.10 ± 14.36
Vegetation		
Bare ground (%)	27.60 ± 21.17	41.26 ± 19.90
Perennial biomass (m^3)	32950.60 ± 7762.47	1384821.75 ± 635192.20

Table 4. Results from principal component analysis on perennial plant species, soil composition, and climate. PC refers to a particular principal component, Variance explained refers to the amount of unique variation accounted for by that component, and Cumulative variance refers to the cumulative variation accounted for by a particular PC and all other PCs extracted prior. Gradient represents the interpretation of a particular PC. Only principal components that were maintained for analyses are presented.

Perennial Plants

PC	Variance explained	Cumulative variance	Gradient
1	18.7	18.7	Forbs versus shrubs and succulents
2	14.1	32.9	Aridity
3	9.3	42.1	Amount of *A psilostachya* and *S. eleagnifolium*
4	8.9	51.0	Amount of tall-statured forbs
5	8.1	59.1	Soil breadth/tolerance
6	6.4	65.6	Disturbance

Soil

PC	Variance explained	Cumulative variance	Gradient
1	91.2	91.2	Proportion sand to proportion silt and clay

Climate

PC	Variance explained	Cumulative variance	Gradient
1	71.7	71.7	Precipitation range versus precipitation extremes
2	16.8	88.5	Summer precipitation versus summer temperature
3	7.3	95.7	Winter temperature versus winter precipitation

Table 5. Summary of trapping success for an additional 15 habitat and rodent survey sites.

Site	*B. taylori*	*C. hispidus*	*N. leucodon*	*P. merriami*	*P. leucopus*	*S. hispidus*
36	0	0	0	0	1	0
37	0	0	0	0	0	0
38	0	0	0	0	0	0
39	0	2	0	0	0	0
40	0	0	1	0	0	0
41	0	0	0	0	0	1
42	0	0	0	0	0	0
43	0	0	0	0	0	0
44	0	0	0	0	0	0
45	0	0	0	0	0	0
46	0	0	0	0	0	0
47	0	1	0	0	0	0
48	2	0	0	0	0	0
49	0	0	0	1	0	2
50	0	1	0	0	0	0
TOTAL	**2**	**4**	**1**	**1**	**1**	**3**

CHAPTER III: UPDATED DISTRIBUTION OF *DIPODOMYS ELATOR* VIA COUNTY ROAD SURVEYS AND PATTERNS OF RODENT SPECIES CO-OCCURRENCE

EXECUTIVE SUMMARY

An updated understanding of the distribution and abundance of *D. elator* is important for informing the decision of whether to list the species under the Endangered Species Act, as well as well as to better inform general conservation strategies. Previous research on the distribution of *D. elator* was carried out via road surveys, with the last such survey taking place over 15 years ago. To improve our understanding of the present-day distribution of *D. elator,* we surveyed over 800 locations along unpaved county roads across the historical range of this species. In addition to identifying sites of presence, we examined patterns of species co-occurrence within the rodent communities from these sites. We determined that *D. elator* presently occurs in five counties in Texas within its historical range (i.e., Childress, Cottle, Hardeman, Wichita, and Wilbarger) and was the eighth most abundant species of the 14 that we encountered. Moreover, we found that the majority of pairwise species associations were random (i.e., there was not strong evidence that pairs of species within the rodent community were aggregating or segregating from each other). For *D. elator,* specifically, we found that most associations with other species were random (i.e., there was no clear pattern that they occurred at the same or different sites). There was, however, a significant positive association with *Peromyscus leucopus*, meaning that the two species occurred together more frequently than expected and two negative associations with Ord's kangaroo rat (*Dipodomys ordii*) and cotton rats (*Sigmodon hispidus*). These findings suggest that interspecific interactions may not play a strong role in influencing the distribution of

D. elator, although future work should examine co-occurrence patterns within the context of the surrounding habitat and across meaningful environmental gradients (e.g., precipitation).

INTRODUCTION

A common hurdle in developing conservation plans for rare and/or threatened or endangered species is lack of basic information on distribution and factors affecting population densities (Price and Endo 1989). This is because effective conservation plans require accurate estimates of the spatial distributions of the species they are trying to protect and rare species are often habitat specialists with a limited number of sites of known occurrence, because they tend to have sparse or restricted distribution patterns (Rabinowitz et al. 1986, Gaston 1997). Thus, determining present-day distribution of a rare species and evaluating how distribution has changed over time are important steps for identifying the underlying mechanisms contributing to rarity of understudied species and identifying effective management strategies.

The present distribution of *D. elator* is not well known and there is some indication that it may be changing. For example, new records that expand the distribution of the species are still being published (Martin and Matocha 1991), while at the same time a number of investigators have returned to sites found to be inhabited by *D. elator* in the earlier part of the 20th century and failed to encounter any individuals (Martin and Matocha 1972, Baumgardner 1987, Moss and Mehlhop-Cifelli 1990). These records suggest that this species may be changing its distribution in Texas, an important consideration for the conservation of this species. As such, a range-wide survey is needed to update our understanding of rarity and conservation status.

A few earlier studies documented the range-wide distribution of this species via county road surveys. Martin and Matocha (1972) first demonstrated that, because *D. elator* can be very active on unpaved county roads at night, trapping along county roads may be an effective means of surveying for the species and discovering new sites of occurrence of *D. elator*. Prior to this study, *D. elator* had been known from six Texas counties: Clay (Merriam 1894, Bailey 1905), Wilbarger (Blair 1949, Dalquest and Collier 1964), Archer (Dalquest and Collier 1964), Foard (Packard and Judd 1968), Wichita (Packard and Judd 1968, Roberts 1969), and Baylor (Baccus 1971). The surveys by Martin and Matocha (1972), which took place primarily between 1969-1970, documented *D. elator* again in Archer, Foard, Wichita, and Wilbarger, as well as discovered the species in two new counties: Hardeman and Motley, both to the west of the prior geographic range. Over a decade later, Jones et al. (1988) updated the distribution of *D. elator* with additional surveys that both revisited earlier localities and searched new areas between 1985-1987. The authors surveyed a total of 14 counties in Texas, and documented *D. elator* in just four of them: Cottle (i.e., a new county record), Hardeman, Wichita, and Wilbarger. Finally, and most recently, Martin (2002) visited all of the counties in the historic range of *D. elator* and documented the species in five of them: Archer, Childress, Hardeman, Motley, and Wichita. While there appears to be some consensus as to the general geographic range of *D. elator*, the results of these various surveys suggest a dynamic distribution, in that the species 1) has appeared sporadically across its geographic range since its discovery and 2) may be shifting towards the west (Martin 2002). This, coupled with a decade-and-a-half long hiatus in distribution-wide surveys, suggests that an update is paramount to understanding the present-day status of this species.

Despite studies on the distribution of this rare species that span a century, we still have limited understanding of the mechanistic factors influencing its distribution, aside from local studies on habitat associations (but see relevant section on habitat surveys). Importantly, despite the documented importance that interspecific interactions can have on distribution and abundance of rodent species (e.g., Brown and Munger 1985), no research has investigated the influence of other rodent species on *D. elator*. Fundamentally, any two species that occur within the same region are either positively, negatively, or randomly associated with each other. Hence, examining patterns of co-occurrence may be useful for inferring patterns of interspecific interactions within the rodent community and identifying and understanding factors that potentially limit the distribution of *D. elator* across its geographic range.

METHODS

Between June 2015 – August 2017 we surveyed dirt and gravel roads at 811 locations across the 11 counties in Texas where *D. elator* has been previously documented (Fig. 1). We also sampled an additional 60 sites in Hall County between 24 – 26 March 2017 (Fig. 1). Although *D. elator* has never been documented from Hall County, Martin and Matocha (1991) and (2002) have suggested that *D. elator* may be experiencing a westward distribution shift and this county is situated directly northwest of the historic range of this species (i.e., north of Motley County and west of Childress County; Fig. 1).

At each site we placed a Sherman live trap (H.B. Sherman Traps, Inc., Tallahassee, Florida) every 10 meters, such that each transect was 100 meters long. Traps were opened for one night and checked the following morning, for a total of 11 trap nights per site. All transects were separated by a minimum of 200 meters. Captured individuals were identified to species. Voucher specimens were deposited in the collection at the Natural Sciences Research Laboratory at Texas Tech University.

We examined spatial structure of rodent species composition based on a Canonical Correspondence Analysis (Ter Braak 1986). Geographic coordinates of latitude and longitude formed the independent matrix and rodent species presence or absence across sites formed the dependent matrix. We examined if the final solution of the CCA accounted for more variation than expected by change based on comparson with applications of the same analysis to 100 permutation of the original data. If the amount of variation accounted for by the CCA based on the real data was greater than in 95% of the applications to permuted data we concluded significance.

We analyzed patterns of co-occurrence among species based on a site by species presence/absence matrix, excluding both sites at which no species was detevcted, as well as the results from the Hall County road surveys because we were primarily interested in species associations within the geographic range of *D. elator*. This left us with 481 sites for the co-occurrence analyses. We used the R package "cooccur" (Griffith et al. 2016), which is based on the probabilistic model of species co-occurrence (Veech 2013). In this model, observed co-occurrence among pairs of species are compared to the expected co-occurrence for each pair, which is the product of the probability of occurrence of the two species multiplied by the number of sampling sites (i.e., 481 in this case). The probabilistic model uses combinatronics to determine the probability that the observed frequency of co-occurrence is significantly large and greater than expected (i.e., a positive association), significantly small and less than expected (i.e., a negative association), or not significantly different and approximately equal to expected (i.e., a random association; Griffith et al. 2016). Because we were only interested in the most important species associations, we removed from analyses all species pairs that according to their probabilities of co-occurrence were expected to share less than one site (i.e., species without sufficient occurrence data; Veech 2013). For all remaining species pairs, the probabilistic model calculated probabilities that those species could co-occur less than or greater than what is observed in the data (i.e., whether the species pairs are significantly associated, positively or negatively).

RESULTS

From the 871 sites that we surveyed, we documented 35 *D. elator* at 26 sites in five counties (i.e., Childress, Cottle, Hardeman, Wichita, and Wilbarger; Fig. 1). No *D. elator* were encountered during the Hall County road surveys (Table 1b). Of the five counties, we captured *D. elator* at one site in Childress, six sites in Cottle, three sites in Hardeman, ten sites in Wichita, and six sites in Wilbarger. Of the 26 total sites that we encountered *D. elator*, seven of the sites had more than one individual (maximum: three individuals). Of the 14 total rodent species that we captured, *D. elator* was the eighth most present species (i.e., occurred at the eighth most sites; Table 1) and the eighth most abundant species (Fig. 2, Table 1).

Although week, significant spatial structure was exhibited by species within out study area. Variation accounted for by the first two canonical analyses was 2.66 percent but this was significantly greater than expected by chance alone. Different spatial structures can be inferred by examining associations of species across the domain identified by the CCA (Fig. 3). For example, *D. elator* exhibits essentially no spatial structure across its geographic range. All other species exhibited varying degrees of spatial structure. *Baiomys taylori*, *Peromyscus attwateri*, and *P. lacianus* exhibited the greatest spatial structure with the former two species more common in the eastern portion of the domain and the latter more common to the west.

Out of 91 possible species pair combinations for the rodent community, 28 pairs (30.8%) were removed from the analysis because the expected co-occurrences of these pairs were less than one site, indicating that many of the species in the community were too rare to use in these analyses. Of the remaining 63 pairs, 35 of the associations were random, 1 was positive, and 27 were negative. For *D. elator* specifically, there were no positive associations with other species but significant negative associations with *D. ordii* and *S. hispidus*, respectively (Fig. 4). This means that the species occurred at the same sites less often than expected (*D. ordii*: expected: 7.4, observed: 1; *S. hispidus*: expected: 10.1, observed: 4). In contrast, *D. ordii* had significant negative associations with eight other species and one significant positive association with *Onychomys leucogaster*, an omnivorous rodent with similar habitat associations (Fig. 4).

DISCUSSION

We conducted roadside surveys for *D. elator* across its historical geographic range (Fig. 1) and found that the species presently occupies less than half of the Texas counties from which it was previously documented. We also found support for the idea that *D. elator* occurs sporadically throughout its distribution. Moreover, we found that a majority of the co-occurrence patterns between rodent species in this region were random, although we identified many negative associations, including two for *D. elator* (i.e., *D. ordii* and *S. hispidus*). Below, we interpret these findings in light of earlier surveys and discuss their significance.

Our road surveys indicate that, although *D. elator* appears to be sporadic throughout its historical geographic range, we encountered it in both the eastern and western portions of the region. Thus, we did not find any support for the hypothesis by Martin (2002) that the species may be shifting its distribution westward (e.g., Motley and Childress counties). For example, although we did not document the species in Clay or Montague counties, where the species likely hasn't occurred for several decades (e.g., Martin 2002), Wichita was the county with the most sites in which we encountered *D. elator*. Similarly, Wilbarger County, in the middle of the range, also had several sites of presence. In contrast, we only encountered *D. elator* on the eastern edge of Childress County, near the border with Hardeman County, and did not encounter any Texas kangaroo rats in Motley County or during our ancillary surveys in Hall County. This suggests that the patterns documented by Martin (2002) may have more to do with a) the sporadic distribution of the species and b) increased sampling effort in the western portion of the region. Nevertheless, given differences in climate and habitat between the eastern and western portions that may diverge further moving forward, continued attention should be given to these difference and how the species is responding over time.

Our findings support growing evidence that *D. elator* exhibits dynamic distribution patterns, given that the species appears to occur sporadically throughout its distribution. Although our results are comparable to those of Martin (2002) in the sense that, like that study, we encountered *D. elator* in five counties, unlike Martin (2002) we did not encounter *D. elator* in Archer County but did document the species in Cottle County. Prior to ours and Martin's (2002) surveys, Jones et al. (1988) documented the species in Cottle, Hardeman, Wichita, and Wilbarger counties. Thus, it appears that the habit of *D. elator* to appear and disappear from counties where it has been documented may be a natural yet unpredictable feature of its natural history.

Despite evidence that *D. elator* appears sporadically throughout its range over time, we did not find any indication that interspecific interactions are strongly influencing these patterns. For example, as with most other species, a majority of the associations with *D. elator* were random, and only two were negative (i.e., *Dipodomys ordii* and *Sigmodon hispidus*; Fig. 3). Moreover, although anecdotal accounts suggest that *D. elator* may be a relatively docile, unaggressive species (Goetze et al. 2008), and that *D. ordii* is comparably more aggressive (e.g., Perri and Randall 1999), it is likely that the negative associations we documented are likely a product of differences in habitat associations between *D. elator* and both *D. ordii* (e.g., soil type) and *S. hispidus* (e.g., vegetation type and cover), as documented in Chapter 2 of this report. Thus, it is unlikely that biotic interactions with other species are having a strong influence on the distribution of *D. elator*.

Finally, the results of our work highlight a number of important tasks for future research on *D. elator*. For example, although we did not encounter *D. elator* in Baylor or Foard county, we believe that these areas should be further surveyed given their proximity of sites of presence of *D. elator* from the last few years. In the case of Baylor County, much of the land is restricted by the presence of the Waggoner Ranch. Future access to this property would be important to determining the extent of the Texas kangaroo rat's distribution in the south-central portion of its range, where the species was previously documented (Baccus 1971). Moreover, although we did not detect many strong co-occurrence patterns in the rodent community, including between *D. elator* and other species, future work should examine the degree to which land-use context (e.g., rangeland versus agriculture) influences patterns of distribution, abundance, and co-occurrence (Kay et al. 2018). Given the prevalence of these land uses in the region, these analyses could be informative for predicting future rodent species dynamics in the region as habitat is increasingly altered.

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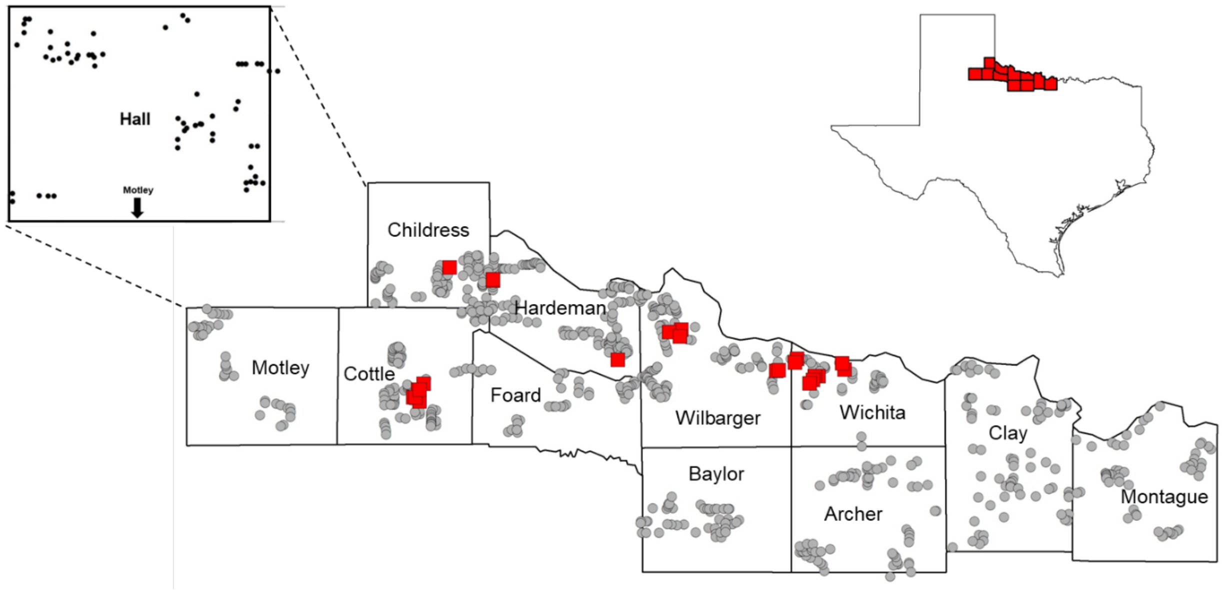

Figure 1. Results of our county road surveys (n = 811 sites) across the historical distribution of the Texas kangaroo rat. Gray circles indicate areas where traps were deployed but the species was not captured (i.e., “absence” sites), whereas red stars indicate areas where the Texas kangaroo rat was captured (n = 26 localities). Inset map shows locations of trapping sites for road surveys in Hall County conducted between 24-26 March 2017. No *D. elator* were encountered during the Hall County surveys.

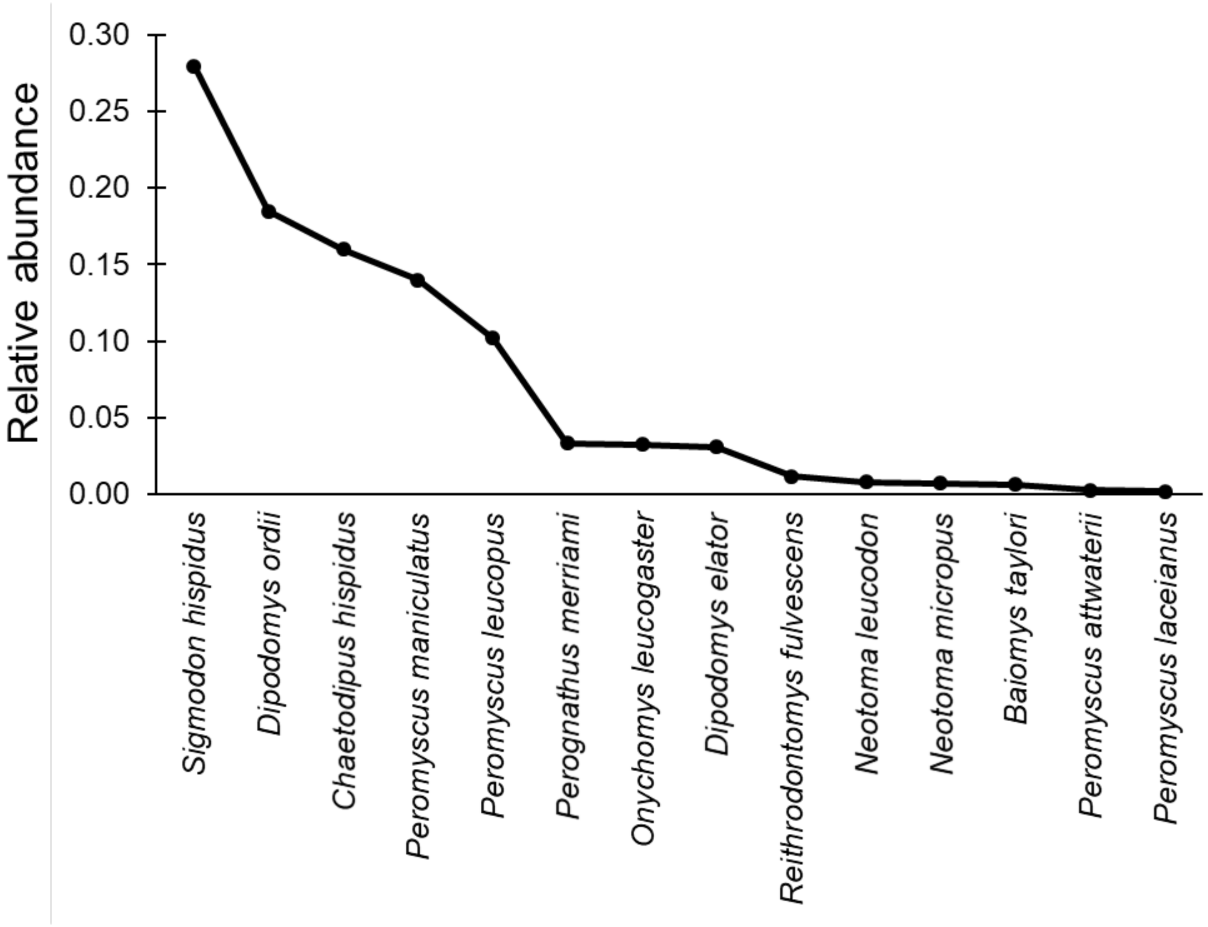

Figure 2. Rank-abundance curve for the rodent community (n = 14 species) based on the county road surveys.

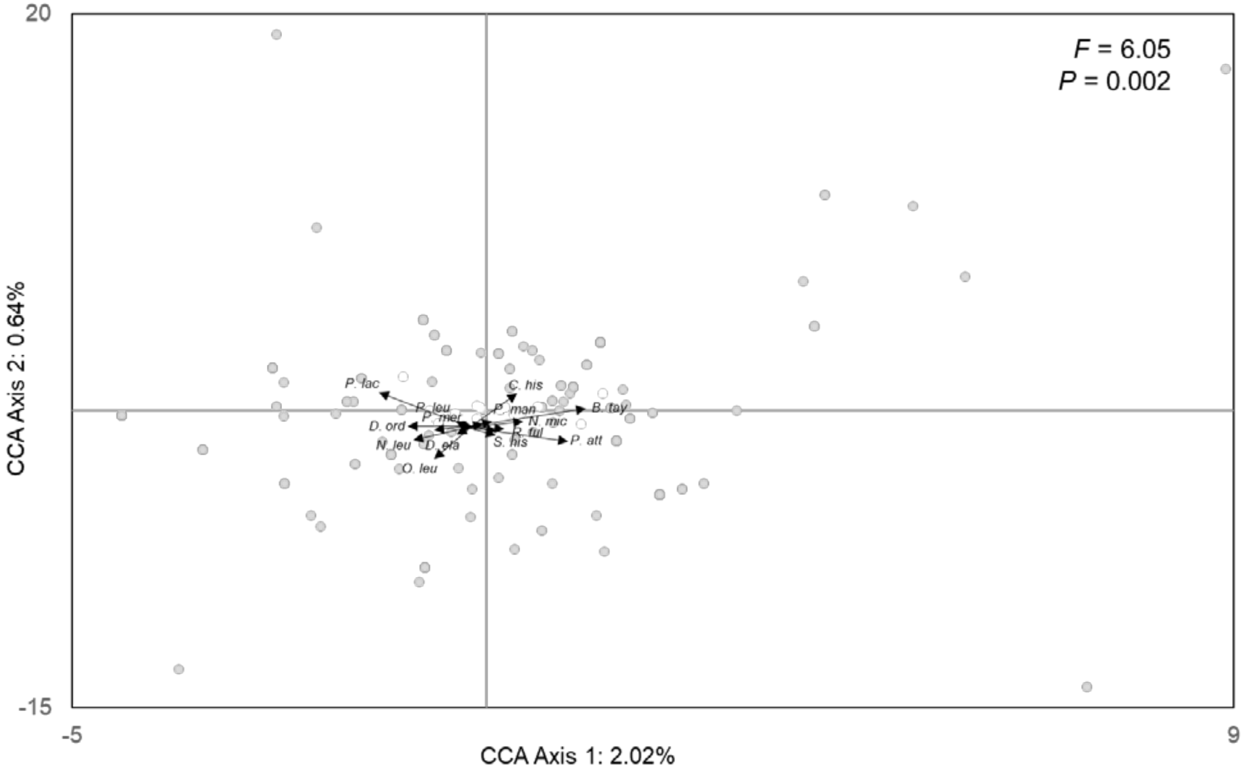

Figure 3. Results from canonical correspondence analysis examining the amount of spatial structure in the distribution of rodent species occurring in the geographic range of *Dipodomys elator*. CCA Axis 1 corresponds to a west to east (small to large values) gradient whereas CCA Axis 2 corresponds to a south to north axis (small to large values). Length and orientation of arrows indicated how correlated species are to a particular axis. A long arrow that is parallel to a particular CCA axis indicates a strong correlation.

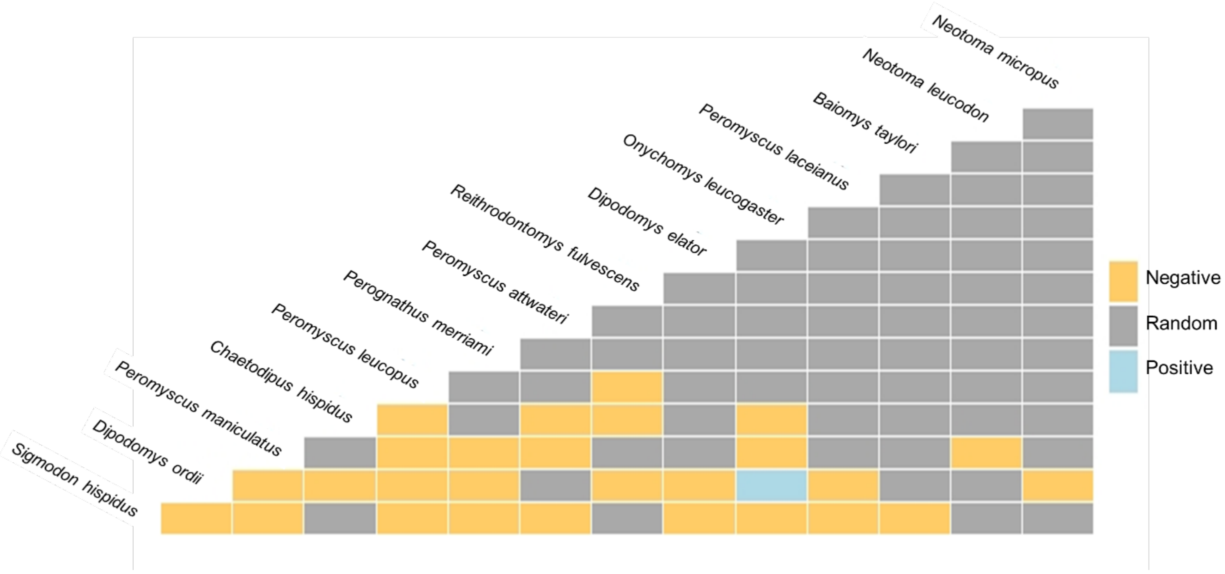

Figure 4. Species co-occurrence matrix from the road survey sites, excluding Hall County, for which there were significant positive, negative, or random associations based on the probabilistic model of species co-occurrence.

Table 1a. Species list from the road surveys indicating the total number of individuals captured, number of sites each species was captured at, the average number of individuals captured per site at sites where the species was present (i.e., not the total number of sites), and the number of sites at which a species co-occurred with *D. elator*.

Species	**Total Individuals**	**Number of sites**	**Abundance per site**	**Co-occurrences w/ TKR**
Baiomys taylori	7	6	1.17	0
Chaetodipus hispidus	182	134	1.36	4
Dipodomys elator	35	26	1.35	--
Dipodomys ordii	210	119	1.76	1
Neotoma leucodon	9	8	1.13	0
Neotoma micropus	8	7	1.14	0
Onychomys leucogaster	37	27	1.37	0
Perognathus merriami	38	32	1.19	0
Peromyscus attwaterii	3	3	1.00	0
Peromyscus laceianus	2	2	1.00	1
Peromyscus leucopus	116	85	1.36	6
Peromyscus maniculatus	159	99	1.61	6
Reithrodontomys fulvescens	13	13	1.00	1
Sigmodon hispidus	318	154	2.06	4

Table 1b. Number of individuals trapped per species from the Hall County road surveys.

Species	Individuals
Peromyscus spp.	45
Dipodomys ordii	28
Mus musculus	2
Perognathus subflavus	2
Reithrodontomys spp.	1
Sigmodon hispidus	1
Chaetodipus hispidus	1
Neotoma spp.	1
Onychomys leucogaster	1
Baiomys taylori	1
Total	**83**

Table 2. Site, latitude, and longitude, respectively, of all road survey sites. Sites in **bold** with an asterisk (*) indicate locations where we encountered at least one *D. elator* individual.

Site	Latitude	Longitude
1	34.02203	-98.76653
2	34.02760	-98.76653
3	34.06727	-98.73150
4	34.07655	-98.73135
5*	**34.10013**	**-98.77162**
6	34.10622	-98.77150
7	34.12032	-98.77873
8*	**34.11943**	**-98.77187**
9	34.08108	-98.73128
10	34.11822	-100.29883
11	34.11909	-100.34380
12	34.12275	-100.33345
13	34.12952	-100.33338
14*	**34.05025**	**-100.21543**
15	34.05977	-100.21773
16	34.08953	-100.09422
17	34.08708	-100.02778
18	34.08913	-100.02912
19	34.10170	-100.01205
20	34.01615	-100.28315
21	34.01542	-100.26940
22	34.01520	-100.25298
23	34.01098	-100.24788
24	34.08513	-99.73113
25	34.08520	-99.73852
26	34.07793	-99.74582
27	34.06033	-99.74947
28	34.04168	-99.75167
29	34.05457	-99.67980
30	34.03467	-99.67990
31	34.01713	-99.68025
32	34.01195	-99.65225
33	34.00560	-99.64560
34	34.22622	-99.73922
35	34.22598	-99.73447
36	34.22555	-99.71632
37	34.22490	-99.68355
38	34.22465	-99.67138
39	34.22437	-99.65612
40	34.22408	-99.63918
41	34.21320	-99.61188
42	34.21157	-99.73695
43	34.21122	-99.71927
44	34.21075	-99.69760
45	34.21012	-99.66950
46	34.19242	-99.61255
47	34.18365	-99.61277
48	34.17960	-99.59098
49	34.17930	-99.57767
50	34.18065	-99.57407
51	34.18053	-99.56970
52	34.18042	-99.56457
53	34.18032	-99.55970
54	34.19343	-99.57568
55	34.18692	-99.57583
56	34.18172	-99.57597
57	34.17802	-99.57610
58	34.17268	-99.57633
59	34.16622	-99.57650
60	34.16390	-99.57717
61	34.15525	-99.57740
62	34.17678	-99.55417
63	34.17745	-99.55112
64	34.17577	-99.54692
65	34.17228	-99.54585
66	34.16793	-99.54457
67	34.15822	-99.54170
68	34.15505	-99.54078
69	34.15417	-99.52278
70	34.15602	-99.52330
71	34.16005	-99.52443
72	34.16985	-99.52718
73	34.17403	-99.52843
74	34.17625	-99.52912
75	34.17970	-99.53013
76	34.18275	-99.52573
77	34.16925	-99.51865
78	34.16633	-99.53295
79	34.35680	-99.59585
80	34.35810	-99.57067
81	34.36357	-99.57057
82	34.37163	-99.57253
83	34.37915	-99.57235
84	34.38537	-99.56830
85	34.38518	-99.55957
86	34.38507	-99.55183
87	34.38500	-99.54662
88	34.38488	-99.53833
89	34.38450	-99.51525
90	34.38443	-99.50978
91	34.37975	-99.50845
92	34.36598	-99.50853
93	34.32568	-99.52787
94	34.32573	-99.53088
95	34.32653	-99.56158
96	34.32662	-99.56955
97	34.08690	-99.44097
98	34.08952	-99.43137
99	34.09035	-99.42850
100	34.09180	-99.42307
101	34.09470	-99.41257
102	34.09800	-99.41040
103	34.04028	-99.37987
104	34.03487	-99.37777
105	34.03180	-99.37575
106	34.01895	-99.38205
107	34.01807	-99.38572
108	34.01643	-99.38882
109	34.01308	-99.38747
110	34.00462	-99.38434
111	34.07048	-99.46188
112	34.07243	-99.45467
113	34.07500	-99.44860
114	34.07348	-99.44800
115	34.07033	-99.44677
116	34.06677	-99.44540
117	34.06270	-99.44383
118	34.05608	-99.44118
119	34.05228	-99.43968

120	34.04778	-99.43793
121	34.04450	-99.43665
122	34.03845	-99.43425
123	34.02763	-99.42998
124	34.00808	-99.42182
125	34.00932	-99.41703
126	34.01107	-99.41042
127	34.01203	-99.40743
128	34.01327	-99.40280
129	33.61823	-99.35788
130	33.60985	-99.37710
131	33.62182	-99.37488
132	33.62558	-99.37415
133	33.63467	-99.37250
134	33.63827	-99.37183
135	33.64843	-99.36995
136	33.61816	-99.39347
137	33.62585	-99.26047
138	33.62505	-99.24607
139	33.59797	-99.23367
140	33.61050	-99.23270
141	33.61047	-99.22905
142	33.61023	-99.21713
143	33.61030	-99.20638
144	33.61023	-99.19502
145	33.61030	-99.17890
146	33.61035	-99.17327
147	33.61038	-99.16897
148	33.61037	-99.16270
149	34.03345	-98.65915
150	34.03912	-98.65918
151	34.04235	-98.65923
152	34.04763	-98.65618
153	34.05182	-98.65378
154	34.05542	-98.65375
155	34.06548	-98.65370
156	34.07420	-98.65318
157	34.07442	-98.64962
158	34.07035	-98.63232
159	34.05975	-98.63235
160	34.05065	-98.62942
161	34.04913	-98.63042
162	34.04775	-98.63998
163	34.09655	-98.82822
164	34.10125	-98.82818
165	34.10522	-98.82918
166	34.10533	-98.83737
167	34.10558	-98.86192
168	34.10563	-98.86655
169	34.13495	-98.89187
170	34.13512	-98.90852
171	34.13520	-98.91798
172*	**34.13527**	**-98.92615**
173*	**34.12080**	**-98.93023**
174	34.12035	-98.88507
175	34.08660	-98.84595
176	34.08323	-98.84607
177*	**34.07275**	**-98.85423**
178*	**34.07283**	**-98.86272**
179*	**34.05812**	**-98.87228**
180*	**34.04430**	**-98.87970**
181	34.01997	-98.87118
182	33.69212	-98.68035
183	33.69870	-98.68638
184	33.74605	-98.68728
185	33.74602	-98.68493
186	33.71322	-98.68503
187	33.71372	-98.63333
188	33.71458	-98.67745
189	33.74590	-98.67733
190	33.74582	-98.67288
191	33.74565	-98.66260
192	33.75570	-98.62912
193	33.78355	-98.60445
194	33.74540	-98.64703
195	33.75848	-98.68793
196	33.96618	-98.90267
197	33.96625	-98.89193
198	33.96623	-98.89073
199	33.86673	-98.70555
200	33.83345	-98.70522
201	33.82500	-98.32290
202	33.82130	-98.29480
203	33.95690	-98.24440
204	33.95880	-98.25940
205	33.94660	-98.32690
206	33.93600	-98.32720
207	33.92970	-98.32720
208	33.97340	-98.34560
209	34.00140	-98.33750
210	33.99960	-98.33110
211	33.99960	-98.32730
212	33.99950	-98.32660
213	34.09780	-98.38550
214	34.09770	-98.37870
215	34.10800	-98.34930
216	34.11200	-98.34920
217	34.10990	-98.33340
218	34.08950	-98.33290
219	34.11500	-98.32720
220	34.09830	-98.30550
221	34.09960	-98.26610
222	34.08490	-98.24700
223	33.92530	-98.10010
224	33.92530	-98.09790
225	33.93350	-98.08810
226	33.95110	-98.06320
227	33.95080	-98.02380
228	33.97210	-97.99970
229	33.98250	-97.99960
230	33.97120	-98.01720
231	33.97390	-98.01720
232	33.97700	-98.01720
233	33.98200	-98.08680
234	33.96960	-98.08720
235	33.95560	-98.09800
236	33.95590	-98.13930
237	33.72190	-98.23180
238	33.62940	-98.26260
239	33.58760	-98.21580
240	33.65940	-98.21360
241	33.69670	-98.21000
242	33.65710	-98.30440
243	33.57330	-98.31810
244	33.52660	-98.30260
245	33.52580	-98.28710
246	33.50700	-98.28730
247	33.49610	-98.29970
248	33.50520	-98.32400

249	33.79080	-98.19250
250	33.78810	-98.18230
251	33.78040	-98.18240
252	33.76550	-98.18260
253	33.72360	-98.18320
254	33.71300	-98.18550
255	33.70860	-98.19220
256	33.69610	-98.19270
257	33.69300	-98.19280
258	33.68730	-98.16740
259	33.66570	-98.15980
260	33.66020	-98.10510
261	33.59840	-97.99180
262	33.57600	-97.99540
263	33.58020	-98.00490
264	33.59810	-98.01640
265	33.76080	-98.12400
266	33.81050	-98.12400
267	33.77770	-98.07080
268	33.75490	-98.03780
269	33.68970	-98.04840
270	33.68570	-98.06020
271	33.67500	-97.99120
272	33.67520	-97.99140
273	33.66220	-97.99140
274	33.65800	-97.99140
275	33.67620	-97.95140
276	33.67170	-97.96300
277	33.97293	-99.73325
278	33.96925	-99.73883
279	33.96927	-99.75830
280	33.96953	-99.76633
281	33.96945	-99.78188
282	33.96295	-99.76815
283	33.92072	-99.88222
284	33.91600	-99.89655
285	33.90775	-99.90052
286	33.87657	-99.92700
287	33.88117	-99.91967
288	33.87547	-99.90030
289	34.25520	-99.39290
290	34.25250	-99.40600
291	34.25030	-99.40840
292	34.23010	-99.40240
293	34.30410	-99.40210
294	34.30650	-99.39260
295	34.32380	-99.39210
296	34.32850	-99.39380
297	34.33600	-99.39650
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303	34.30360	-99.37050
304	34.29230	-99.34910
305	34.30120	-99.35180
306	34.30450	-99.35270
307	34.30930	-99.35420
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311	34.38660	-100.14410
312	34.36380	-100.15380
313	34.37300	-100.16230
314	34.38020	-100.16020
315	34.38300	-100.16190
316	34.40090	-100.16120
317	34.39180	-100.17910
318	34.37340	-100.17920
319	34.36590	-100.17020
320	34.36580	-100.16690
321	34.14230	-99.21550
322	34.13080	-99.19150
323	34.13750	-99.19420
324	34.14140	-99.17730
325	34.14700	-99.17960
326	34.15130	-99.18120
327	34.16180	-99.18540
328	34.17330	-99.15330
329	34.16230	-99.14900
330	34.10620	-99.13040
331	34.10170	-99.12330
332	34.10720	-99.09730
333	34.12750	-99.06510
334	34.13060	-99.06950
335	34.14180	-99.07400
336	34.33042	-99.47107
337	34.33363	-99.47237
338	34.33692	-99.47357
339	34.34057	-99.47492
340	34.34933	-99.47665
341	34.35285	-99.47650
342	34.35435	-99.46663
343	34.35412	-99.45323
344	34.35423	-99.46223
345	34.35488	-99.44483
346	34.31097	-99.42585
347	34.30447	-99.42347
348	34.30093	-99.42223
349	34.29957	-99.41943
350	34.30093	-99.41412
351	34.30202	-99.41040
352	34.30282	-99.40707
353	34.30397	-99.40252
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355	34.27742	-99.42615
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358	34.44790	-99.95230
359	34.44790	-99.94850
360	34.44790	-99.94590
361	34.44760	-99.93020
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363	34.46120	-99.88410
364	34.46110	-99.87290
365	34.46090	-99.86660
366	34.46080	-99.86180
367	34.46230	-99.85610
368	34.46220	-99.85090
369	34.46220	-99.85180
370	34.46800	-99.84310
371	34.46810	-99.83980
372	34.46810	-99.83480
373	34.46460	-99.82920
374	34.46400	-99.82110
375	34.45170	-99.82220
376	34.12772	-100.31601
377	34.13462	-100.31602

378	34.14061	-100.31925
379	34.14063	-100.32499
380	34.14059	-100.33213
381	34.14601	-100.33359
382	34.15390	-100.33366
383	34.16296	-100.33263
384	34.16850	-100.33249
385	34.17202	-100.33065
386	34.17540	-100.32349
387	34.17810	-100.31808
388	34.17798	-100.31293
389	34.17071	-100.30749
390	34.16799	-100.30608
391	34.14694	-100.30317
392	34.14065	-100.30337
393	34.14064	-100.30654
394	34.14062	-100.31292
395	34.12262	-100.31599
396	33.90335	-100.18301
397	33.90680	-100.18300
398	33.92100	-100.18290
399	33.95770	-100.18280
400	33.96150	-100.19520
401	33.95200	-100.20020
402	33.94370	-100.20030
403	33.93600	-100.20030
404	33.93090	-100.20040
405	33.91910	-100.20050
406	33.91590	-100.20060
407	33.91160	-100.20060
408	33.90990	-100.20060
409	33.90290	-100.20100
410	33.94510	-100.23960
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462	33.73165	-97.86166
463	33.72773	-97.86209
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475	33.89067	-97.74693
476	33.92296	-97.73221
477	33.93341	-97.71603
478	33.97284	-97.67609
479	33.85575	-97.96666
480	33.82327	-97.49871
481	33.83332	-97.50803
482	33.84663	-97.53197
483	33.84129	-97.53689
484	33.80939	-97.55213
485	33.78952	-97.55729
486	33.77555	-97.53152
487	33.75996	-97.53391
488	33.76418	-97.56209
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502	33.53694	-97.64809
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515	33.74053	-98.74340
516	33.72834	-98.74516
517	33.71282	-98.80552
518	33.71915	-98.80553
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520	33.73056	-98.78150
521	33.69684	-98.85384
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524	33.73032	-98.76693
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529	33.43650	-98.86087
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539	33.48420	-98.91438
540	33.48667	-98.92310
541	33.49615	-98.89861
542	33.49615	-98.87809
543	33.46807	-98.86978
544	33.46929	-98.84986
545	33.47783	-98.81753
546	33.54565	-98.55412
547	33.53366	-98.54374
548	33.52710	-98.54385
549	33.51447	-98.54446
550	33.50217	-98.54458
551	33.43032	-98.54504
552	33.42478	-98.54557
553	33.42477	-98.58188
554	33.42477	-98.58805
555	33.42068	-98.59914
556	33.38300	-98.60949
557	33.39835	-98.53812
558	33.43475	-98.58598
559	33.44998	-98.59062
560	33.45821	-98.59940
561	33.46269	-98.60232
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563	34.44620	-100.00172
564	34.43624	-100.00207
565	34.35066	-100.23408
566	34.35094	-100.25298
567	34.33410	-100.33492
568	34.32789	-100.33514
569	34.32739	-100.39283
570	34.34968	-100.35201
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572	34.36513	-100.35144
573	34.36834	-100.34795
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578	34.44986	-100.07169
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587	34.48361	-100.03542
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589	34.49354	-100.04228
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591	34.42382	-100.15521
592	34.42450	-100.16029
593	34.46052	-100.16921
594	34.44167	-100.17723
595	34.43289	-100.17763
596	34.42134	-100.17803
597	34.44127	-100.03693
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599	34.38390	-99.98618
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608	34.46263	-99.98365
609	34.48049	-99.98297
610	34.48724	-99.98274
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612	34.31833	-100.00694
613	34.31858	-100.01797
614	34.31886	-100.03603
615	34.31927	-100.05364
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617	34.42028	-100.01643
618	34.39957	-100.00327
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623	34.48384	-100.07044
624	34.47578	-100.07076
625	33.99012	-100.77515
626	33.98288	-100.77513
627	33.98028	-100.73647
628	33.97628	-100.72163
629	33.96516	-100.69711
630	33.96518	-100.68787
631	33.95154	-100.68610
632	33.93521	-100.68822
633	33.90735	-100.71137
634	33.90333	-100.72145
635	33.94075	-100.79316

636	34.07644	-100.88359	679	34.29904	-100.09450	722	33.51973	-99.21432
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638	34.08186	-100.91565	681	34.29815	-100.05717	724	33.53500	-99.19821
639	34.09938	-100.90710	682	34.30424	-100.02876	725	33.55621	-99.18018
640	34.20611	-101.02000	683	34.30116	-100.00227	726	33.56552	-99.17721
641	34.14361	-101.02167	684	34.42413	-100.09013	727	33.56364	-99.15662
642	34.08861	-101.02444	685	34.43222	-100.14242	728	33.56370	-99.14285
643	34.28557	-100.03802	686	34.42638	-100.14276	729	33.56779	-99.18045
644	34.25730	-99.83467	687	34.28949	-100.90363	730	33.54815	-99.18066
645	34.25783	-99.86665	688	34.28963	-100.91701	731	33.53682	-99.18077
646	34.27317	-99.89378	689	34.29015	-100.93355	732	33.56286	-99.32411
647	34.27352	-99.91235	690	34.29247	-100.94368	733	33.55997	-99.34868
648	34.27393	-99.93267	691	34.29237	-100.95789	734	33.56258	-99.36842
649	34.26670	-99.94587	692	34.30891	-100.97717	735	33.56436	-99.38210
650	34.26710	-99.97323	693	34.25211	-100.98474	736	33.57344	-99.45098
651	34.31777	-99.90593	694	34.24881	-100.96819	737	33.57415	-99.45614
652	34.31862	-99.95078	695	34.25359	-100.93769	738	33.57318	-99.47376
653	34.31892	-99.97675	696	34.23755	-100.97762	739	33.56779	-99.47469
654	34.31902	-99.98847	697	34.24478	-100.99374	740	33.53357	-99.47050
655	34.29847	-99.97905	698	34.24477	-101.00391	741	33.53432	-99.45605
656	34.31322	-99.94355	699	34.24835	-101.01938	742	33.53410	-99.41124
657	34.29808	-99.91672	700	34.24619	-101.02512	743	33.53531	-99.28427
658	34.32972	-99.89860	701	34.21739	-101.00772	744	34.17780	-99.00060
659	34.41270	-100.39360	702	34.21576	-100.99765	745	34.16090	-99.00270
660	34.41660	-100.39370	703	34.21930	-100.99023	746	34.12640	-98.99860
661	34.42020	-100.39390	704	33.99706	-99.52837	**747***	**34.13560**	**-98.95470**
662	34.42600	-100.39380	705	34.01757	-99.53639	748	34.06230	-99.01860
663	34.43110	-100.39360	706	34.02637	-99.53980	749	34.05440	-99.02040
664	34.43670	-100.38830	707	34.03110	-99.54163	**750***	**34.04170**	**-99.01660**
665	34.43660	-100.38400	708	34.03981	-99.54503	751	34.03020	-99.01310
666	34.43720	-100.37600	709	34.05783	-99.49708	752	34.28351	-99.64156
667	34.44370	-100.37580	710	34.04628	-99.49474	753	34.12429	-99.08088
668	34.45750	-100.37410	711	34.04293	-99.50706	754	34.12170	-99.09316
669	34.45640	-100.36540	712	34.04131	-99.51324	755	34.10033	-99.13041
670	34.44700	-100.34830	713	34.03954	-99.51960	756	34.08892	-99.14311
671	34.26891	-100.05006	714	34.02504	-99.52093	757	34.06675	-99.13629
672	34.26438	-100.04704	715	34.01279	-99.51624	758	34.05647	-99.24125
673	34.27102	-100.02021	716	33.57798	-99.13424	759	34.04873	-99.23264
674	34.29931	-100.00221	717	33.58045	-99.17157	760	34.05458	-99.22270
675	34.31883	-100.03203	718	33.58027	-99.20170	761	34.07224	-99.22791
676	34.31885	-100.03617	719	33.55062	-99.23920	762	34.14828	-99.72898
677	34.29929	-100.04037	720	33.54940	-99.21609	763	34.14810	-99.72386
678	34.29274	-100.09502	721	33.53068	-99.21445	764	34.14806	-99.71574

765	34.14237	-99.67795	780	34.26390	-99.57362	795	34.19218	-99.39089
766	34.14465	-99.66154	781	34.27821	-99.57321	796	34.21091	-99.39650
767	34.21193	-99.74681	782	34.29185	-99.57088	797	34.21011	-99.55781
768	34.20513	-99.75835	783	34.24570	-99.28638	798	34.24043	-99.55676
769	34.19746	-99.75428	**784***	**34.23764**	**-99.33315**	799	34.24368	-99.55666
770	34.18272	-99.73609	785	34.23138	-99.36361	800	34.16721	-99.52664
771	34.18266	-99.73060	**786***	**34.22896**	**-99.37510**	801	34.15888	-99.52432
772	34.16055	-99.14831	787	34.33890	-99.38068	802	34.13036	-99.55157
773	34.16369	-99.14951	788	34.26014	-99.35739	**803***	**34.13356**	**-99.55254**
774	34.11201	-99.05579	789	34.21406	-99.33821	804	34.19776	-99.57571
775	34.10860	-99.05478	790	34.20115	-99.29849	805	34.20755	-99.57541
776*	**34.09517**	**-99.00391**	791	34.20250	-99.31189	806	34.21138	-99.57532
777*	**34.09680**	**-98.99613**	792	34.17360	-99.38528	**807***	**34.08338**	**-98.73146**
778	34.09764	-98.99201	793	34.15962	-99.38108	**808***	**34.05272**	**-98.71546**
779	34.28244	-99.59676	794	34.18313	-99.38818			

CHAPTER IV: PREDICTING THE PRESENT AND FUTURE DISTRIBUTION OF THE TEXAS KANGAROO RAT

EXECUTIVE SUMMARY

For rare species such as *D. elator*, identifying present-day distribution can be important for better understanding their conservation status. Species distribution modeling is a technique that produces predictive models for the probability of occurrence of a focal species at unsampled sites based on habitat characteristics of sites of documented presence. We used Maxent to build historical, present-day, and future species distribution models for *D. elator*. The historical model was generated using all known museum records for *D. elator*. The present-day distribution model was created based on occurrence records from our field surveys between 2015-2017, whereas the future distribution model was created using the present-day occurrence records but with future climate projections. For the present-day potential distribution of *D. elator*, we found that most suitable habitat is present in Wichita and Wilbarger Counties. Moreover, little to no suitable habitat was predicted in Archer, Baylor, Motley, Clay, and Montague Counties, all counties where the species has been previously documented, whereas the model suggests suitable habitat could exist in two previously undocumented counties: King and Dickens. This suggests that future surveys in these two additional counties may be worthwhile for accurately identifying the present distribution of *D. elator*.

Main takeaways: 1) Soil texture is an important predictor of the present-day distribution of the species; 2) many of the counties where *D. elator* was previously encountered presently appear to lack suitable habitat conditions; 3) the present and future niche models suggest that Wichita and Wilbarger, and potentially Cottle, may be the most important areas for prioritizing conservation and management efforts.

INTRODUCTION

Understanding species distributions is essential to ecology, evolution, and conservation biology (Guisan and Thuiller 2005, Elith and Leathwick 2009). For example, in light of the planet’s growing biodiversity crisis (Ceballos et al. 2015), range size has become one of the most important characteristics for assessing the vulnerability of species to environmental change (Lee and Jetz 2011). Developing effective conservation strategies therefore requires an understanding of species distributions and habitat associations (Kremen et al. 2008, Hochachka et al. 2012), because effective conservation plans require accurate estimates of the spatial distributions of the species they are trying to protect.

Species distribution models can be useful in this regard to both estimate habitat suitability or quality and test hypotheses about the relationship between a species and its abiotic and biotic environment (Peterson et al. 2011). Distribution models use measures of climate, soils, and vegetation at the precise locations of presence of a species and combines this information with GIS (Geographic Information Systems) layers of the same kinds of information but over extensive geographic areas to predict unsampled areas of probable occurrence (Franklin 2010). In doing so, species distribution models are becoming an indispensable tool to conservation planning (Guisan and Zimmermann 2000, Loiselle et al. 2003). For rare and/or threatened or endangered species, these models can be particularly useful for assisting in identifying previously unknown populations, guiding additional surveys, informing selection and management of areas to protect or manage, and even predicting how the species may shift its distribution in the future (Graham et al. 2004, Hijmans and Graham 2006).

Here, we estimated the past, present, and future potential distribution of *D. elator*. Despite its threatened status, more than 500 museum specimens have been collected that represent a valuable tool for documenting the historical potential distribution of this species. Despite this, the distribution of *D. elator* is not well known and there is some indication that it may be changing. New records that expand the distribution of Texas kangaroo rats are still being published (Martin and Matocha 1991, Martin 2002), while at the same time a number of investigators have returned to sites found to be inhabited by *D. elator* during the earlier part of the 20th century and failed to encounter any (Martin and Matocha 1972, Baumgardner 1987, Moss and Mehlhop-Cifelli 1990). Given the uncertainty surrounding distribution of *D. elator*, and the importance of accurate estimates for informing conservation considerations, we created distribution models for *D. elator* to 1) estimate the present-day geographic extent of this species, 2) identify the degree to which the distribution has changed over time, and 3) predict how the distribution may change in the future in response to climate change.

METHODS

Advances in species distribution modeling has generated new techniques and ideas about best practices for the methodology. Accordingly, after producing an initial prediction of the present-day distribution of *D. elator*, we then provided an updated prediction with refined methodology (Fig. 2), and then ultimately one in which we incorporated soil texture as an additional predictor variable in addition to the refined methodology (Fig. 3). Soil texture proved to be an important addition to the model (Table 1). Although the particular details of these updates will be provided within the Methods section, along with appropriate justification, we report the general changes here. All of the refinements that have been made have been done to better reflect ecology of the species. Specifically, we have 1) changed the area of extent over which the model was created from the entire state of Texas to a much more ecologically-meaningful extent, 2) incorporated land cover and soil as predictor variables to better characterize the distribution of the species, 3) removed highly-correlated predictor variables to reduce the potential for overfitting, and 4) thinned the occurrence data used in the model to reduce spatial bias.

We used the software package Maxent, a presence-background model, to model the present-day potential distribution of *D. elator*. Maxent uses a maximum entropy approach to estimate the most uniform distribution of a species' occurrence across the study area, minimally constrained by the provided environmental data (Phillips et al. 2006). Importantly, Elith et al. (2011) showed that Maxent performs well with sparse datasets (e.g., rare species), relative to other modeling approaches.

Present-day potential distribution

We modeled the present-day potential distribution of *D. elator* using 26 occurrence records obtained via county road surveys conducted between 2015-2017 across the historical geographic range of the species. Martin and Matocha (1972) and Jones et al. (1988) demonstrated that, because *D. elator* can be very active on unpaved county roads at night, road surveys are an effective means of discovering sites of occurrence of this species. We used SDM Toolbox v. 2.2 (Brown et al. 2017) in ArcMap 10.5.1 to remove points within 1 kilometer of each other, reducing the dataset from an initial 44 occurrence points to the 26 described above. Given the importance of choice of study extent (Barve et al. 2011), we limited our area to a buffer of 100 km from all occurrence records (Bean et al. 2014), with the Red River providing a natural cutoff to the north since *D. elator* is believed to be extirpated from its previous geographic range in Oklahoma (Martin 2002, Oklahoma Department of Wildlife Conservation 2016).

We considered 21 environmental variables as potential predictors of the present-day distribution of *D. elator*. Nineteen bioclimatic variables were obtained from the WorldClim database at a 1-km^2 spatial resolution (http://www.worldclim.org/bioclim.htm). These climatic variables (Hijmans et al. 2005) are frequently used in distribution modeling as independent variables (Hijmans and Graham 2006). Soil texture class data (e.g., sandy loam, clay loam, sand; see Chapter 2, Fig. 4) was obtained from the CONUS-SOIL database at 1-km^2 resolution. Land cover data from 2011 was obtained from the National Land Cover Database at 30 m^2 resolution. Because the climate layers were the coarsest resolution layers (i.e., ~1 km^2), the land cover data was aggregated to match this resolution.

To reduce redundancy in environmental variables, we used SDM Toolbox v. 2.2 (Brown et al. 2017) in ArcMap 10.5.1 to assemble a correlation matrix for the 19 bioclimatic variables across our spatial extent of analysis. We retained only a single variable for variables that were correlated at $r > 0.9$, using Bean et al. (2014) as a guide to determine which variables to retain for the model. This procedure reduced the initial dataset to 11 variables (i.e., 9 bioclimatic variables, land cover, and soil). Of the bioclimatic variables, we retained annual mean temperature (BIO1), Minimum temperature of the coldest month (BIO6), mean temperature of the warmest quarter (BIO10), annual precipitation (BIO12), precipitation of wettest month (BIO13), precipitation of driest month (BIO14), precipitation of driest quarter (BIO17), precipitation of warmest quarter (BIO18), and precipitation of coldest quarter (BIO19). Reducing the number of variables to those considered ecologically relevant and nonredundant makes hypothesis testing and interpretation of results more straightforward (Elith et al. 2011) and decreases the potential for overfitting (Warren and Seifert 2011).

Validation is necessary to assess the predictive performance of the model. Ideally, an independent dataset should be used for validation; however, given the nature of our study an independent dataset is not available. Consequently, we followed the most commonly-used approach, which is to partition the data randomly into training and testing sets, thus creating quasi-independent data for validation of models (Guisan and Thuiller 2005). We used 80% of the data points to build the model and the remaining 20% of the data points for model validation. To evaluate model performance, we used a receiver-operating-characteristic (ROC) analysis, which plots sensitivity (y-axis, ommission error) against 1-specificity (x-axis, commission error). Omission error is defined as known presences that are predicted absent, and commission error is defined as locations predicted suitable for which no presences are known. The area under the ROC curve (AUC), which is an indicator of model prediction accuracy, was calculated. The analysis was conducted for the testing dataset (20% of the data points) to assess the average performance of the resulting models with a fixed threshold of 0.10 (10% omission error), which rejects the lowest 10% of possible predicted values.

RESULTS

The ultimate present-day distribution model created in Maxent (Fig. 3), which incorporated soil as a predictor variable, produced an Area Under the Curve (AUC) of 0.94, indicating that the model has good predictive ability. All other models similarly produced AUCs above 0.9. In the present-day prediction, most suitable habitat was predicted to occur in northern Wichita County and northeastern Wilbarger County, as well as central and south-central Cottle County (Fig. 3). Soil texture, annual mean temperature (BIO1), minimum temperature of the coldest month (BIO6), land cover, precipitation of the driest month (BIO14), and mean temperature of the warmest quarter (BIO10) were the variables that contributed most to the prediction in the model (Table 1).

In contrast, the historical distribution model predicted relatively high probability of habitat suitability in two areas: Wichita and Wilbarger Counties, as well as Hardeman and Foard Counties (Fig. 1). The future distribution model predicts a single relatively high probability of habitat suitability in Wichita and Wilbarger Counties (Fig. 5).

DISCUSSION

The results of our past, present, and future distribution models for *D. elator* highlight a number of important things. Below, we discuss a number of items related to the present-day distribution model, including the geographic arrangement of suitable habitat, variable importance, and future directions.

In the updated present-day distribution model, it appears that there is little to no suitable habitat in five of the 11 counties from where *D. elator* has been documented (i.e., Archer, Baylor, Clay, Montague, and Motley; Fig. 3), a distinct contrast with the results of the historical potential distribution (Fig. 1). However, it is important to note the presence of present-day suitable habitat in Cottle County. From a management standpoint, this suggests that conservation plans in these counties may be most practical, whereas restoration practices in Hardeman and potentially Foard Counties may be critical for trying to reconstruct the recent historical distribution of the species, especially in light of the findings in Weber et al. (2016) suggesting that habitat suitability determined by distribution models could correlate with the abundance of a species across its geographic range. Considering this species may already be abundance limited, this could have significant impacts on the future persistence and even population genetics of *D. elator*.

In the updated present-day model, soil texture was the most important predictor of *D. elator* distribution. This agrees with the findings from Chapter II of this report and provides more evidence, albeit at a larger scale. Given the limited availability of soil types in which we encountered *D. elator* during our surveys (Fig. 4B), especially relative to land cover types (4A), this suggests that soil may be an important limiting factor contributing to the overall rarity of this species. More specifically, this indicates that the distribution of loamy soils across the region may be an important predictor for the success of conservation practices. As discussed in earlier sections and in the concluding section on conservation recommendations, locations for habitat restoration and/or management practices (e.g., prescribed burns, woody shrub removal) should therefore likely be dictated by areas with suitable soil types in order to maximize their effectiveness.

Moreover, in the present-day distribution model, as with the historical (Fig. 1) and future (Fig. 5) models, a suite of climatic variables, both extremes (e.g., minimum temperature of the coldest month) and averages (e.g., annual mean temperature; Table 1), were important for predicting the distribution of *D. elator*. The impacts of climate change can be complex and have idiosyncratic effects on species' ranges (Parmesan 2006). This suggests that ongoing changes in climate could drive shifts in the distribution of *D. elator* due to climate-mediated shifts in habitat suitability. For example, due to the precipitation gradient that runs across the region, future climate shifts could create increasingly divergent habitat characteristics in the eastern and western subregions of the distribution if precipitation and temperature patterns were to become more variable. Indeed, results from our future distribution model suggest that suitable climate conditions may predominantly occur in the eastern portion of the region under future conditions

(Fig. 5). Given this potential, understanding interactions between climate and different habitat features, such as the role of soil texture in buffering against aboveground climate shifts, may be an important future line of research for this species and its future persistence.

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Figure 1. Map of species distribution model based on historical occurrence records of *D. elator* in Texas, Arkansas, Louisiana, Oklahoma, and New Mexico using climate and 2011 land cover data. Warm and more-red colors indicate areas of high probability of occurrence, whereas cool and more-blue areas indicate low probability of occurrence.

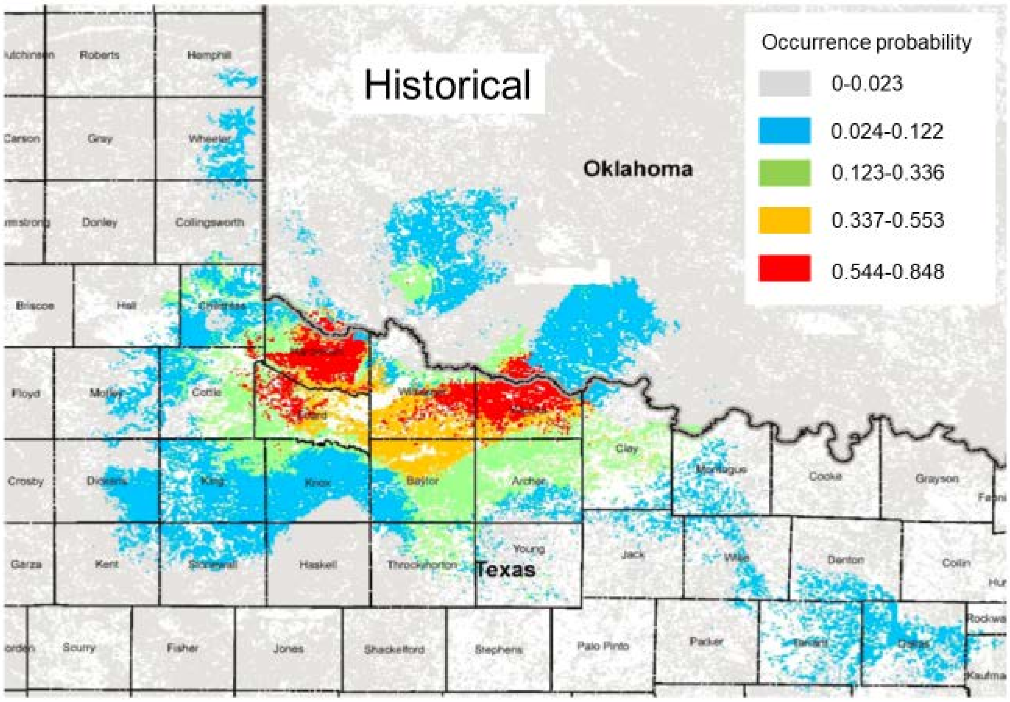

Figure 2. Preliminary present-day potential distribution for *D. elator* based on climate and land cover characteristics.

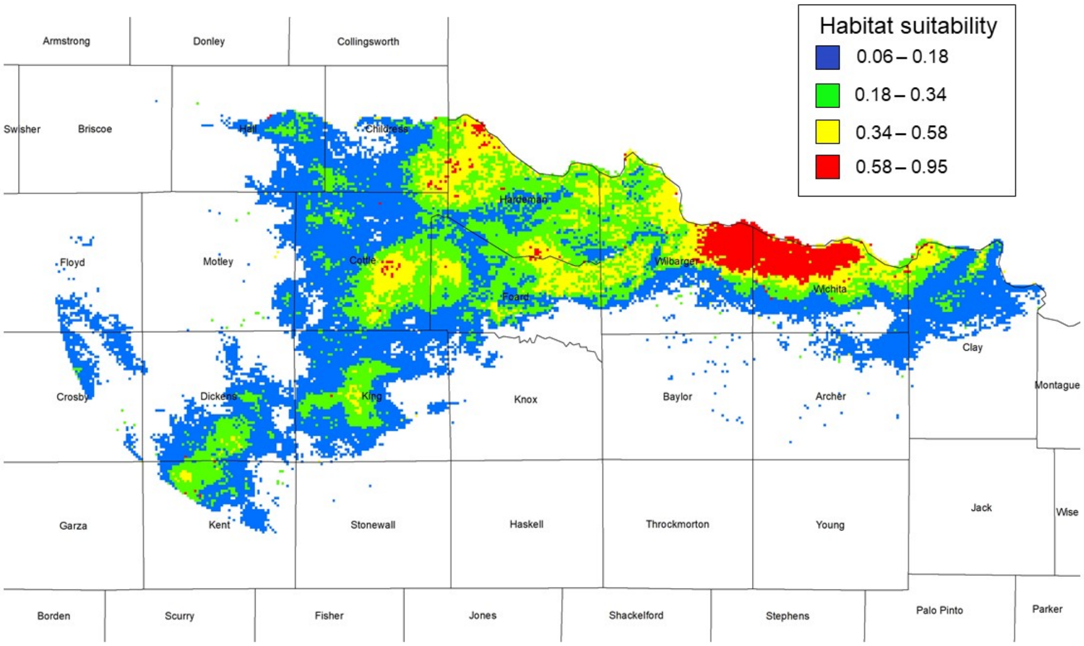

Figure 3. Updated present-day potential distribution for *D. elator* based on climate, land cover, and soil characteristics.

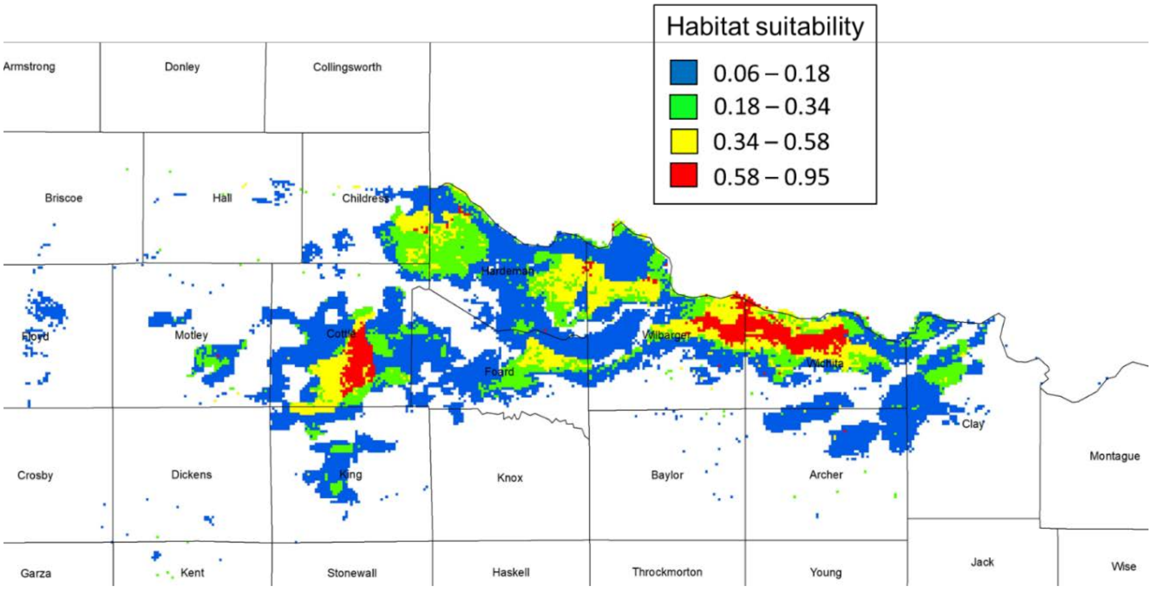

Figure 4. Maps of the geographic extent of a) land cover and b) soil types across the historical range of *D. elator* in which we encountered the species during road surveys.

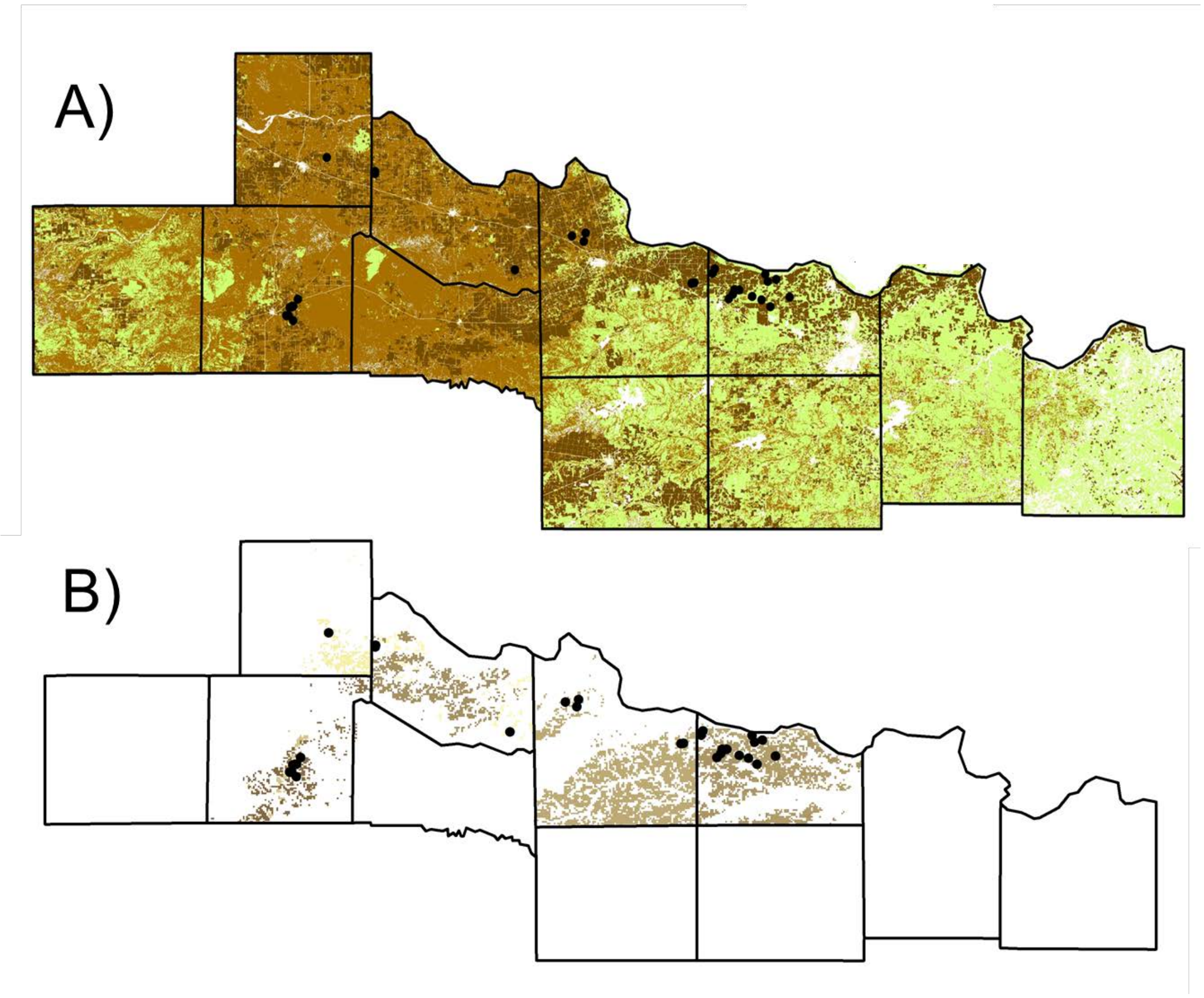

Figure 5. Map of potential future distribution of *D. elator* in Texas based on occurrence data obtained via field surveys and 2011 land cover data, as well as climate data that represent projections of future climate conditions.

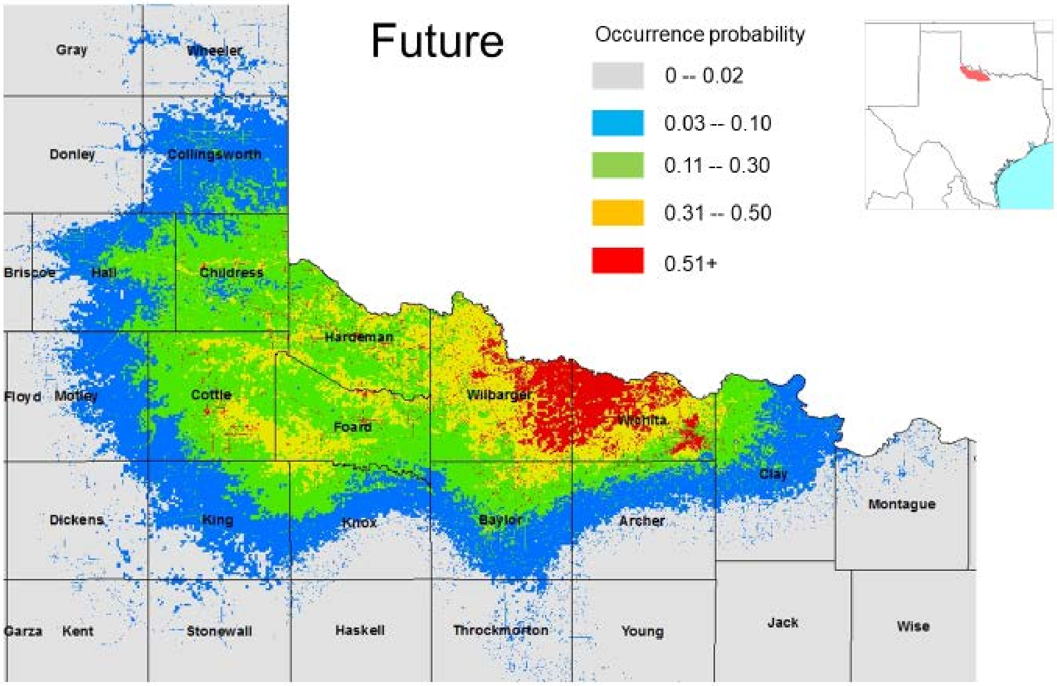

Table 1. Selected environmental variables and their percentage of contribution in the Maxent model for the predicted potential distribution of *D. elator* in Texas. Only variables with a relative contribution greater than five percent are included.

Environmental Variable	**Contribution (%)**
Soil Texture	39.8
Annual mean temperature	16.5
Min. temperature of coldest month	11.5
Land cover	9
Precipitation of driest month	8.6
Mean temperature of warmest quarter	7

CHAPTER V: POPULATION GENETICS OF *DIPODOMYS ELATOR*

EXECUTIVE SUMMARY

Investigations into the natural genetic variation of non-model organisms has increased since the boom of next generation molecular and sequencing techniques. Many studies take advantage of these tools in the context of “fresh” samples, and due to the high-quality DNA needed, decline to measure genetic variation in conspecific historical samples. *Dipodomys elator* is a rare kangaroo rat that is threatened in the state of Texas. Based on field surveys from the past 50 years, it appears that its distribution shifts over time. In the present paper, we analyze the genetic variation of 28 historical samples and 37 contemporary samples using 3RAD, a modified restriction site associated sequencing approach amenable to low input or degraded samples. We demonstrate that, currently, there are at least two subpopulations and contemporary populations deviate from Hardy-Weinberg equilibrium. These samples have an excess of heterozygotes which is symptomatic of small, isolated populations experiencing coalescence, though it is unclear how recently this has occurred. Because of this result, we are confident classifying the *D. elator* population as a metapopulation in the closed system of north-central Texas. Importantly, since subpopulations can vanish very rapidly, it is important for managers to pay close attention to the presence and genetic variability of the species for long-term population viability.

INTRODUCTION

Despite its arcane nature, measuring genetic variation within a species of concern has been integral to conservation efforts (Frankham et al. 2010). Accurately quantifying this population genetic variability has important implications for management of rare, threatened or endangered species. Moreover, use of population genetic summary statistics can be used to delimit management units based on significant allele frequencies (Moritz 1994), examine population structure (Rousset 2004), or assess connectivity of demographically disparate subpopulations (Slatkin 1987).

From a molecular standpoint, few markers work on highly degraded DNA samples (Bayona-Vásquez 2019). Conservation biologists have relied on both microsatellites and mitochondrial DNA (mtDNA). Microsatellites are popular because their use, even in small numbers, is informative, and mtDNA can achieve reciprocal monophyly more rapidly than nuclear markers (Avise et al. 1987). However, in organisms where very little is known about the genomic architecture, there is considerable preparation needed to prepare microsatellite primers. Additionally, differentiation in mtDNA does not necessarily reflect differentiation in the nuclear genome (Zink & Barrowclough 2008). Thus, an alternative to using microsatellites or mtDNA for studies in conservation is the utilization of next-generation sequencing (NGS).

Advances brought forth in the -*omics* era offers conservation biologists the unique ability to predict the evolutionary potential of a species from hundreds of polymorphic loci (Seeb et al. 2011). With NGS, researchers can get base pair data resolution for whole genomes. Additionally, more data can be generated from NGS, which can increase statistical power (Felsenstein 2005, Garner et al. 2016). Also, with NGS the tedious process of primer development is circumvented, and the process is more amenable to degraded DNA than microsatellites (Schlotterer 2004, Shafer et al. 2015). Thus, use of museum specimens and DNA collected from minimally invasive means can more easily be included in NGS datasets to further quantify the impacts of habitat fragmentation on gene flow in threatened species (Wandeler et al. 2007, Bradley et al. 2014).

Restriction site associated DNA sequencing or RAD-Seq was developed in 2008 for genetic mapping (Baird et al. 2008). Since then, it has been co-opted for use in studies of ecology and evolution, including conservation genetics (Peterson et al. 2012, Narum et al. 2013, Andrews et al. 2016). While there is a remarkable abundance of conservation-minded studies using either single RAD or ddRAD protocols, very few to our knowledge has implemented 3RAD, a variation of RAD-Seq that allows for reduced input of DNA (Graham 2015, Glenn 2016, Bayona-Vásquez 2019) as is typical in studies using historical specimens loaned from museums of natural history.

The Texas kangaroo rat (*Dipodomys elator*) is a seemingly rare heteromyid rodent that has a limited distribution in north-central Texas (Dalquest & Collier 1964, Baccus 1971, Martin & Matocha 1972, Carter et al. 1985, Martin & Matocha 1991). Though historically found in two counties in Oklahoma, it appears to have been extirpated from that state (Baumgardner 1987). Moreover, the Texas kangaroo rat has a small range sizes and low dispersal (Garner 1970, Stangl et al. 1992) which increases potential isolation from nearby subpopulations. The distribution of the Texas kangaroo rat appears dynamic (Nelson et al. 2009); for instance, though the species was described from a specimen in Clay county, it has not been spotted in that county in more recent surveys. Furthermore, the last population genetics analysis of *D. elator* published before 2019 was conducted in 1987 (Hamilton et al. 1987) making it critical to assess modern genetic variability. However, inquiry into genetic variation of the species should not only rely on contemporary samples differentiated by geographic space, but also on historical samples separated by time.

Here, we compare *D. elator* samples from two time periods (pre- and post-2000), and for contemporary samples, investigate differences in genetic diversity across the distribution. These samples allow us to make interesting predictions regarding genetic diversity within *D. elator*, and how new molecular techniques can make these types of inquiries feasible. Our historical samples were concentrated in one region of the distribution, which is currently the “middle” of the contemporary predicted distribution. Contrarily, our contemporary samples were taken from either side of this region (i.e. to the west and east). Because of such setup, we make several predictions regarding these samples: 1) there is no population subdivision in the historical samples, but there is subdivision in the contemporary samples; 2) there will be higher genetic diversity among contemporary samples, as they are taken from across the distribution, compared to the historical samples, all taken from a single county; 3) despite evidence of divergence, contemporary samples do not show low levels of genetic diversity, indicating that gene flow or other factors, has counteracted such separation.

METHODS

Collection of samples

To acquire samples necessary for our population genomics analysis, we live-trapped kangaroo rats using Sherman live traps (23x9x8 cm; Tallahassee, Florida) during road and habitat surveys within the historical range of *D. elator* from 2015 to 2017. When a *D. elator* individual was caught, it was either 1) taken as a voucher specimen for deposition into the Natural Science Research Laboratory at the Museum of Texas Tech University or 2) had between two to four whiskers extracted from each side of the rostrum (Halsey et al., *submitted*). We selected thicker whiskers (i.e., macrovibrissae) and kept the follicle intact. Whiskers were stored in a sterile vial with 1% sodium dodecyl sulfate (SDS) lysis buffer (Longmire et al. 1997).

Other methods of collecting DNA from rats included tail salvages and from toe clips from museum specimens. *D. elator* tail lengths average about 196 mm (Schmidly & Bradley 2016), and at times the end of the tail (i.e., the plume) would be severed by the door of an activated Sherman trap. These salvaged tail plumes were placed similarly in sterile vials of 1% sodium dodecyl sulfate (SDS) lysis buffer (Longmire et al. 1997). We also sampled toe clippings that had been collected from rats from 1986 to 1995 by Martin and Matocha. After initial population genetic analysis, we discovered a region within our sampling range where no individuals had been caught. We termed this area a "sampling hole," and is in Foard, Baylor and Wilbarger counties. To "fill" this hole, we have requested three *D. elator* toe clips from museum specimens Midwestern State University in Wichita Falls, Texas and three from the Southwestern Museum of Biology in Albuquerque, New Mexico to run genetic analysis. These samples were collected in the late 1960s and early 1970s. In total, we analyzed 71 *D. elator* samples from five tissue types (liver, whisker, tail, buccal swab and toe clips) and two time periods (late 20th century and our contemporary surveys from 2015 to 2017). Throughout the manuscript, we will refer to samples either being historical (prior to 2000) or contemporary (after 2000) and belonging to the western (Cottle, Childress and Hardeman counties) or eastern portion of the study region (Baylor, Wilbarger and Wichita counties). All sampling followed guidelines established by the American Society of Mammalogists (Sikes & Mammalogists 2016). These protocols were approved by the Institutional Animal Care and Use Committee at Texas Tech University (#T14083).

DNA extraction

We performed DNA extraction using the Qiagen DNeasy Blood and Tissue spin column protocol (Qiagen; Venlo, the Netherlands). For liver, toe clips and tail salvages, we followed the manufacturer's recommendations. For whisker and buccal swab samples, the following adjustments were implemented. For each whisker, the follicle was cut away from the whisker shaft using scissors cleaned with 50% bleach and 70% ethanol. We added 180 µL of the SDS lysis solution from each sample to a 1.5 mL microcentrifuge tube and 20 µL of proteinase k. After an incubation period of at least 8 hours, the remaining follicle (if present) was removed to avoid clogging the column during isolation.

For buccal swabs, approximately 20 µL of DNA was pipetted directly from the swab and placed in a microcentrifuge tube and 180 µL of the lysis solution of the sample was added. Also, we decreased the final elution volume from 200 µL to 100 µL in attempts to recover as much concentrated DNA as possible. DNA concentration was fluorometrically quantified using the Qubit 3.0, high sensitivity assay (Invitrogen, Life Technologies, Carlsbad, CA).

3RAD and data management

The 3RAD protocol is a variation of RAD-Seq that uses 3 restriction enzymes. Here, we used the following three restriction enzymes: EcoRI from the Ry13 strain of *Escherichia col*i, MSpI from *Moraxella* and ClaI from *Caryophanon latum*. We mixed 5 µl of genomic DNA per sample, restriction enzyme adapters, restriction enzymes, dH2O and NEB 10x CutSmart Buffer and incubated this mix at 37°C for 2 to 4 hours. Immediately following incubation, we added a ligation mix that joined DNA strands. The ligation mix was comprised of DNA ligase, rATP, ligase buffer and dH20. Samples were incubated at 22°C for 20 minutes, then at 37°C for 10 minutes. This process was repeated 3 times. Then samples were incubated at 80°C for 20 minutes, then were held at 10°C.

In the first PCR step, we used the Kapa HiFi DNA polymerase, Kapa HiFi buffer, dNTPs, iTru5 8N primer and linker ligated DNA fragments. Our volume for each sample was 50 µL. The thermocycler profile was 1 cycle of 98°C for 60 seconds, 60°C for 30 seconds, 72°C for

6 minutes and then held at 15°C. Reactions were purified with speedbeads and then resuspended with 33 µL of dH2O.

The second PCR step employed the same DNA polymerase and master-mix ingredients, but with a P5 primer and an iTru7 primer. We implemented the following thermocycler profile: 98°C for 40 seconds, then 6 cycles of 98°C for 20 seconds, 60°C for 15 seconds, 72°C for 30 seconds, followed by 72°C for 5 minutes. Samples were held at 15°C.

All samples were pooled, purified with speedbeads, and resuspended with 60 µL of dH2O. Liquid was removed from the beads using a magnet. Finally, samples were run on an agarose gel for visualization purposes and quantified using Qubit fluorometer. We conducted size selection (525 bp +/- 10%) using Pippen after normalization and pooling all samples. Samples were sent off for sequencing using TruSeq sequencing primers and an eight base pair index.

We used the Stacks v1.48 software platform (Catchen et al. 2013) to demultiplex, analyze, and export data into other formats. We demultiplexed the data using the ‘process_radtags’ module by Stacks and the in-line barcodes for each sample using default settings.

After the data were demultiplexed, we filtered out poor reads using the AfterQC ‘after.py’ pipeline (Chen et al. 2017). Poor reads were those that: had a low quality score (evaluated by base-calling logarithmic probabilities), had bad overlaps (too little of the complementary reads lined up), had too many ambiguous nucleotides (reported as N), had reads that were too short, or reads with polyX (a long sub-sequence of a same base ‘X’) regions. Approximately 1.5% of reads per sample were classified as ‘bad’ and were removed from the dataset. After filtration, we aligned reads using the Burrows-Wheeler aligner within Stacks to the

Dipodomys ordii reference genome (version 1), which was sequenced in 2008 by the Broad Institute (https://www.ncbi.nlm.nih.gov/genome/772).

With filtered and aligned reads, we ran the ‘pstacks’ module where data are grouped into putative loci, and polymorphisms were identified. Next, we ran the ‘cstacks’ module that catalogued all loci. This catalogue was continually referenced during population genetic analysis. Then the ‘sstacks’ module was called, which compared each individual to the catalogue to determine allelic states at the requested loci. Finally, we ran the ‘populations’ module which computes some commonly used population genetic measures such as expected and observed heterozygosity and π. These data, as genotypes, were outputted into readily accessible file formats that were used by population genetic programs outside of Stacks such as Genepop (.gen) and Structure files (.str).

Population Genetic Metrics

We used the *Stacks* ‘populations’ module to output a series of population genetic summary stats for polymorphic and all positions, including expected and observed heterozygosity, nucleotide diversity, and .We ran the ‘populations’ module more than once to test for differences in SNP counts when adjusting for missingness in the datasets. Though the “gold standard” is include loci where 75 to 80% of the individuals in a population have that locus (Mastretta-Yanes 2015), this has been shown to bias population genetic measures, especially in cases where data are not plentiful. This influences biological implications dependent on these data (Gautier et al. 2013, Huang & Knowles 2014, Hosner et al. 2015, Hodel et al. 2017). We ran the ‘populations’ using this 75% rule (-r 0.75), a more liberal filter (-r 0.5) and a more conservative filter (-r 0.95). Furthermore, to account for differences in sample size among our hypothesized subpopulations, we randomly selected 6 individuals from the west and 6 individuals from the east deme and reran ‘populations’ with an -r value of 0.75.

For Hardy-Weinberg analysis for both historical and contemporary samples, we conducted a Hardy-Weinberg global test heterozygote excess method in Genepop 4.7.0 (Rousset 2008), then, if faced with a significant result, followed the considerations to correct for multiple tests (Waples 2014).

STRUCTURE

A useful role for genotyping is to assign individuals to certain groups based on genetic similarities. This is especially useful when population structure is cryptic or not readily identifiable. While many clustering approaches use distance-based measures, these measures were too dependent on the specific distance measure utilized (Pritchard et al. 2000). Structure 2.3.4 is a software program that incorporates a Bayesian framework to assign individuals to clusters (K). K is typically unknown. Users iterate through the model, specifying different prior estimates for K. The software calculates a likelihood score, which determines the most probable value of K. The model can be run with or without assuming admixture, which is the interbreeding of previously isolated subpopulations (or its converse, the beginning of population isolation). In practice, individuals are assigned membership coefficients for each K, which adds up to one across all clusters.

For Bayesian analysis using *Structure*, we removed all loci that were out of Hardy-Weinberg equilibrium, then randomly selected 1,000 of these “whitelisted” loci to reduce the amount of data run through the model. Only one randomly selected SNP from each locus was used to minimize the possible effects of linked data, which is known to cause Structure to behave inappropriately. Because the population structure within *Dipodomys elator* is unknown, we used the following to increase the chance of detecting cryptic population structure. We selected a lambda value of 0.9 instead of the default value of 1 and the standard admixture model. We initially ran the program with the LOCPRIOR option as location identifiers (east/west) to improve weak data. After several runs, our r value largely exceeded one, suggesting that the location data was not improving estimates by the program, so we dismissed the LOCPRIOR model. For all runs, we executed 50,000 burn-in iterations and 200,000 Markov Chain Monte Carlo (MCMC) repetitions with 10 replicates at each K, which ranged from 1 to 7.

Principal Components Analysis

To visualize genetic structure of the population without assigning individuals to clusters *a priori*, we conducted a principal components analysis using R package 'adegenet' version 2.1.1 (Jombart 2008) on historical and contemporary samples. We converted our individual by locus matrix into a 'genlight' object, then ran the glPca function. We chose to retain 4 axes for the historical dataset and 3 for the contemporary dataset based on the scree plots generated by glPca.

RESULTS

3RAD analysis for the overall dataset of 65 individuals produced over 34 million reads. Before filtering within the 'populations' module, there were 330,326 loci suitable for analysis. The mean sample coverage (depth) was approximately 8x.

Summary population genetics

The historical samples did not show any departure from Hardy-Weinberg based on the heterozygote excess method with a global *p*-value was 0.996. Contrarily, the contemporary samples showed deviation from Hardy-Weinberg equilibrium. The global *p*-value for the test was $p < 0.0001$; correcting for multiple tests, the proportion of significant outcomes exceeded the false discovery rate of 0.05.

Using a high missingness value (-r), both contemporary subpopulations showed higher observed heterozygosity and nucleotide diversity than historical samples (Table 1). *F*-statistics indicate that *D. elator* is not experiencing much inbreeding within its subpopulations and that there is not much genetic differentiation among subpopulations (Table 2).

Determination of population substructure

We used the program DISTRUCT v1.1 to visualize the final output of our structure runs, as they provide more flexibility concerning aesthetic choices (Rosenberg 2004). For k=2, many individuals appear to be admixed (Figure 1). Three individuals in the east subpopulation had membership coefficients of 1 for the pink cluster, and all individuals collected that year (2015) had membership coefficients dissimilar to the remaining samples (greater than 0.75 membership for pink). The plot for k=3 recreates the plot for k=2 in that most individuals are admixed (Figure 1). However, membership for the third cluster (yellow) is very low. Only 2 individuals from 2015 had assumed memberships for this third cluster.

Principal components analysis can be used to visualize data, as it reduces the number of variables in an orthogonal manner to a new set of variables derived from the original variables. The PCA for contemporary samples (Figure 2) follows the spatial distribution of the samples (Cottle to the far left/west, Wichita to the far right/east). The colors assigned in ‘adegenet’ refer to the relatedness of the samples, therefore, it appears there are 3 groups with at least 3 individuals per group. The PCA for historical samples (Figure 3) largely confirms that all individuals were taken from the same region (Hardeman County), but with a few exceptions. The green sample at the extreme end of principal component 2 could be considered an outlier, whereas the three pink individuals at the positive end of principal component 1 could be transients (two of the three were male individuals).

DISCUSSION

This study evaluates change in genetic diversity over time and across space in a rare species with constantly shifting distribution. This is the second population genetic study on *Dipodomys elator* in over 30 years, making the study valuable for conservation efforts moving forward. Hamilton et al. (1987) used allozyme markers to conclude that there was moderate genetic differentiation among three *D. elator* localities (Hardeman, Wilbarger and Wichita counties). While seemingly incongruent with our results, this may not be the case. It is very possible that these samples were collected as recently diverged subpopulations were beginning to coalesce. That would explain the genetic differentiation found in 1987 but has decreased 30 years later.

Genetic analysis revealed there has not been a substantial loss in genetic diversity though there seems to be a decrease in the distribution of *Dipodomys elator*. Using the strictest ‘populations’ run (missingess value of 0.95), current samples showed higher heterozygosity than historical samples. The contemporary east deme also exhibited negative F_{IS} scores. Evidence of an excess number of heterozygotes and high negative inbreeding scores indicate demographic fluctuations occurring within the *D. elator* metapopulation (Levins 1969, Hanski & Gilpin 1991, Nathan et al. 2017). Diverged, small subpopulations that have been previously isolated appear to currently engage in gene flow. This supports the idea that the *D. elator* population overall can be considered a classic metapopulation, given surveys from 1987 to the time of this writing. We do realize that our historical samples were taken from one county (Hardeman), effectively ignoring the diversity displayed by members across metapopulation at the time, but these were the only samples available. Nevertheless, these results support our hypothesis that *D. elator* has not experienced a decrease in heterozygosity. Due to the unclear nature of the *D. elator* metapopulation, the overall population must be monitored (Lindenmayer & Lacy 1995). Managing the metapopulation must be concerned with maintaining dispersal and gene flow and other population dynamics among the subpopulations. Should managers elect for extreme measures to manage *D. elator* populations, such as translocations or reintroductions, knowledge that the population is a metapopulation is critical. Lastly, it is important to note that the metapopulation context in a conservation context has several assumptions. One assumption is the "equilibrium" between colonization and extinction across long time scales (i.e., if one patch goes extinct, another is colonized). This seems unlikely in many natural populations (Akçakaya et al. 2007), including that of *D. elator*, but this type of assumption can be used to appropriately model changes in demography and genetics of the Texas kangaroo rat.

Results from our contemporary samples confirm that though population differentiation is not substantial ($F_{ST} < 0.05$), there is evidence for population substructure. Principal components analysis suggests that though Cottle, Hardeman and Childress counties are more related to each other than the counties in the east there is still enough genotypic variance to further separate them into subpopulations.

For our contemporary samples, the algorithm determined the best value of K to be somewhere between k=2 and k=5. We felt that more clusters, while not impossible, do not strike us as biologically practical. It may just be an artefact of our sampling scheme (for example, k=5, one for each county). Secondly, it is known that newly colonized or subpopulations on the fringes exhibit lower levels of genetic diversity than expected (Eckert et al. 2008). For our contemporary samples, this is not the case; the high level of genetic diversity does not seem to support cluster sizes of k=4 and k=5. Based on ecological characteristics across the study area, our *a priori* supposition was that there are 2 subpopulations (east and west). However, three distinct groupings are revealed, suggesting some divergence in the west deme. We reran Structure on our west subpopulation and found that indeed there appears to be 2 subpopulations in the west. However, there is no geographic explanation for the substructure, indicating that this might not present a real phenomenon.

Though there is not a lack of research on life history traits such as reproduction and dispersal habits of *Dipodomys elator*, which greatly improves our understanding of this elusive rodent (Garner 1970, Roberts & Packard 1973, Stangl et al. 1992, Goetze et al. 2007, Nelson et al. 2009), we are still in the dark when it comes to basic biological answers. We do know, however, that its metapopulation tracks favorable habitat, albeit in a more restricted range than previously.

Overall, the population of *Dipodomys elator* shows no decrease in genetic diversity from historical samples. Additionally, current subpopulations display low levels of inbreeding and very little genetic differentiation among subpopulations. There appears to be at least two *D. elator* subpopulations. However, because the two subpopulations are on opposite sides of cline, separated by the Waggoner Ranch in Wilbarger and Baylor counties, it is difficult to determine if the differentiation is due to that distance or if there is true population substructure and isolation from other habitat patches (Audzijonyte & Vrijenhoek 2010). Continued implementation of historic samples from museum collections is critical to solving this problem. We are confident that our results will empower others to use the 3RAD approach on degraded or low input sample because it is valuable for making population genetic inferences for management in a variety of spatial and temporal contexts.

Given the current advances in molecular technologies and analyses, it is no longer necessary to limit samples in terms of time. Doing so, especially in species that remain understudied, will prove detrimental to any plan long-term plan for management. We advise continued use of reduced representation sequencing (ddRAD, 3RAD) but with inclusion of historic samples to fully encapsulate temporal genetic variability within an imperiled species. This is especially important for species that are rarely seen or captured. Our findings suggest that the population of *Dipodomys elator* is a classic metapopulation that must be vigorously monitored so that managers can detect any great losses in evolutionary potential.

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Table 1: General summary statistics calculated in Stacks on 65 *D. elator* samples. Private alleles are those alleles not shared with any other subpopulation. Observed and expected heterozygosity are the proportion of loci that are heterozygous. π is a measure of nucleotide diversity. F_{IS} indicates the inbreeding coefficient.

		Private alleles	Observed heterozygosity	Expected heterozygosity	π	F_{IS}
Missingness value of 0.95; 2,230 SNPs	Historical	7	0.021	0.025	2.53×10^{-4}	+0.063
	Contemporary: East	20	0.175	0.112	1.1×10^{-3}	-0.095
	Contemporary: West	9	0.135	0.143	1.4×10^{-3}	+0.066
Missingness value of 0.75; 17,811 SNPs	Historical	4,550	0.130	0.130	1.1×10^{-3}	+0.022
	Contemporary: East	2,115	0.054	0.056	5.7×10^{-4}	+0.030
	Contemporary: West	1,688	0.082	0.089	9.3×10^{-4}	+0.040
Missingness value of 0.50; 60,166 SNPs	Historical	7,371	0.122	0.132	1.4×10^{-3}	+0.079
	Contemporary: East	4,520	0.104	0.111	1.1×10^{-3}	+0.048
	Contemporary: West	2,292	0.100	0.108	1.2×10^{-3}	+0.052
Random; 1,864 SNPs	Contemporary: East	674	0.140	0.131	1.5×10^{-3}	+0.022
	Contemporary: West	837	0.150	0.148	1.6×10^{-3}	+0.042

Table 2: Wright's F-statistics for 65 *D. elator* samples. Values were generated using the STACKS 'population' module with an -r (missingness) value of 0.75.

	F_{ST}		
	Historical	Contemporary: East	Contemporary: West
Historical		0.020	0.026
East Deme			0.024

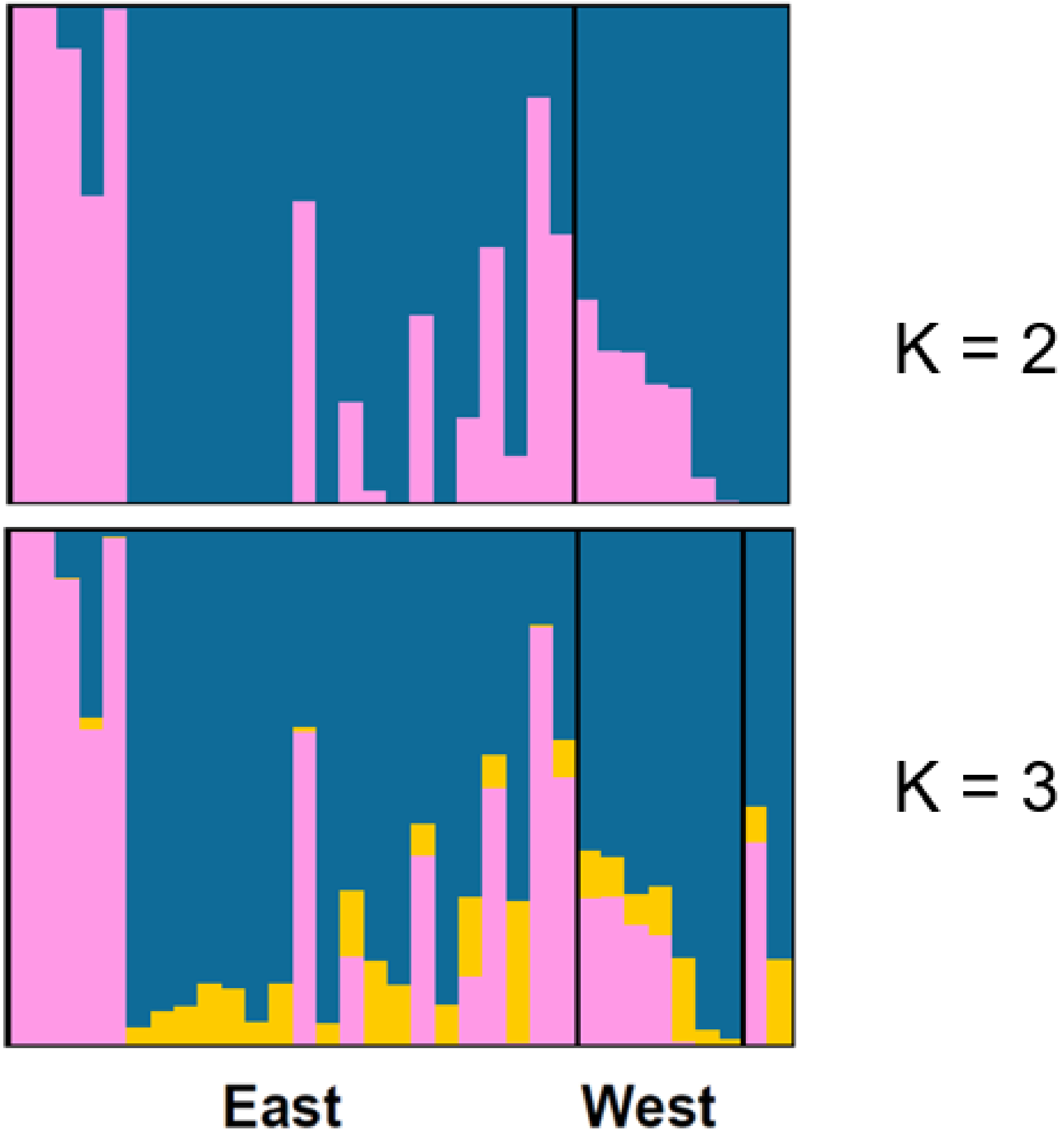

Figure 1: Structure plot for clusters k=2 and k=3 for contemporary D. elator samples. Each colored vertical bar represents an individual and the colors comprising of each individual represent coefficients of membership (from 0-1) for each k-colored cluster. Vertical bars with more than one color are individuals modeled to be admixed.

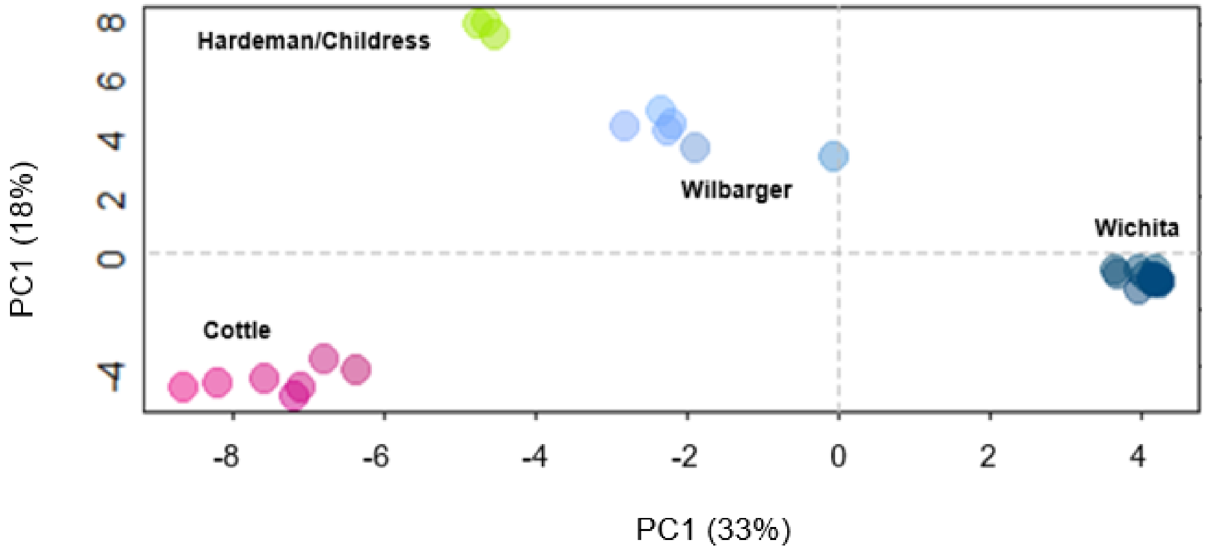

Figure 2: Principal components analysis on the genotypes of contemporary samples using the glPCA function in R package ‘adegenet’. Three axes were retained.

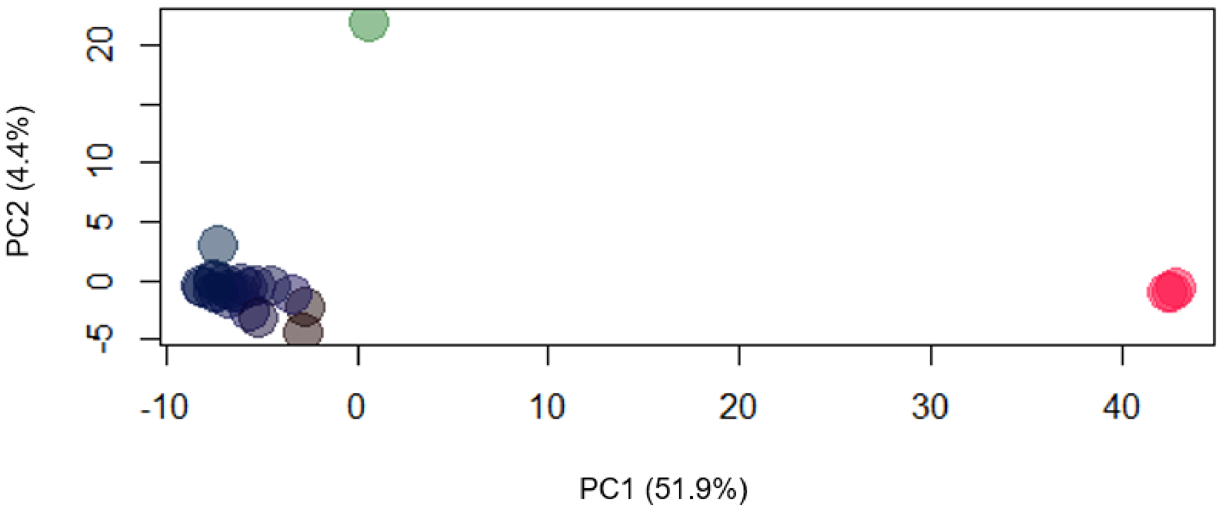

Figure 3: Principal components analysis on the genotypes for historical samples from Hardeman County using the glPCA function in R package ‘adegenet’. Four axes were retained.

Table 3: Supplemental. Sixty-five samples used in the genetic analysis including temporal (historical, contemporary) subpopulation, spatial (east or west) subpopulation, the specific county the individual was found, and tissue type.

Sample Name	Temporal Deme	Spatial Deme	County	Tissue
MM_099	Historical	West	Hardeman	toe
MM_101	Historical	West	Hardeman	toe
MM_102	Historical	West	Hardeman	toe
MM_103	Historical	West	Hardeman	toe
MM_104	Historical	West	Hardeman	toe
MM_121	Historical	West	Hardeman	toe
MM_124	Historical	West	Hardeman	toe
MM_125	Historical	West	Hardeman	toe
MM_126	Historical	West	Hardeman	toe
MM_127	Historical	West	Hardeman	toe
MM_128	Historical	West	Hardeman	toe
MM_134	Historical	West	Hardeman	toe
MM_135	Historical	West	Hardeman	toe
MM_138	Historical	West	Hardeman	toe
MM_139	Historical	West	Hardeman	toe
MM_140	Historical	West	Hardeman	toe
MM_142	Historical	West	Hardeman	toe
MM_149	Historical	West	Hardeman	toe
MM_150	Historical	West	Hardeman	toe
MM_151	Historical	West	Hardeman	toe
MM_152	Historical	West	Hardeman	toe
MM_158	Historical	West	Hardeman	toe
MM_159	Historical	West	Hardeman	toe
MM_168	Historical	West	Hardeman	toe
MM_169	Historical	West	Hardeman	toe
MM_170	Historical	West	Hardeman	toe
MM_174	Historical	West	Hardeman	toe
MM_177	Historical	West	Hardeman	toe
RDSLAB_8510	Contemporary	East	Wichita	whisker
RDSLAB_8550	Contemporary	East	Wichita	whisker
RDSLAB_8557	Contemporary	East	Wichita	whisker
RDSLAB_8563	Contemporary	East	Wichita	whisker
RDSLAB_8574_Ta	Contemporary	East	Wichita	tail
RDSLAB_8580	Contemporary	East	Wichita	whisker
TK_163651	Contemporary	East	Wichita	whisker
TK_163652	Contemporary	East	Wichita	whisker
TK_163654	Contemporary	East	Wichita	whisker
TK_163655	Contemporary	East	Wichita	whisker
TK_163656	Contemporary	East	Wichita	whisker
TK_163658	Contemporary	East	Wichita	whisker
TK_163659	Contemporary	East	Wichita	whisker

TK_163670	Contemporary	East	Wichita	whisker
TK_163671	Contemporary	East	Wichita	whisker
TK_163672	Contemporary	East	Wichita	whisker
TK_163679	Contemporary	East	Wichita	whisker
TK_163680	Contemporary	East	Wichita	whisker
TK_163686	Contemporary	East	Wichita	whisker
TK_199281	Contemporary	East	Wichita	liver
TK_163660	Contemporary	West	Cottle	whisker
TK_163661	Contemporary	West	Cottle	whisker
TK_163662_Ta	Contemporary	West	Cottle	tail
TK_163675	Contemporary	West	Cottle	whisker
TK_163676_Ta	Contemporary	West	Cottle	tail
TK_199274	Contemporary	West	Cottle	whisker
TK_199275_Ta	Contemporary	West	Cottle	tail
TK_163663	Contemporary	West	Hardeman	whisker
TK_199282	Contemporary	West	Hardeman	liver
TXRODX_1003	Contemporary	West	Childress	buccal
TK_199276	Contemporary	East	Wilbarger	liver
TK_199277	Contemporary	East	Wilbarger	liver
TXRODX_1040_Ta	Contemporary	East	Wilbarger	tail
TXRODX_1047	Contemporary	East	Wilbarger	whisker
TXRODX_1048	Contemporary	East	Wilbarger	whisker
TXRODX_1049	Contemporary	East	Wilbarger	whisker
TXRODX_1054	Contemporary	East	Wilbarger	whisker

Table 4: Supplemental. Mean ln likelihood and variance values for structure run for k=1 to k=7. The runs plateaued between k=2 and k=5.

Clusters	Ln likelihood (Variance of ln likelihood)
1	-49875.0 (969.3)
2	-34064 (1338.4)
3	-34504.9 (2062.1)
4	-33488.3 (2241.4)
5	-32848.8 (2452.9)
6	-52182.8 (41786.6)
7	-35600.3 (13225.8)

CHAPTER IV: EFFICACY OF REMOTE SENSING TECHNOLOGIES TO DETECT BURROWS OF DIPODOMYS ELATOR AND ULTIMATELY ESTIMATE ABUNDANCE ACROSS ITS GEOGRAPHIC DISTRIBUTION IN TEXAS

EXECUTIVE SUMMARY

Effective management of rare species requires an understanding of both distribution and abundance, although estimating abundance of a species is often more challenging logistically. Here, we determined the potential for using high-resolution imagery to count *D. elator* burrows across its entire range and discuss the implications for landscape level detection and mapping. Specifically, we surveyed a private property located in Wichita County, TX for *D. elator* burrows and used an Unmanned Aerial System (UAS) to collect very high-resolution RGB imagery and digital elevation models (2.5 mm pixel size) over active and inactive burrows located in mesquite mounds and anthropogenic features (roadsides, fences, etc.). We used 26 identified burrows locations to characterize these based on topography and vegetation density. Circular and linear mounds used by *D. elator* for construction of burrows were characterized by prominent slope and aspect ranges in the digital surface models. Burrow entrances and disturbed soils from trails outside burrow entrances were observed using the RGB imagery. We found that *D. elator* burrows can only be identified with <5 cm pixel resolution data which rules out the possibility of using high-resolution imagery data currently available at the state level. Alternatively, we propose that use of NAIP imagery at 0.5 and 0.6 m pixel resolution in combination with resampled Digital Elevation data can provide effective means for identifying potential TKR burrow locations at the county level. We present three different spatial models at the county, landscape and site scale that combine topographic and vegetation fractional cover information using a weighted overlay approach. These modeling approaches have strong

predictive capabilities and can be integrated with UAS data for visual confirmation of active or inactive burrows. The study concludes that very high resolution imagery and topographic information at pixel resolutions <5 cm collected by airborne systems can effectively help locating active *D. elator* burrows. However, to remain cost effective, upscaling to county level will require reducing the sampling area to the most suitable habitat areas. Modeling approaches, such as the ones proposed in this study, can help locating these sampling areas effectively.

INTRODUCTION

Understanding ecology of rare species requires thorough understanding of both distribution and abundance. For example, a species may be rare based on its distribution, abundance, and/or habitat specificity (Rabinowitz et al. 1986). Characterizing patterns of rarity of species has fundamental implications for conservation (Gaston 2010, 2012), because rarity is associated with extinction risk, although the significance of particular threats for rare taxa are likely to vary based on the ways in which a species is considered rare (e.g., small distribution and/or low abundance). Thus, quantifying both distribution and abundance of a rare species is important to characterize the conservation status of a species (Hartley and Kunin 2003) and develop effective conservation and management plans.

The Texas kangaroo rat (*Dipodomys elator*) is a rare species that currently has been documented in just five counties in north-central Texas (see Chapter 3). Although we now have a better understanding of the distribution of *D. elator*, it is still unclear how abundant this species is across its distribution. The paucity of information on Texas kangaroo rat abundance is due in part to the fact that much of the land in this region is privately-owned, such that access to habitat is limited. Importantly, while it is feasible to estimate distribution of a species by determining presence or absence opportunistically (e.g., surveying via county roads and creating predictive distribution maps), estimating abundance requires more rigorous sampling within habitat patches.

Given the increasing use of Unmanned Aerial Systems (UAS) based technology in ecological field studies due to their novel spatial perspectives of ecological phenomena (Anderson and Gaston 2013), harnessing high-resolution aerial imagery to count *D. elator* burrows across the distribution of this species may provide a novel method to estimate the present-day abundance of Texas kangaroo rats. Texas kangaroo rats have distinctive features of their burrows. In particular, there is a propensity to make them of a unique size and orientation (Roberts and Packard 1973, Stangl et al. 1992) as well as in locations with elevated, open areas (e.g., fence rows, decaying brush piles, or beneath mesquite shrubs; Stangl et al. 1992, Goetze et al. 2007, Nelson et al. 2009). Moreover, as these rodents excavate their burrows they bring to the surface soil that has different color and texture than the soil at the surface. Thus, there appears to be promise in using the unique architecture and features of active burrows to count their actual number across the entire range of the Texas kangaroo rat via high-resolution imagery. Such an ability would be a huge advance in conservation efforts of this species because it would provide the first range-wide estimate of the abundance of *D. elator* and would likely be the most important information guiding such decisions for this species available to date.

Low-cost UAS have grown quickly in the last decade with applications in recreational use, wildlife ecology and agricultural research (Birdsong et al 2015, Everacerts 2008, Bryson 2014, Rango 2010). UAS technology consists of integrated quadcopter or fixed-wing vehicle with intelligent flight planning and data processing capabilities. UAS systems can generate multispectral imagery at millimeter resolution for any desired target in the landscape in addition to digital surface models for elevation data.

Here, we attempted to determine the efficacy of using high-resolution imagery as a tool to count *D. elator* burrows across the distribution of this species in order to obtain a range-wide estimation of its present-day abundance. In doing so, we established the degree to which it is possible to use UAS technology, digital elevation models and high-resolution imagery to identify Texas kangaroo rat burrows and therefore estimate population size.

We performed this study in three stages. The first stage consisted of an initial characterization of burrows using imagery collected from an Unmanned Aerial System and the evaluation of the impact of pixel size on burrow detection. This information allowed us to determine the minimum pixel resolution needed to effectively identify a TKR burrow in the landscape. The next set of stages were intended to evaluate the effectiveness of modeling approaches using publicly available datasets (e.g NAIP imagery, digital elevation models, flooding datasets) and UAS imagery to predict the location of TKR burrows in the landscape. These models are intended to serve as an example of a potential workflow for TKR burrow mapping that could start with a landscape scale model or a county scale model to locate suitable areas of burrow presence and follow up with a campaign of UAS data collection over most suitable areas. In this last stage, UAS imagery would help to detect and count active and inactive burrows from aerial surveys. In this context, the second stage consisted of a landscape scale approximation tof burrow presence and a county level approximation to burrow presence based on multiple sources of data. The third stage consisted of generation of a predictive model to locate common types of burrows using UAS imagery.

METHODS

Study area

Field data collection was restricted to the Goetze Ranch in Wichita County, Texas (Fig. 1). Sections of this ranch have been used extensively for previous research on *D. elator* (e.g., Stangl et al. 1992, Goetze et al. 2007, Nelson et al. 2009). Different sections of this ranch vary in land-use practices, and therefore vary in grazing intensity and the presence of agriculture (i.e., wheat) and/or oilfield infrastructure. As such, there is also considerable variability in vegetation cover, although the dominant woody vegetation in all sections of the ranch is mesquite (*Prosopis glandulosa*). Soils across the area are categorized as clay loams (e.g., Kamay soils; Goetze et al. 2007).

Ground survey

Field visits were made during the spring, summer and fall of 2018 in order to locate active and inactive TKR burrows within the Goetze Ranch. The field survey campaign consisted of locating mounds under mesquite or other raised surfaces (e.g., fence line), registering their geographic coordinates (using a GPS) and taking notes on the orientation of the entrances to the burrow, the amount of vegetation and the presence of excavated soil in the surface (i.e., trails). We identified 26 burrows in nine different locations within the Goetze Ranch (See Figure 1 for a map of burrow locations within the Goetze Ranch). Photo examples of some of the burrows identified are shown in Figure 2.

Surveys to locate and identify burrows conveyed what other efforts have confirmed: TKR burrows are usually found in mounds formed by tall mesquite shrubs or elevated ground formed by anthropogenic features (such as road sides and fences) in well-drained areas (usually higher ground to avoid occasional flooding). Tall mesquite shrubs help block the sunlight that reaches the understory and therefore decreases grass density in the understory. These less vegetated mounds with a predominance of bare soil in the base of the mesquite shrub are preferred by TKR. We also noticed the prevalence of bare soil around entrances to burrows in anthropogenic land features. In the areas surveyed, TKR burrows could only be confused with mounds used by wood rats. However, woodrats use deadwood (trunks, branches) which are accumulated on the mound and show a pattern distinct from TKR burrows.

First stage: TKR burrow characterization using UAS imagery

Remote sensing imagery acquisition was performed using an Unmanned Aerial System (UAS). The vehicle used for this study was a DJI Phantom 4 Pro (DJI Technology Co., Ltd.). The Phantom 4 Pro is a quadcopter vehicle that carries an RGB (red, green and blue) camera capable of acquiring photography at 12 megapixels with scenes of 4,000 x 3,000 pixels each. The camera is mounted on a motorized gimbal allowing the camera to be redirected and stabilized during flight. The UAV is capable of using GPS and GLONASS satellites for positional accuracy. It is flown using a controller included with the UAV capable of reaching about a half mile from the hand-held controller. This distance provided adequate coverage of the site from a single launch point. The DJI batteries lasted for approximately 17 minutes of flight time. Four batteries were used to conduct all flights during field visits.

Image processing was performed using the Pix4D platform (Pix4D S.A.) which includes a mobile app and a desktop software. The Pix4D Capture mobile application was used on an Apple iPhone for creating flight plans and automatically flying the Phantom 4 quadcopter. The app allows users to customize altitude, speed, and image overlap for each flight. This application allows for the creation of flight plans without having flown over the site previously. Flight plans can also be saved and re-used for repeat flights.

At the Goetze Ranch, the nine different locations where TKR burrows were found were surveyed using the UAS. The flight plans for the Phantom 4 over these locations allowed us to create orthomosaic photos with a pixel resolution of 0.25 cm or 2.5 mm. Orthomosaic photos are aerial photos geometrically corrected such that distortions are minimized and equal across the dataset. We use Pix4D to design flight plans at 10 m altitude with >75% overlap between collected imagery during clear weather and low wind speed conditions. Each scene had an average size of 1 acre. Once imagery were collected on site, Pix4D desktop software was used for post-processing. The software allowed us to generate three main products: a) an RGB orthomosaic photo, which consists of the geometrically corrected image at 2.5 mm pixel resolution with three spectral bands of blue, green and red range wavelengths; b) a Digital surface model or Digital Terrain model at 2.5 mm pixel resolution which consists of ground elevation measurements generated through automated photogrammetric methods; and c) a 3D terrain model generated by combining the surface model and the orthomosaic photo. See Figure 3 for an example of the products generated per flight.

We performed a two-step approach for characterizing TKR burrows using the UAS imagery collected. The approach consisted on characterizing TKR burrows using topographic and RGB image products and then evaluating the impact of pixel resampling on the detection ability of TKR burrow features.

First, we used a geographic information system (ArcGIS, ESRI) to generate topographic layers from the DEM. We used ArcGIS Spatial Analyst tools to generate slope and aspect topographic layers from the Digital Surface Model product. We also generated a green foliage map by applying a threshold to values from a vegetation index derived from the RGB imagery. The index used was the Visible Atmospherically Resistant Index (VARI) which allows estimating vegetation fraction quantitatively with only the visible range of the spectrum (Gitelson et al. 2002). The formula for VARI is: (Green - Red) / (Green + Red – Blue). From these UAS-derived products, descriptive statistics of elevation range, slope, aspect and % foliage cover were calculated for each burrow. Measurements are given for surfaces within a 1.5 m radius from the center of the mesquite mounds with visible burrow entrances. The same measurements were done on burrows found in anthropogenic features. We also compared the ability to discriminate between excavated or disturbed soil (trails) and non-disturbed soil in UAS imagery.

Spatial resolution (pixel size) of the imagery collected at the site level can impact the ability to observe features related to TKR burrow presence; therefore, for this stage we evaluated the effect of pixel resampling on our ability to visualize entrance to burrows in mounds and TKR trails by resampling original RGB imagery and DEM to 2.5 cm, 5 cm, 8 cm, and 15 cm pixel size on three of the most conspicuous mounds with TKR burrow presence.

Second stage: Landscape scale and county level predictive models

We investigated the hypothesis that TKR burrows are usually found in mounds formed in well-drained areas of higher elevation (to avoid occasional flooding) where sparse vegetation is predominant. We believe that if this hypothesis is true, landscape level modeling using digital elevation models and vegetation maps can be used to approximate to the distribution of TKR burrows at the county or at the state scale.

Landscape scale model generation

For deriving the landscape scale model, we used a Digital Elevation Model (DEM) at 10 m resolution and High Resolution Imagery (NAIP multispectral imagery, 0.5 m pixel resolution) acquired from the Texas Natural Resources Information System (TNRIS) data portal and explored the relation between TKR burrows, topography and vegetation within the boundaries of the Goetze Ranch.

We applied an unsupervised classification of the NAIP multispectral imagery using the IsoCluster method in ArcGIS to derive a map depicting the distribution of two classes: vegetation and bare soil, for the entire extension of the Site #1 of the Goetze Ranch. We also reclassified the Digital Elevation Model in five different altitudinal ranges from low to high. Higher elevation and low vegetation density grid cells will be classified as ‘High potential for TKR burrow areas’ while the lower elevation and high vegetation density grid cells will be classified as ‘Low potential for TKR burrow areas’.

We used a GIS-based “weighted overlay” approach to combine both datasets. The weighted overlay approach is a tool in ArcGIS that allows computation of a weighted sum of categorical maps where each class has been ranked by importance and each dataset has been ranked by degree of influence. The final output is a suitability map where higher values depict areas of higher importance and influence. In this case, both vegetation and elevation were given the same degree of influence on the overall model. Given that the vegetation density map was calculated at 10 m pixel resolution, the output of this model was presented at the same resolution.

A diagram showing the methodology for the derivation of the landscape scale model is shown in Figure 4.

A ground validation effort was carried out in August 2019 to test the predictive power of this model. Six (6) students were trained to identify and locate potential TKR burrows on the ground and register their locations using a GPS unit. Site #1 was surveyed but because of time constraints, transects in Site #1 were limited to the eastern portion of the study area. A total of 41 burrow locations were recorded during this validation survey.

County scale model generation

For deriving the county level model, a predictive model was generated under the same rationale and methodology for the entirety of Wichita County, however, this time all datasets were analyzed at the 0.6 m pixel resolution, which is the spatial resolution of the NAIP imagery mosaic available for the entire county. To obtain this map, we mosaicked 121 NAIP scenes from 2016 into one single four-band product (Visible, Near-Infrared) using the ERDAS Imagine Software. We also acquired a Digital Elevation Model (10 m spatial resolution) for the entire county, and a land cover/ land use map from Cropscape (USDA) at 30- m pixel resolution. This time all data were resampled to the highest spatial resolution of 0.6 meters of spatial correspondence.

Vegetation and bare soil land cover classes were derived from the NAIP imagery through an unsupervised classification approach (IsoCluster, ArcGIS). The Cropscape dataset was used to discriminate natural vegetation cover and urban land uses from active crops under the assumption that frequently disturbed areas like crops will have a lower probability of burrow presence. Given that the NAIP imagery is able to expose mounds formed by fence lines and other structures, this assumption does not excludes the agricultural areas entirely but only excludes the productive areas covered by crop plants.

The model was derived through a weighted overlay approach in ArcGIS. Both vegetation and DEM datasets were given similar influence (45% and 45%, respectively) while the Cropscape data was given lower influence (10%). Finally, we used the National Flood Hazard Layer (NFHL) from FEMA (https://www.fema.gov/national-flood-hazard-layer-nfhl) to allow the model to run for areas with lower probability of flooding (X Class in NFHL dataset). A diagram showing the methodology for the derivation of the County level model is shown in Figure 4.

Third stage: Predictive model of burrow occurrence using UAS imagery

In the third stage, we developed a spatial model (based on a weighted overlay approach) for detecting burrows by integrating neighboorhood statistics of slope, aspect, elevation and percent green foliage cover derived from UAS imagery.

Circular or linear mounds where TKR burrows might be present are characterized by broader ranges in elevation and aspect, with overall high slopes and different degrees of vegetation density. Spatial indicators of these characteristics can be achieved through the application of neighborhood statistics called ‘focal statistics’ on RGB imagery and Digital Surface Models derived from UAS imagery.

The focal statistics tool performs a neighborhood operation that computes an output raster where the value for each output cell is a function of the values of all the input cells that are in the adjacent pixels (neighborhood) around that location (ArcGIS, ESRI). Focal statistics for elevation and aspect classes were based on range (difference between the smallest and largest value) while focal statistics for slope and vegetation were based on mean. The statistic used in these two processes were selected based on evaluation of several preliminary iterations of the model with different statistics. The use of focal statistics allow enhancing terrain features with circular or linear patterns. These statistics provided a better depiction of the variability of the land biophysical properties.

Focal statistics were applied using a radius of 1 m. The products were reclassified in categorical maps of five classes each. A weighted overlay model was implemented by setting weights to the values of each variable based on the information collected in the first and second stage. For the DSM, slope and aspect products, the categorical classes (1-5) were ranked in importance where 5 represents the highest importance. For the vegetation categorical classes (1-5), the class 1 and 2 (lowest vegetation abundance) were given the highest importance instead. The influence of the DSM and the vegetation were higher than slope and aspect, but these varied from site to site.

The model output was evaluated at three independent locations were UAS surveys were performed and where there were multiple TKR burrows present. For reference, we will refer to these locations as site A, site B and site C which all correspond to the eastern portion of Site#1. The validation consisted of observing the correspondence of highly suitable areas in the model with confirmed burrow entrances and trails. These were identified with the help of the RGB imagery. Figure 5 and 6 show flowcharts of the spatial modelling process.

RESULTS

First stage: Burrow characterization

Imagery collected at 10 m altitude over mounds with TKR burrows allowed us to identify physical properties associated with the mounds used by TKR. From all 26 burrows characterized in the ground, eighteen (18) had visible features that allowed proper characterization with UAS imagery. Figure 7 shows (Only for sample C1) a summarized graph that integrates: a) the relative position of the burrow entrances in each mound; b) a snapshot of the RGB imagery from Nadir (90° from the surface plane), as well as views of the mound from 45° and 315° from the surface plane; c) representation of the mounds with the digital surface model (elevation in m), slope and aspect products; d) summary statistics of elevation, slope, aspect and vegetation fractional cover and e) a 3D surface model of the mound shape as detected by the UAS photogrammetry processing. In Appendix 1, we show the same data for all eighteen (18) samples.

Elevation ranges from the surface plane to the top of the mound ranged from 20 cm to 1.85 m with an average of 60 cm. The average in degrees was 65° around samples and aspect range was high with almost slopes facing in opposite directions most of the time. Vegetation fractional cover averaged 23% around mounds with TKR burrows. Most mounds with high levels of vegetation cover, however, had smaller bare soil patches where the burrow entrance was visible. No TKR burrows were found in continuous grassland patches.

Entrances to burrows (holes) of different shape and diameters are visible from UAS imagery. Most of these holes are 10-cm in diameter. In some cases, entrances are obscured by shadows from branches and vegetation. The same occurs in anthropogenic mounds. As seen in these digital characterizations, some of the TKR burrows were found in anthropogenic features below rails or metal poles. These rails and metal poles are also detected by the UAS photogrammetry processing and can be observed in the digital surface models and slope and aspect products which makes it difficult to observe the location of the holes and the shape of the mound itself. However, the 3D modeling capabilities of UAS data allows generating different angles and perspectives of the same location and therefore allows the user to identify features not seen at nadir. Off-nadir images in Appendix 1 show different viewing perspectives of the same mounds.

Trails located outside the entrances (holes) to the burrows are easily observed from UAS imagery at 2.5 mm pixel resolution. In addition, loose soil in the surface as a result of TKR excavation has a distinctive spectral signature when compared to bare soil. As seen in Figure 8, loose soil usually is darker (lower brightness values) than the surrounding soil. This makes both trails and excavated soil easily identified from UAV imagery at this resolution.

Figure 9 demonstrates the impact of pixel resampling to lower resolution on the ability to detect key features of mounds with TKR burrows such as the presence of entrances, vegetation cover fraction, topographic features and the presence of trails. In the samples here evaluated, the capability to identify most of the features is lost in lower pixel resolutions (>5cm). In fact, at 15 cm pixel resolution, mesquite or anthropogenic mounds are impossible to detect.

Second stage: landscape scale and county level predictive models

In this stage, we used spatial data to map well-drained areas of higher elevation (to avoid occasional flooding) where sparse vegetation is predominant, as a proxy to find suitable areas for TKR burrow presence within the Site #1 of Goetze ranch.

Figure 10 shows the result of this simple weighted overlay approach. Both vegetation and elevation data were assigned the same level of influence on the model. In the resulting model, areas with elevation and vegetation characteristics suitable for TKR burrows were highlighted in red. These areas correspond to the northeastern portion of the Site#1 of the Goetze ranch, a narrow strip or corridor of the land adjacent to the crop area in the southeastern portion of the site, and the southwestern portion of the site. Other locations around the roads were also indicated by the model as suitable. During the validation process, entrances to burrows were observed and recorded across for the western portion of the site. Only a total of 7 of 41 burrows were far outside of the area predicted as suitable by the model, meaning that 83% of the points fell within the predicted suitable area or in its adjacencies in Site#1. The most striking correspondence was located in the lower right corridor which was delineated by the landscape model. Eleven different burrows were found in these strips of land with low vegetation/bare soil in higher ground identified by the model as suitable burrowing area. We believe the low vegetation/bare soil areas that were not identified by our model and therefore missed to represent the location of 7 different burrows might be the result from the loss of detail from resampling the vegetation cover from 0.5 m pixel resolution to 10 m.

The output from the County level model is presented in Figure 10. The County level model for Wichita County was performed at the original resolution of the NAIP imagery used in the assessment (0.6 pixel resolution). The model shows a clear concentration of highly suitable areas for TKR burrow presence in the northwestern and north central portions of Wichita county. Interestingly, highly suitable areas highlighted in red shows strong similarities to the suitable areas predicted by the MAXENT model in Chapter III for northwestern Wichita County (Fig 3 and Fig 5 of Chapter III). Figure 10 also shows the relative location of TKR burrows identified during ground surveys and its correspondence to highlighted areas. Although the model incorporates land cover/land use data from Cropscape at 30-m resolution the output still conveys a level of detail that allows identification of specific natural and anthopogenic features at high spatial resolution (Figure 11). Further aerial or field surveys can use these models to locate specific land properties to survey in the ground.

Third stage: Predictive model of burrow occurrence using UAS imagery

In this stage, we generated a slope and aspect product from the digital surface model (elevation) at the 2.5 mm spatial resolution as well as a vegetation cover product from unsupervised classification of the UAS RGB imagery. This assessment was done at three locations in the eastern portion of Site#1 (Site A, B and C). Focal statistics were performed on slope, vegetation, aspect and elevation products and then reclassified into five categories, which were ranked in levels of importance during the weighted overlay parameterization process. The implementation of the focal statistic tool is a computing intensive process. At first, other statistics related to flow accumulation and flow direction were attempted, however, these properties were correlated with slope and elevation and therefore excluded from the analysis. Other neighborhood statistics such as ‘majority’ or ‘variety’ were also attempted, but in order to shorten the computing time of such high resolution datasets we implemented similar metrics such as mean and range that gave similar results in less processing time.

For Site A (Figure 12), the model predicted highly suitable areas adjacent to and along a corridor of elevated ground where a fence line was built. Low probability areas were predicted for vegetated areas and cotton cropland. As indicated in Figure 12, four out of five TKR burrows identified in field surveys in the same area corresponded spatially to highly suitable areas indicated by the model. In Figure 13, we show results for Site B and Site C. In Site B, the model also predicted the exact location of the three TKR burrows observed during field surveys, while the model for Site C predicted the exact location of three out of six TKR burrows. In general, the majority of TKR burrows were within or near high suitability areas predicted by the model, while only one was far-off a predicted area. In the case of Site B, the best outputs were achieved without the DSM layer. After inspecting this layer, we noticed that it showed very little variation across the landscape which could have affected the final model given its high relative influence.

DISCUSSION

The use of UAS products for TKR habitat characterization allowed us to understand the potential of airborne RGB imagery and topographic data for locating and identifying active or inactive TKR burrows in a landscape and the possibility of using publicly available datasets for county level and landscape scale assessments. The summary graphs (Appendix 1, Figure 7) show that TKR burrows can be present in a wide variety of topographic and vegetation cover spatial configurations, including anthropogenic features such as road sides, the base of tanks, oil pipes, fence lines, among others. The association of TKR burrows with mesquite shrubs was recorded in samples C1, C2, C4, C6 for example, while C3, 15, C17 and C20 are clear examples of TKR burrows located in anthropogenic features (See Appendix 1). As seen in the slope products derived for each sample and the 3D surface models, the predominance of steep slopes is a common attribute of all TKR burrows. Circular mounds show high concentration of steep slopes plus a higher range of aspect values than the surface plane, and although not circular, other types of mounds such as the ones created by fence lines (linear mounds), also generate a concentration of steep slopes and high range of aspect values (Samples C10-C16). These topographic features make suitable areas for TKR burrow presence prominent and detectable in the landscape.

In addition, mounds with patches of bare soil and surrounded by low vegetation cover can be found by calculating the fraction of vegetation cover across a landscape using the UAS

RGB imagery. The Visible Atmospherically Resistant Index (VARI) was an effective index for separating vegetation from bare soil. However, we did find some commission and omission errors in the vegetation maps that could be improved. There is potential for improvement in the quantification of vegetation fractional cover by using other methods for vegetation classification such as object-oriented image classification and rule-based approaches (Ruiz et al. 2018). No TKR burrows were found in continuous grassland patches, but although low vegetation cover is important, burrow entrances were also observed in small bare soil patches around high levels of vegetation cover in some cases (C16, C17). This highlights the importance of improving vegetation cover maps for further modeling. It is also important to consider the season for UAS imagery data collection given that differences in vegetation greenness can affect the accuracy of vegetation fraction estimates.

Although it is impossible to detect activity within a burrow system using a UAS with an RGB camera, we can use proxy features to determine whether a burrow is active or not. At 2.5 mm pixel resolution, changes in soil texture caused by soil disturbance in adjacency of burrow entrances are easily observed. Some of these disturbances extend over almost a square meter in front of the burrow entrance (C3, C10, See Figure 8).

The features and properties of TKR burrows could be observed at the pixel resolution obtained from flying the UAS at 10-m altitude. However, given that most dimensions of holes and soil disturbances (trails) occur in the scale of centimeters and that mound themselves have a diameter of ~1 meter, the detection probability of key features are lost when pixel size is increased. Based on our assessment, products derived at >5 cm pixel resolution are not able to detect holes and trails in the landscape. This rules out the possibility of using currently available datasets from the Texas Natural Resources Information System (TNRIS) at 15 cm pixel resolution for detecting and mapping TKR burrows, which was a primary purpose for this assessment. Although this information has not been made official, next generation Texas imagery datasets from airborne sources are targeting pixel resolutions of 5 cm and 8 cm (TNRIS, 2018) which is promising. No time frame is available on these projects.

As an alternative, estimating distribution and abundance of TKR populations could be supported by a combination of county level models and landscape scale models (such as the ones proposed in this study) and UAS derived products. For example, a user could generate a county level model for identifying suitable areas in Wichita County and then use a stratified random sampling approach to plan flight missions with an UAS. The UAS imagery could then be further analyzed to find mounds or exposed slopes in the landscape, and RGB imagery inspected for trails and burrow entrances at these specific locations. Costs associated with such an approach are low and can provide an approximation to abundance and distribution of a rare species. During the modeling approach, further refining and adapting the importance ranks and percents of influences of each available information layer can help improving the outputs. The modeling approach here applied is a simple method with limited data transformation required which highlights the predictive power of topographic and spectral information collected at high spatial resolutions.

Alternatively, an agency can also hire a specialized remote sensing company to plan an airborne campaign using a small airplane over suitable areas as detected by the County level approach. Although this a more expensive option, a customizable airborne campaign can collect <5 cm imagery over the entire suitable area and in addition, provide topographic information from advanced laser based systems such as LiDAR. Point-cloud products from LiDAR can provide multiple return data that can help separate vegetation structure from mound topographic properties and automatically detect holes in the ground.

Sub-surface information is possible to obtain using ground-penetrating radar (GPR) technologies. Radar penetrating technologies can provide information about the three-dimensional structure of burrow systems underground (Chandra and Tanzi, 2018). Data analysis of GPR data is more complex and requires higher computing processing capabilities, especially if generated over large areas. Drone-based GPR technologies are recent and few research teams and/or companies offer this as an integrated tool. However, such system will most likely be widely accessible in a few years and the possibilities will be greater for efficiently identifying and mapping TKR burrows at the landscape scale on a routine basis.

CONCLUSION

Our study highlights the importance of surface topographic information and vegetation fractional cover information for TKR burrow detection. Unmanned aerial systems are able to provide a customizable solution for providing the necessary datasets at the best spatial resolution possible. In our study, we found that TKR burrows can only be identified with <5 cm pixel resolution data which rules out the possibility of using high-resolution imagery data currently available at the state level. Alternatively, the use of NAIP imagery at 0.5 and 0.6 m pixel resolution in combination with resampled Digital Elevation data can provide effective means for identifying potential TKR burrow locations at the county level. This information can help when designing aerial campaigns or field surveys for efficient sampling. The data transformations for predicting the locations of potential TKR burrows are minimal and this is possible due to the high predictive power of topographic information such a slope and aspect in determining the location of mounds and exposed slopes. In addition, the integration of such data with 3D modeling capabilities enhances the user’s ability to explore the imagery and further inspect for the presence of holes and trails in terrain. We recommend the use of the county level approach as a supporting tool for understanding the potential TKR distribution in Texas and the use of UAS imagery for predicting and detecting TKR burrows at the site level. We also recommend testing alternatives approaches such as LiDAR and ground-penetrating radar technologies to further explore TKR habitats in Texas.

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FIGURES AND LEGENDS

Figure 1. Relative location of TKR data collection sites in north-central Texas. A total of 26 burrows have been visited and characterized from the Goetze Ranch. Sites #1 and Site #2 corresponded to properties managed by Dr. Jim Goetze.

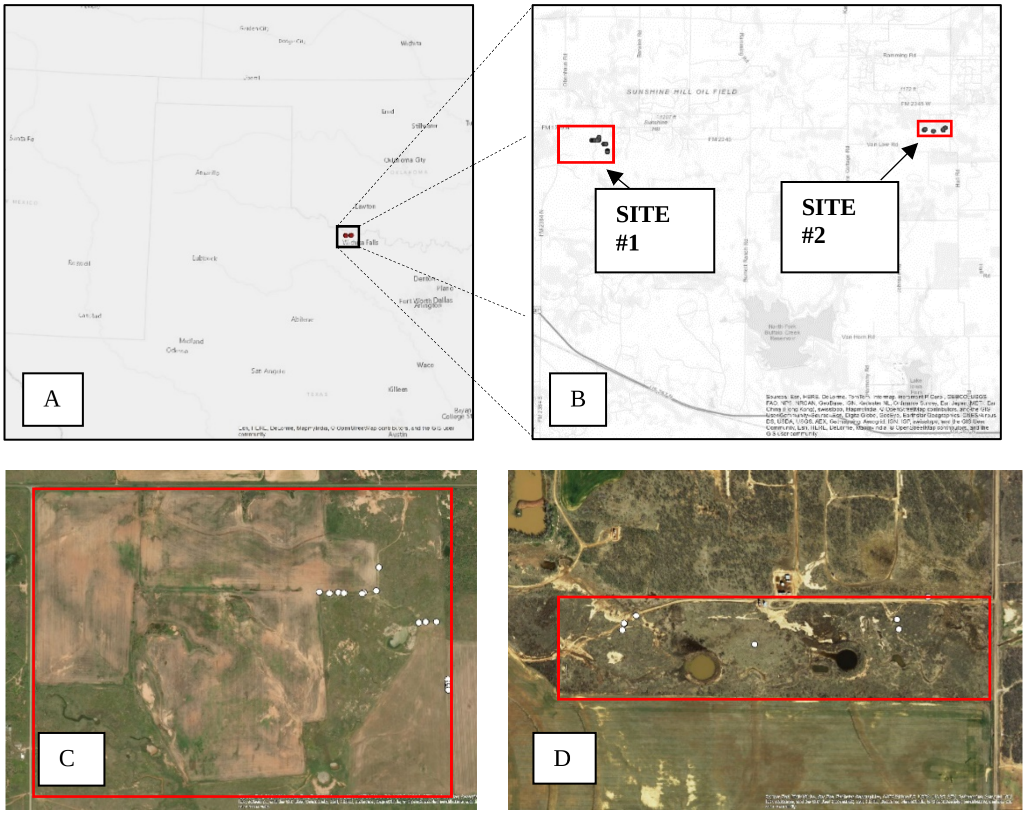
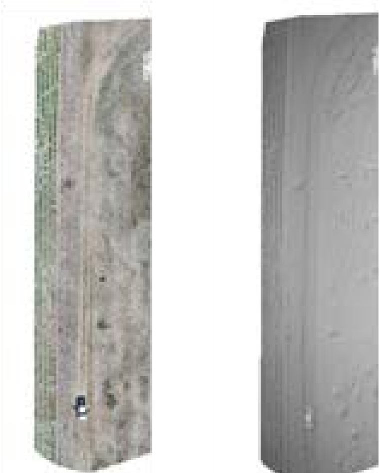
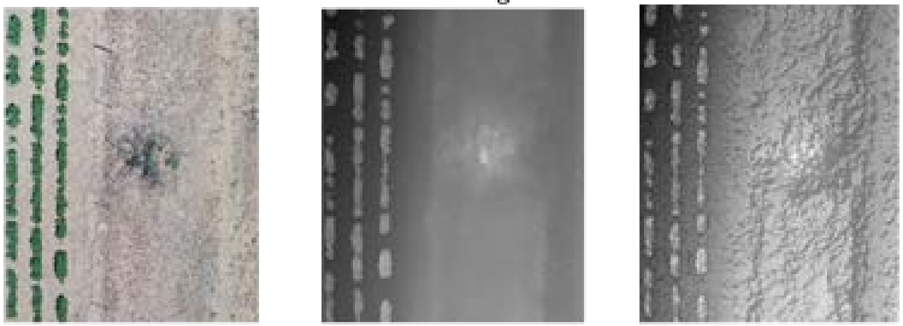


Figure 2. Examples of active burrows located at the Goetze Ranch showing entrances (holes), the predominance of exposed bare soil, and the occurrence of trails and loose or disturbed soil near the entrance.

Figure 3. Products generated from the UAV system: the RGB (red, green and blue) imagery and the digital surface models or DSM (elevation datasets). A hillshade model was derived from the DSM using ArcGIS. At the original pixel resolution (2.5 mm), trails and loose soil in the surface from TKR active burrows are evident. The DSM allows for the identification of mounds over the landscape.



RGB Scene from UAV   Digital Surface model



Subset of scene over a   Digital Surface model   Digital Surface model

TKR active burrow as seen from the UAV-   Digital Surface model   Digital Surface model

Figure 4. Diagram showing the workflow of the methodology for generating the landscape scale and the county level spatial models.

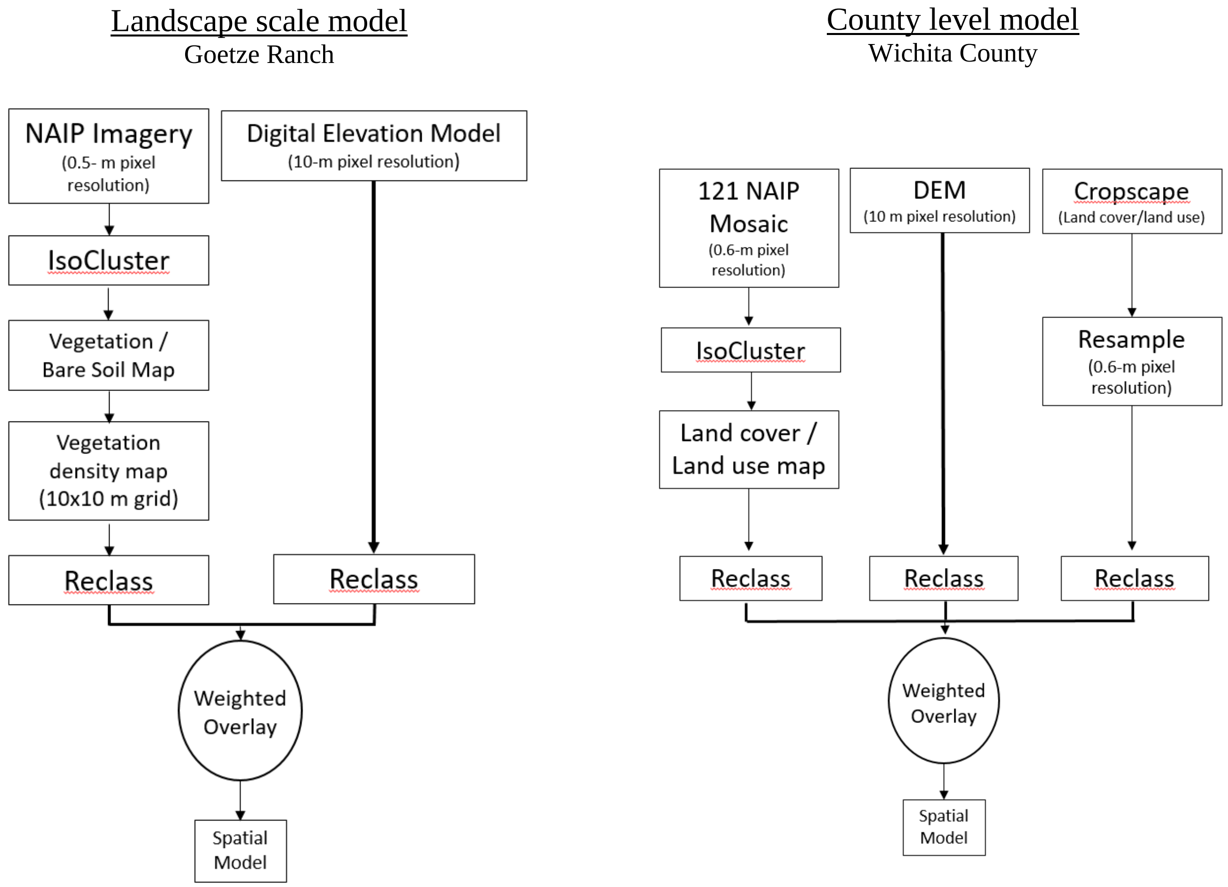


Figure 5. Flowchart showing the model framework based on UAS imagery. The model output is intended to predict potential locations of burrows from very high-resolution imagery (RGB and DEM).

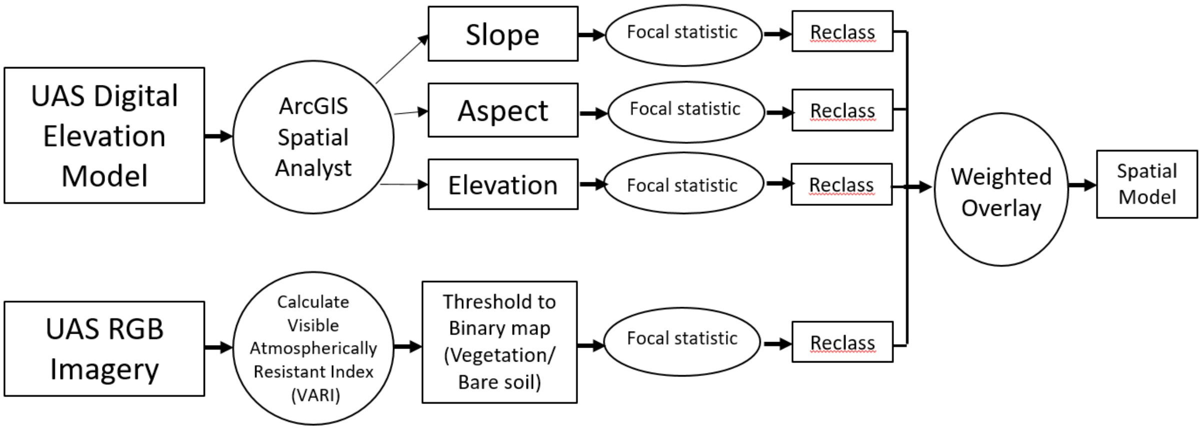


Figure 6. Modelling framework for detecting TKR burrows using UAS-derived RGB imagery and Digital Surface Model. Focal statistics were applied using a radius of 1 m. A weighted overlay model was implemented by setting weights to the values of each variable based on the information collected in the first stage. Red region in the output model graph shows the mound detected by automated methods.

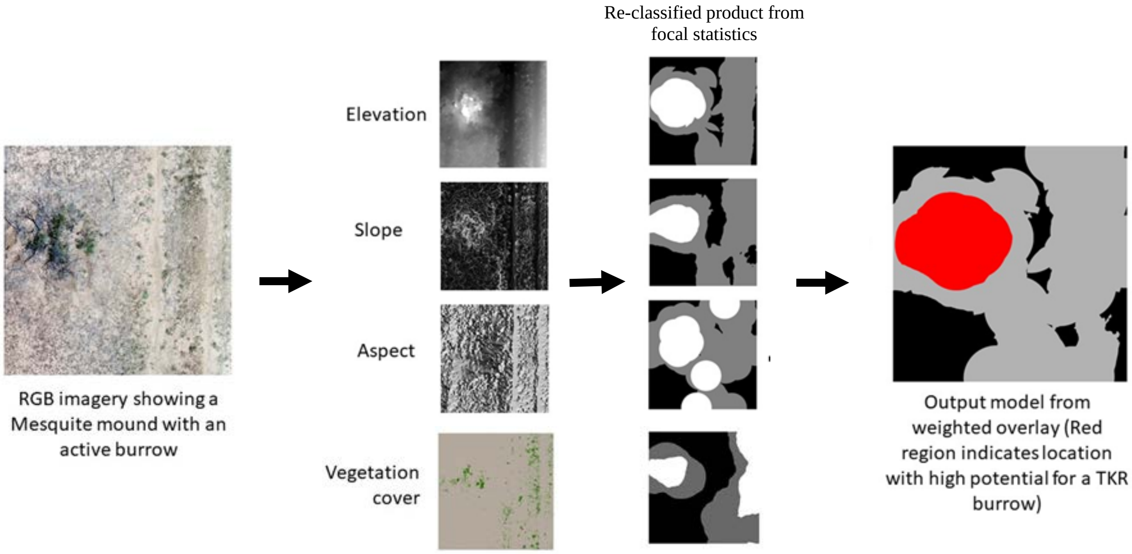
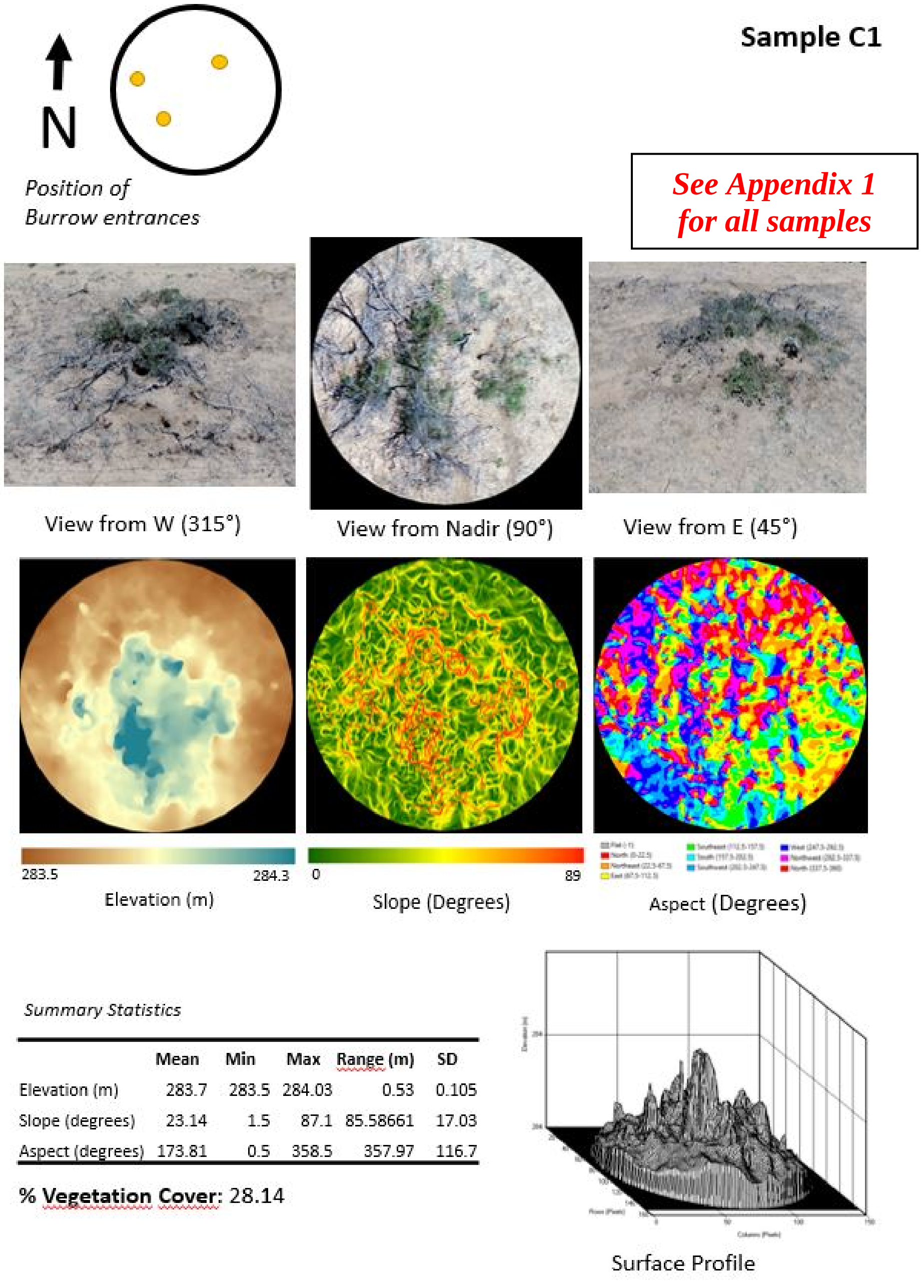


Figure 7. Graph summarizing the main characteristics of each sample. This graph integrates: a) the relative position of the burrow entrances in each mound; b) a snapshot of the RGB imagery from Nadir (90° from the surface plane), as well as views of the mound from 45° and 315° from the surface plane; c) representation of the mounds with digital surface model (elevation in m), slope and aspect products; d) summary statistics of elevation, slope, aspect and vegetation fractional cover and e) a 3D surface model of the mound shape as detected by the UAS photogrammetry processing. In Appendix 1, we show the same data for all eighteen (18) samples.



Summary Statistics

	Mean	Min	Max	Range (m)	SD
Elevation (m)	283.7	283.5	284.03	0.53	0.105
Slope (degrees)	23.14	1.5	87.1	85.58661	17.03
Aspect (degrees)	173.81	0.5	358.5	357.97	116.7

% Vegetation Cover: 28.14

Figure 8. Images of disturbed or loose soil by *D.elator* activity near the burrow entrance. These images correspond to UAS derived RGB imagery at 2.5 mm pixel resolution.

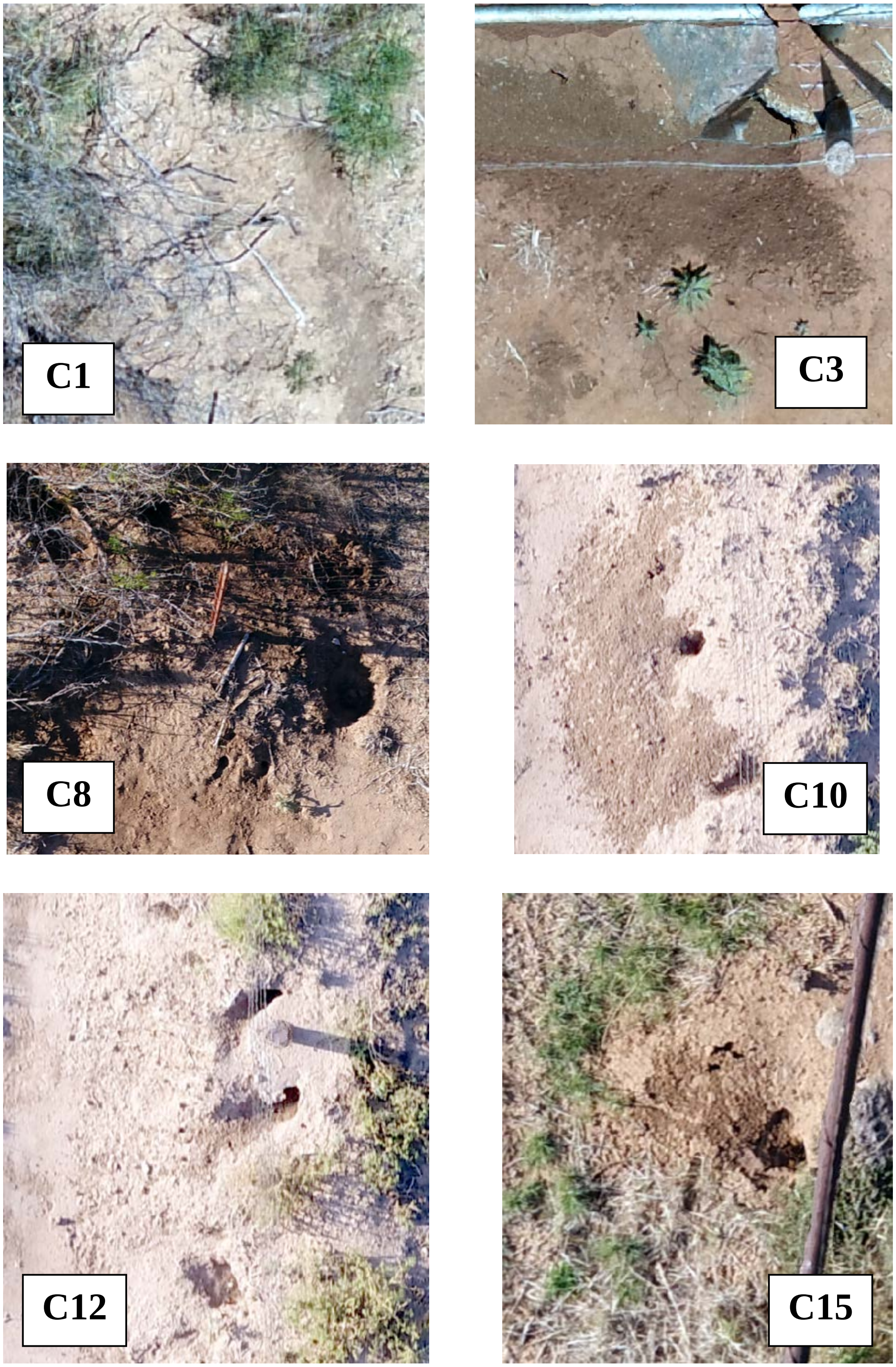


Figure 9. Remote sensing of mounds with active burrows at different spatial resolutions. In this figure, we show three conspicuous TKR burrows mapped at the UAV-based imagery at its original resolution (0.25 cm or 2.5 mm) and compared to resampled versions of coarser spatial resolution. At the 15 cm pixel resolution (same resolution of imagery provided by TNRIS), most physical features for identification of active burrows are lost.

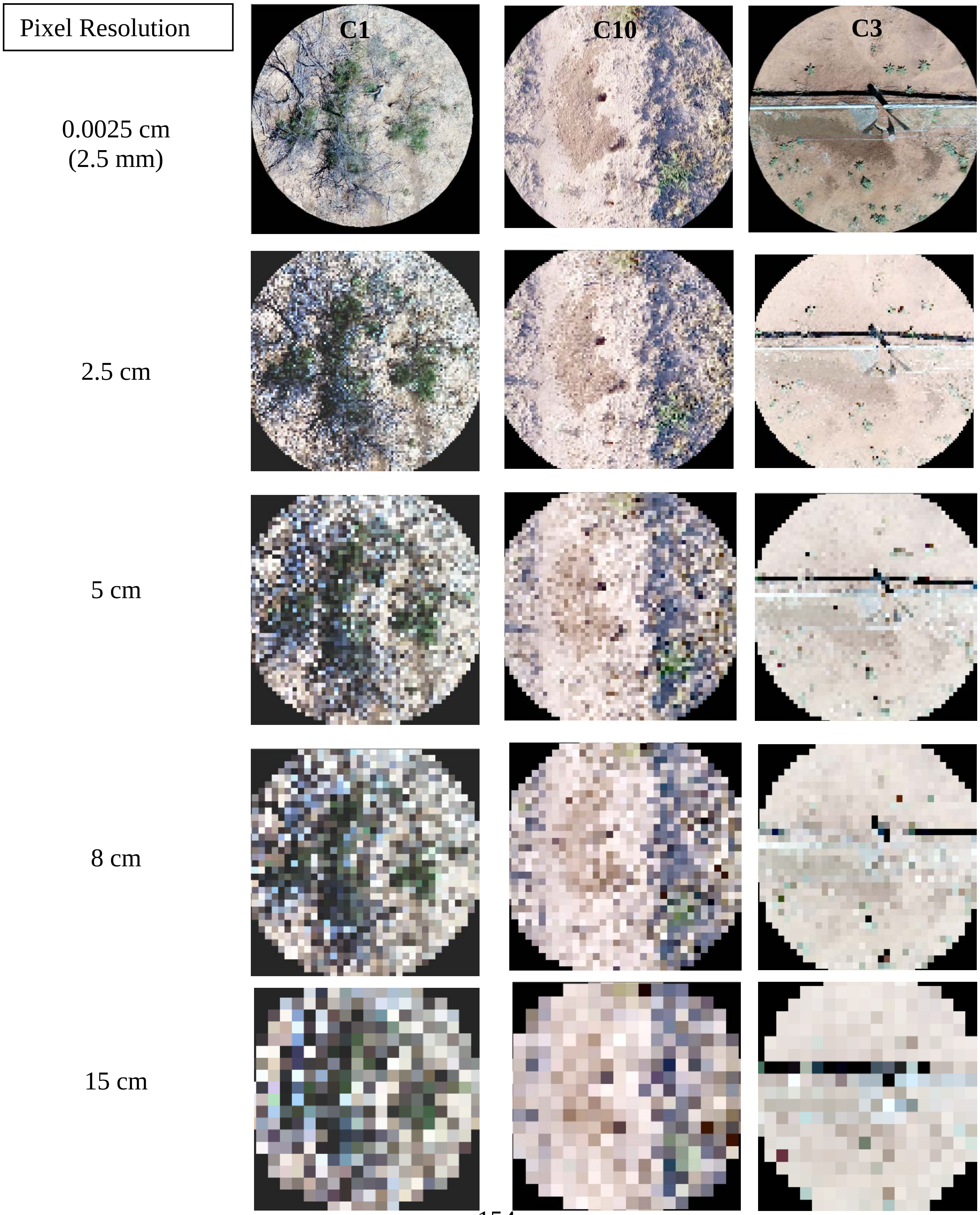


Figure 10. The landscape level model integrates vegetation cover and digital elevation to locate areas at higher elevation with sparse vegetation. The model is obtained by applying the weighted overlay layering method in ArcGIS using categorical maps of elevation and vegetation cover. Only a total of 7 from 41 burrows were far outside of the area predicted as suitable by the model, meaning that 83% of the points fell within the predicted suitable area or in its adjacencies in Site#1.

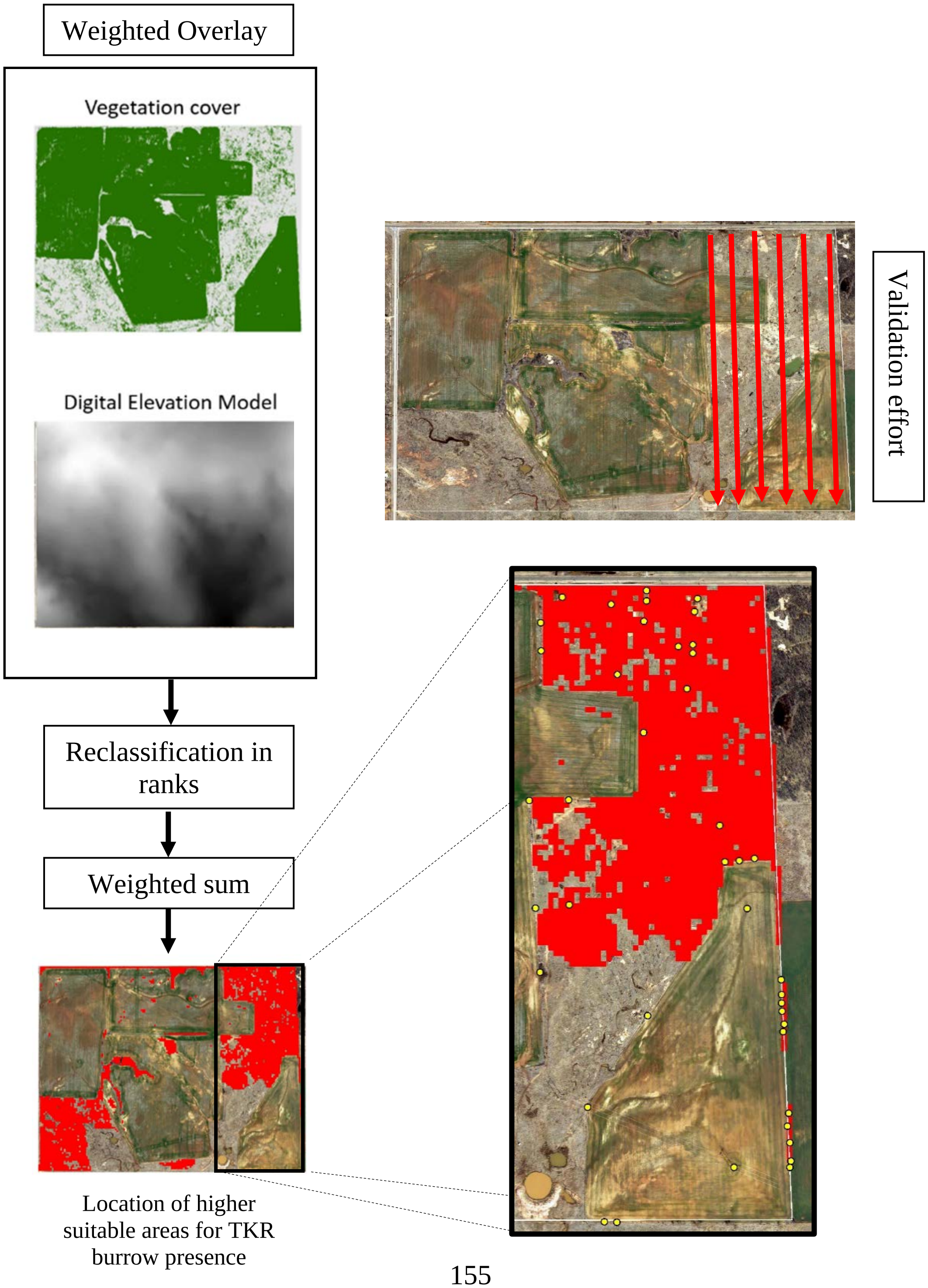
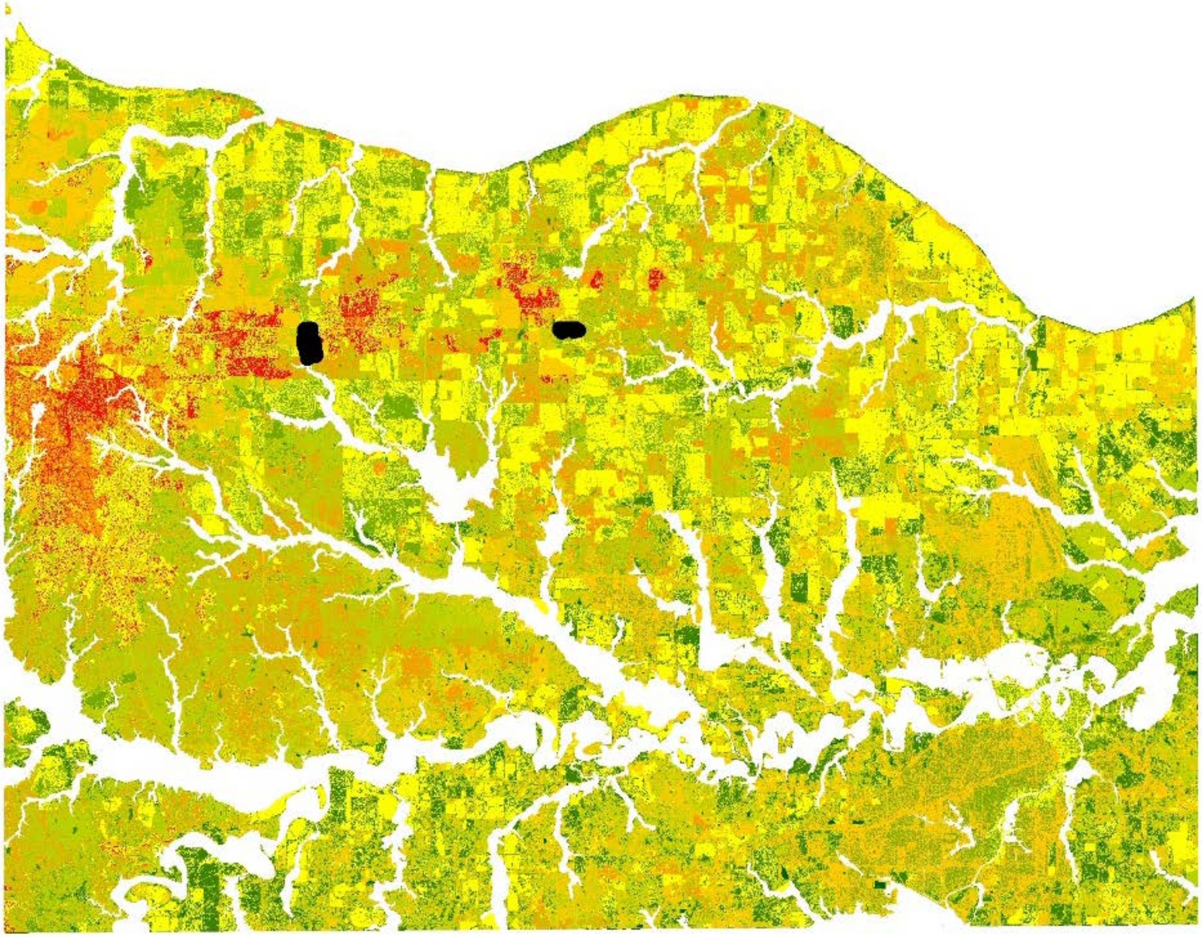


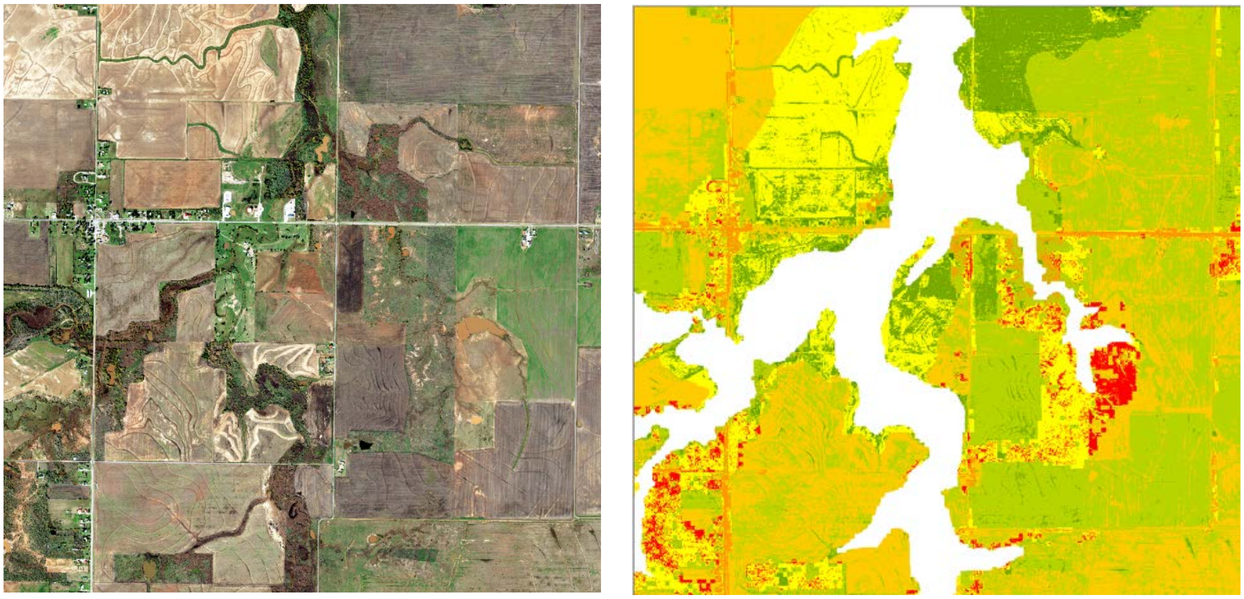
Figure 11. Output from county level model derived from land cover/land use maps generated from 121 NAIP imagery (mosaic) at 0.6 pixel resolution, Cropscape crop type data, digital elevation model products, and the National Flood Hazard Layer. The model predicts highly suitable areas in the northwestern portion of Wichita County demonstrating similarities with models generated in Chapter III. These areas can be surveyed using UAS imagery and topographic products at higher resolutions.



Suitability for Burrow presence (low[1] > high [9])

Wichita County

Figure 12. Depiction of a complex landscape at very high resolution using the county level model derived from land cover/land use maps generated from 121 NAIP imagery (mosaic) at 0.6 pixel resolution, Cropscape crop type data, digital elevation model products, and the National Flood Hazard Layer. The model highlights exposed terrain with low or sparse vegetation as suitable for TKR burrows.



Suitability for Burrow presence
(low[1] > high [9])

Figure 13. UAS derived predictive model based on a weighted overlay of the digital surface model, slope, aspect and vegetation fractional cover products. The site here presented corresponds to flight over the southeastern portion of the Site #1 in Goetze ranch. Four out of five confirmed TKR burrows appear in line with the predicted locations detected by the model.

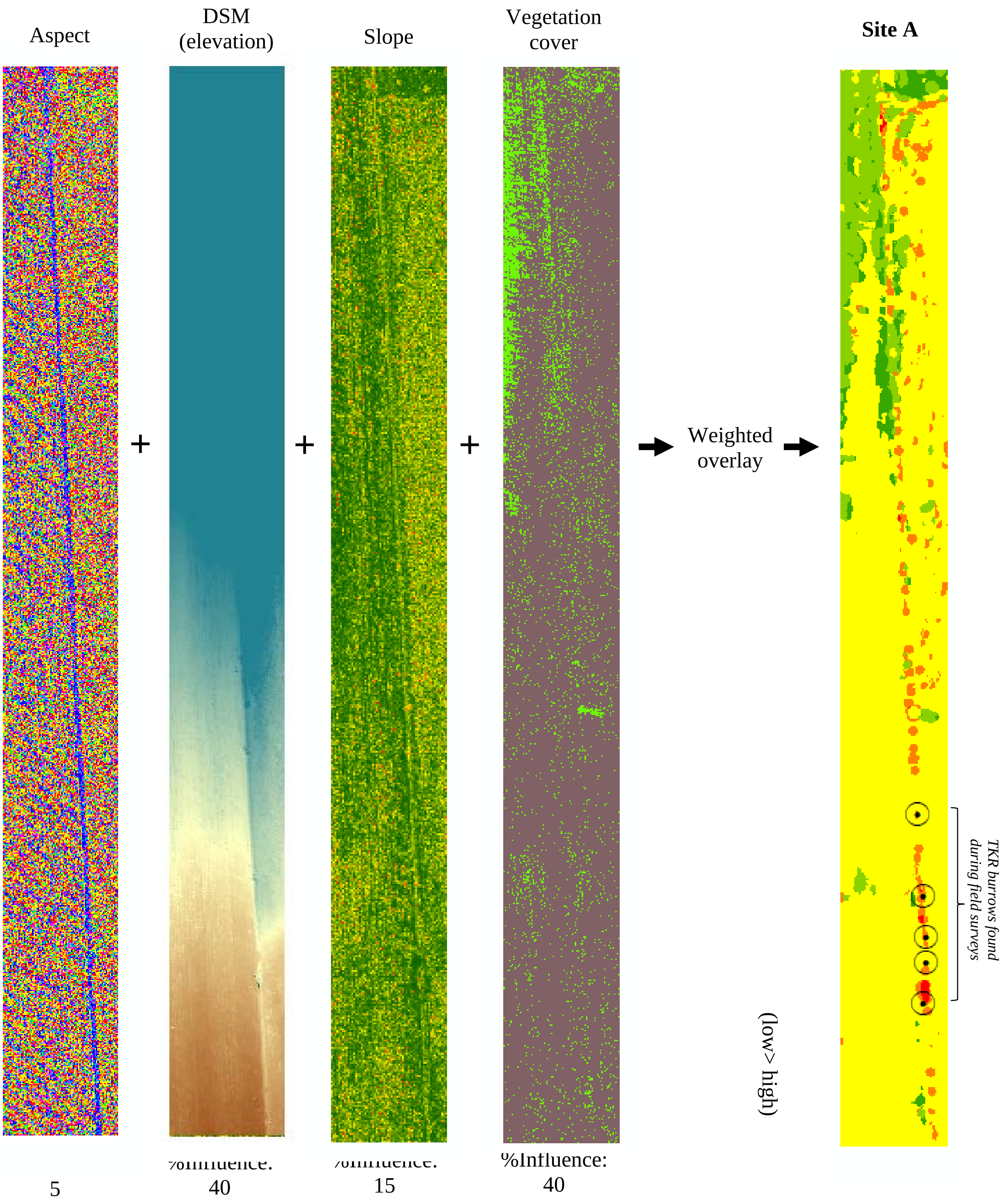
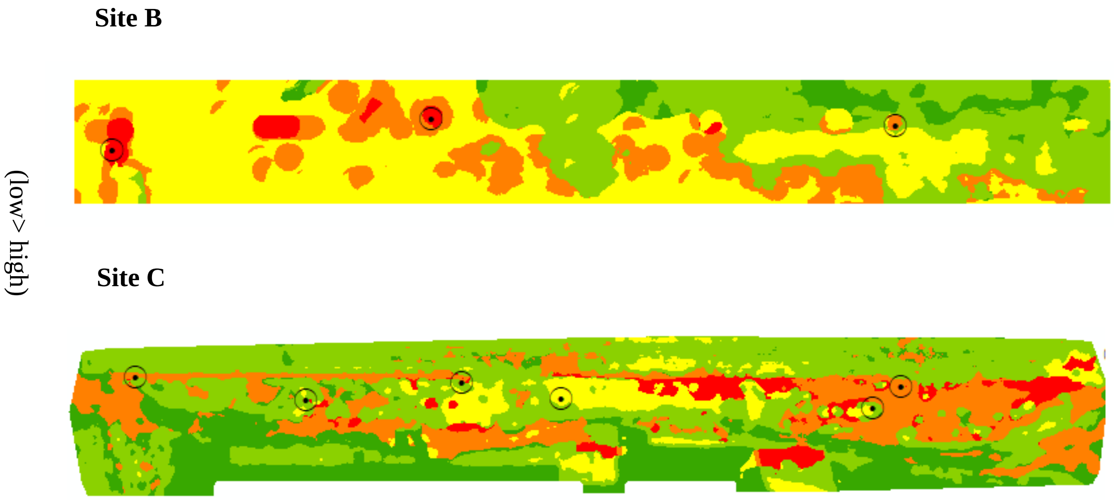
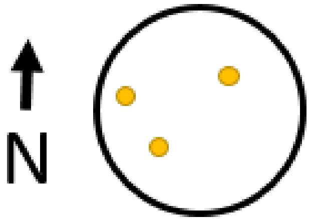


Figure 14. UAS derived predictive model based on a weighted overlay of the digital surface model, slope, aspect and vegetation fractional cover products. The site here presented correspond to UAS flights over Site B and Site C of the Site #1 in Goetze ranch. The model generated for Site B predicted the locations of three TKR burrows as observed in field inspections, while model generated for Site C only predicted the location of three out of six TKR burrows. Two of those, however, were located very close to highly suitable areas. The model generated for Site C was run without the DSM layer, and therefore, the variables available for this model were less than the other models here presented.



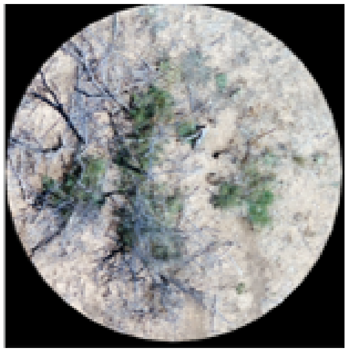
APPENDIX 1. SAMPLES C1-C20.

Sample C1

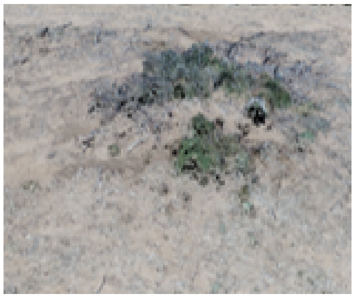


Position of Burrow entrances

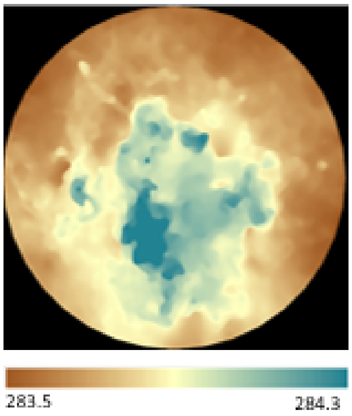
View from W (315°)



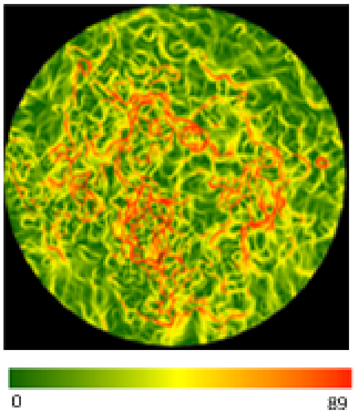
View from Nadir (90°)



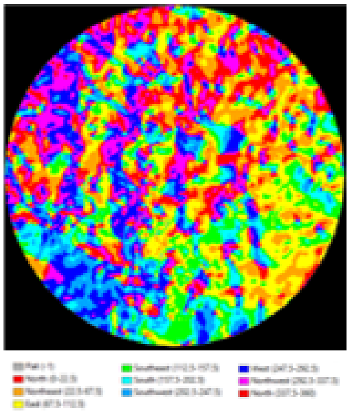
View from E (45°)



Elevation (m)



Slope (Degrees)

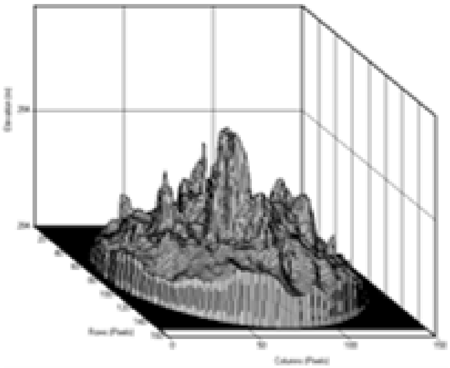


Aspect (Degrees)

Summary Statistics

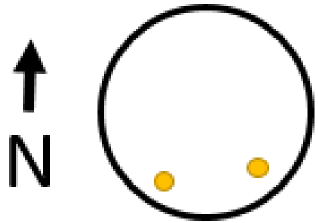
	Mean	Min	Max	Range (m)	SD
Elevation (m)	283.7	283.5	284.03	0.53	0.105
Slope (degrees)	23.14	1.5	87.1	85.58661	17.03
Aspect (degrees)	173.81	0.5	358.5	357.97	116.7

% Vegetation Cover: 28.14



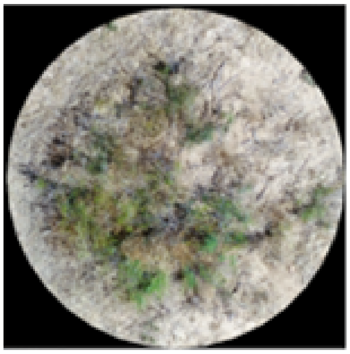
Surface Profile

Sample C2



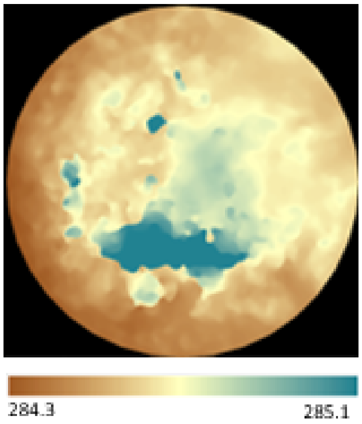
Position of Burrow entrances

View from W (315°)

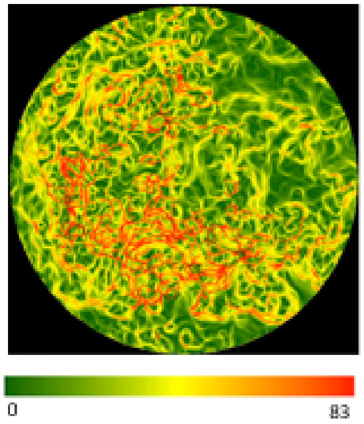


View from Nadir (90°)

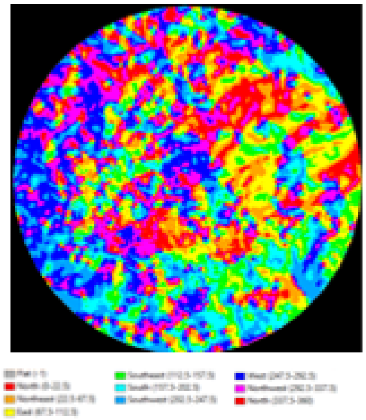
View from E (45°)



Elevation (m)



Slope (Degrees)

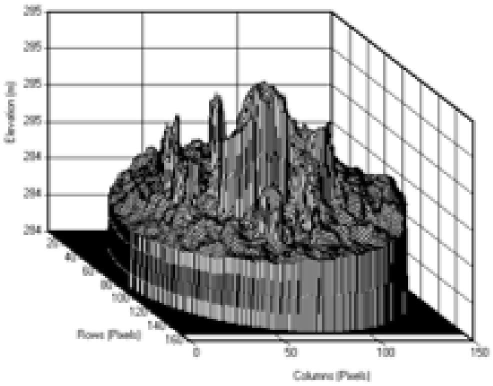


Aspect (Degrees)

Summary Statistics

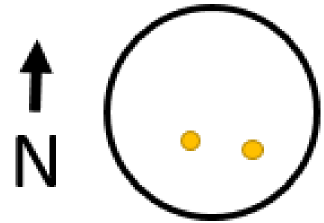
	Mean	Min	Max	Range (m)	SD
Elevation (m)	284.56	284.32	285.1	0.78	0.14
Slope (degrees)	20.43	0.53	82.87	82.34	17.26
Aspect (degrees)	198.2	5.09	351.89	346.8	99.96

% Vegetation Cover: 15.7



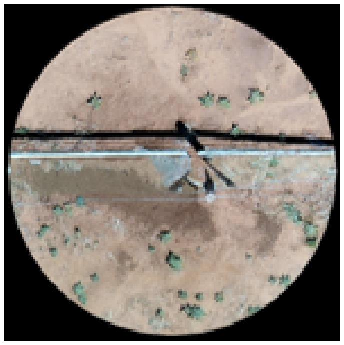
Surface Profile

Sample C3

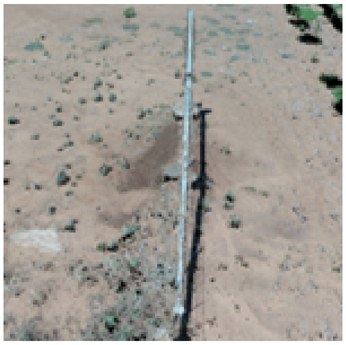


Position of Burrow entrances

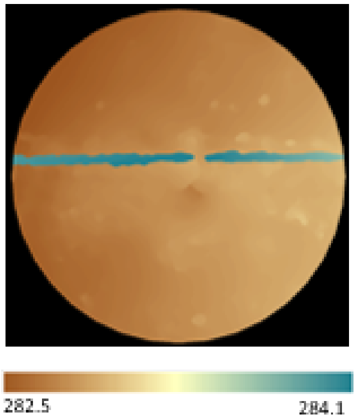
View from W (315°)



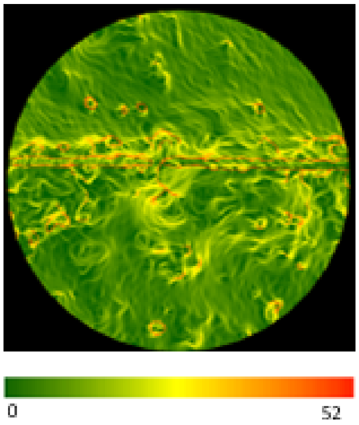
View from Nadir (90°)



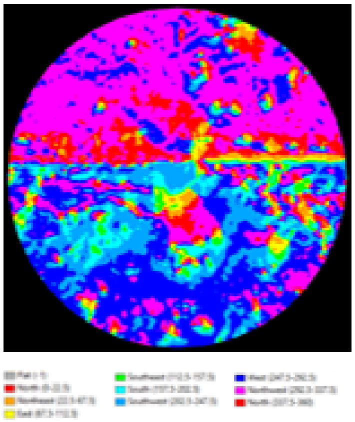
View from E (45°)



Elevation (m)



Slope (Degrees)

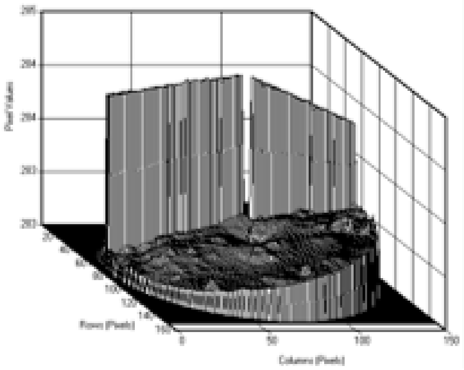


Aspect (Degrees)

Summary Statistics

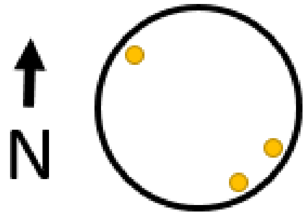
	Mean	Min	Max	Range (m)	SD
Elevation (m)	282.8	282.5	284.1	1.6	0.21
Slope (degrees)	14.47	0.43	51.64	51.21	10.11
Aspect (degrees)	266.04	8.42	340.4	332.04	64.95

% Vegetation Cover: 15.14

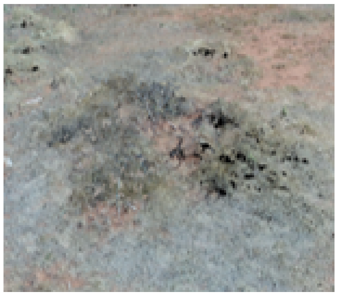


Surface Profile

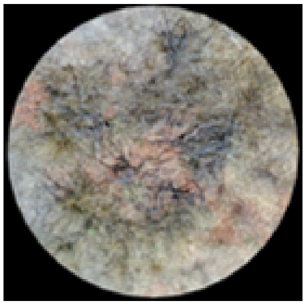
Sample C4



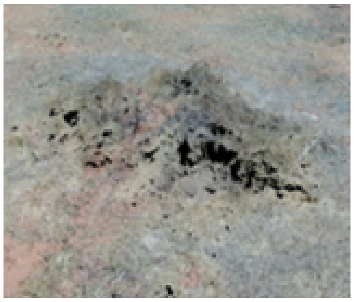
Position of Burrow entrances



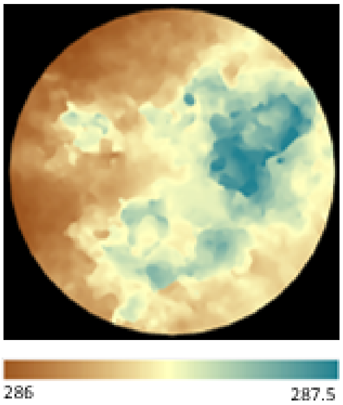
View from W (315°)



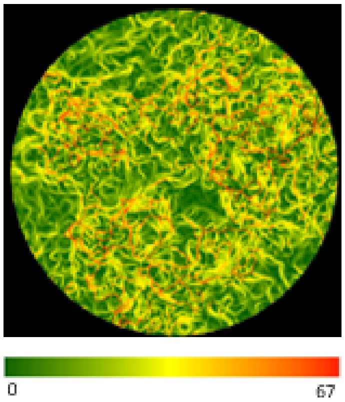
View from Nadir (90°)



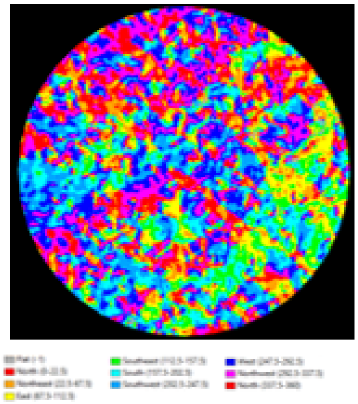
View from E (45°)



Elevation (m)



Slope (Degrees)

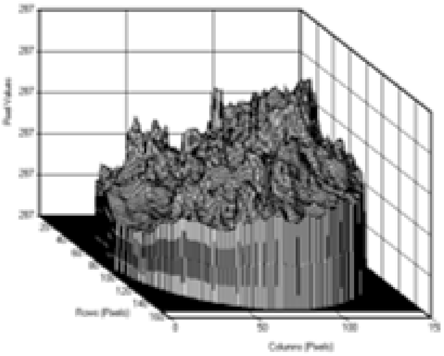


Aspect (Degrees)

Summary Statistics

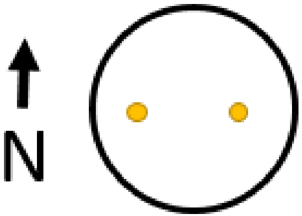
	Mean	Min	Max	Range (m)	SD
Elevation (m)	286.99	286.75	287.3	0.55	0.13
Slope (degrees)	22.7	0	67	67	22.7
Aspect (degrees)	235	0	355.78	355.78	95

% Vegetation Cover: 40.42



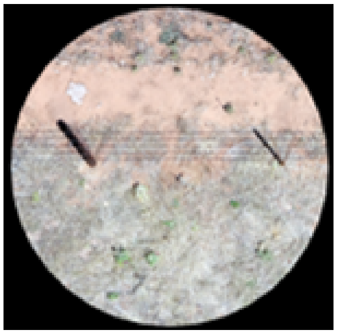
Surface Profile

Sample C5



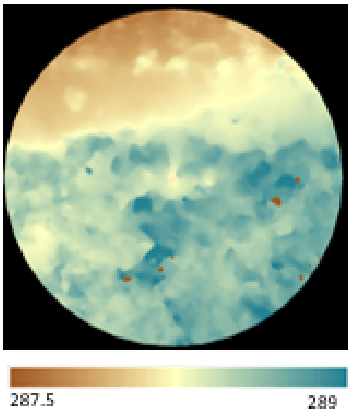
Position of Burrow entrances

View from W (315°)

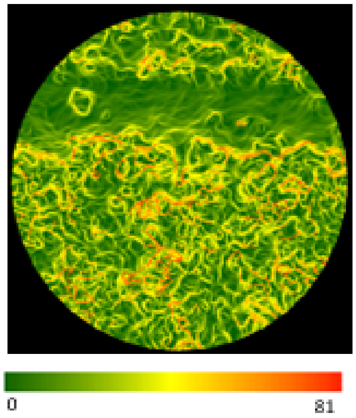


View from Nadir (90°)

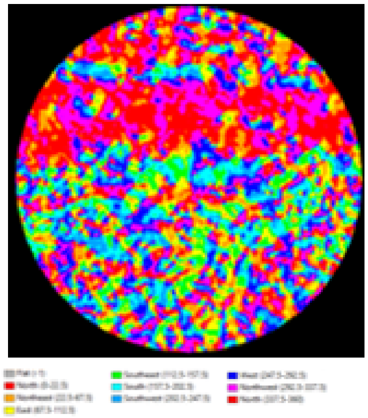
View from E (45°)



Elevation (m)



Slope (Degrees)

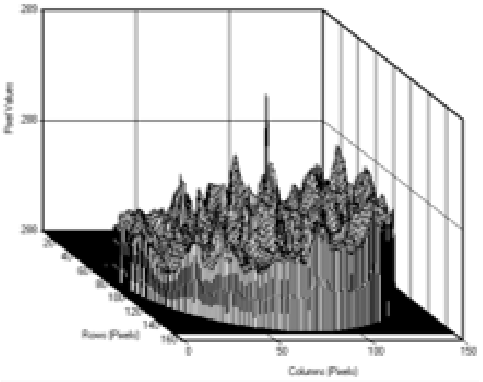


Aspect (Degrees)

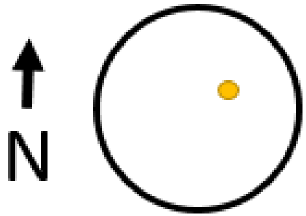
Summary Statistics

	Mean	Min	Max	Range (m)	SD
Elevation (m)	288.1	287.89	288.23	0.34	0.08
Slope (degrees)	19.38	1.23	80.07	78.84	15.25
Aspect (degrees)	211.02	3.91	356.67	352.76	126.2

% Vegetation Cover: 38.7



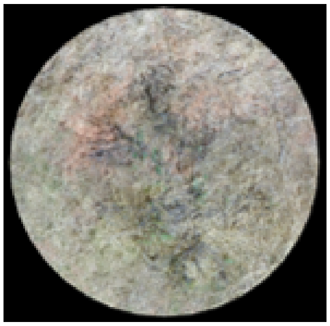
Surface Profile



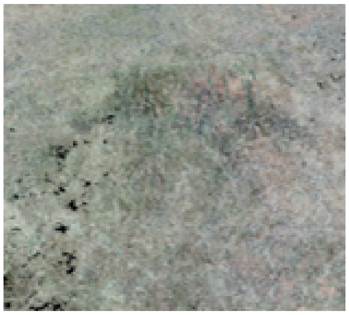
Position of Burrow entrances

Sample C6

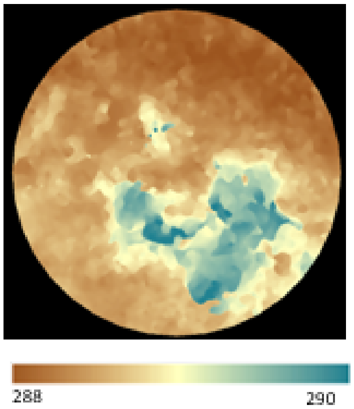
View from W (315°)



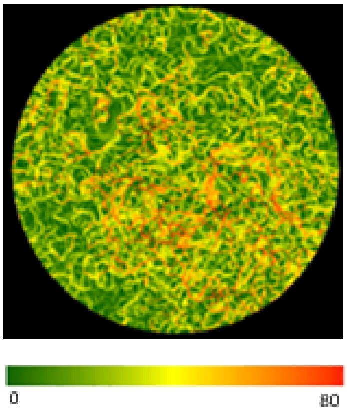
View from Nadir (90°)



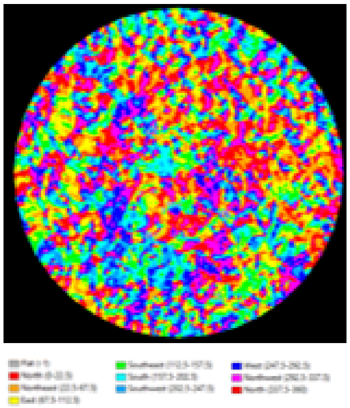
View from E (45°)



Elevation (m)



Slope (Degrees)

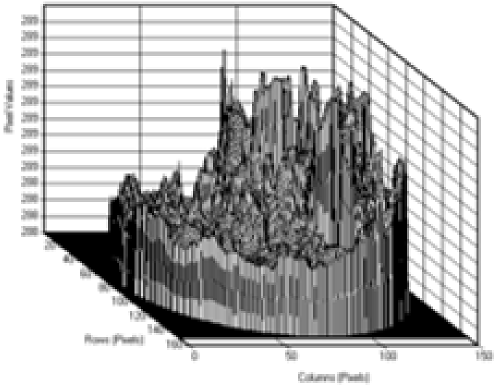


Aspect (Degrees)

Summary Statistics

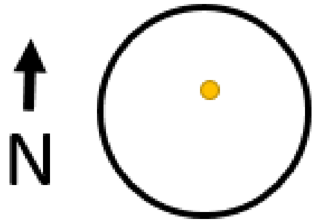
	Mean	Min	Max	Range (m)	SD
Elevation (m)	288.5	288.35	288.87	0.52	0.11
Slope (degrees)	22.3	1.09	77.93	76.84	18.66
Aspect (degrees)	180.01	2.48	359.49	357.01	108.94

% Vegetation Cover: 52



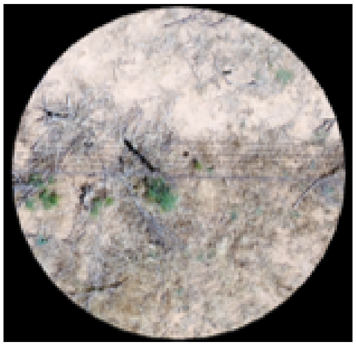
Surface Profile

Sample C7

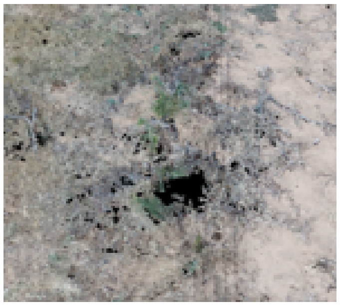


Position of Burrow entrances

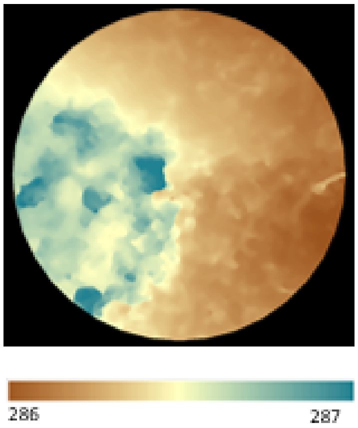
View from W (315°)



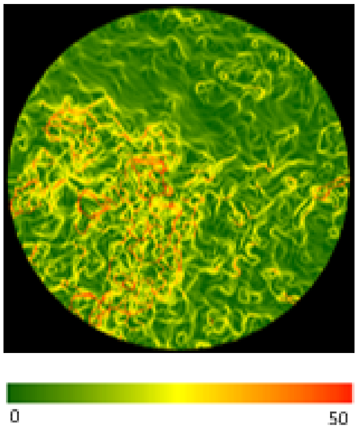
View from Nadir (90°)



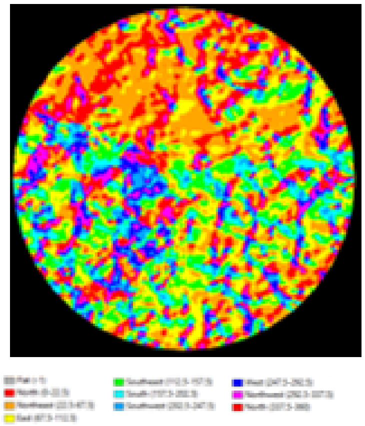
View from E (45°)



Elevation (m)



Slope (Degrees)

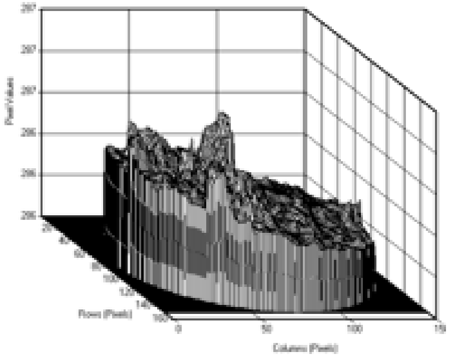


Aspect (Degrees)

Summary Statistics

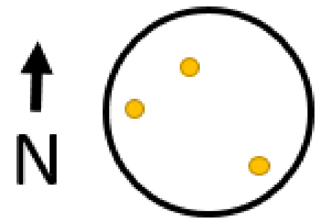
	Mean	Min	Max	Range (m)	SD
Elevation (m)	286.35	286.19	286.68	0.49	0.09
Slope (degrees)	17.68	1.85	47.68	45.83	12.96
Aspect (degrees)	132.71	2.2	351.07	348.87	103.11

% Vegetation Cover: 11.57



Surface Profile

Sample C8

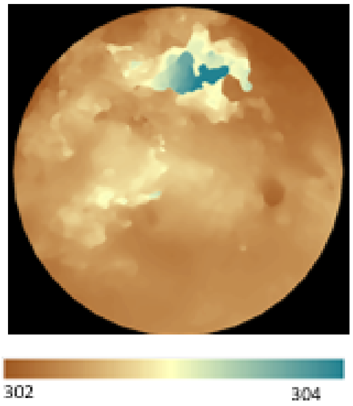


Position of Burrow entrances

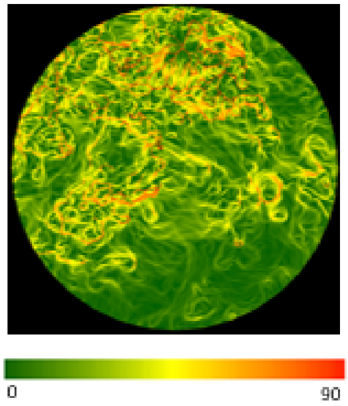
View from W (315°)

View from Nadir (90°)

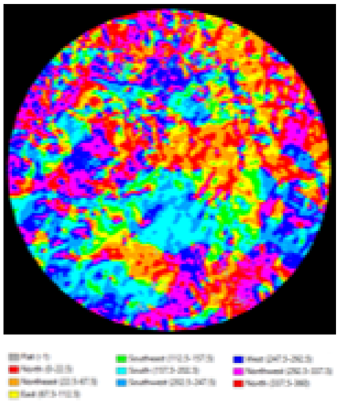
View from E (45°)



Elevation (m)



Slope (Degrees)

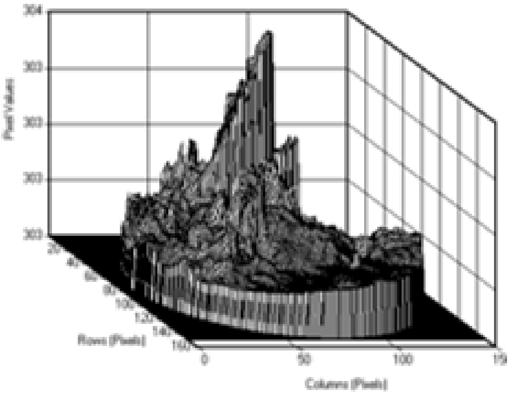


Aspect (Degrees)

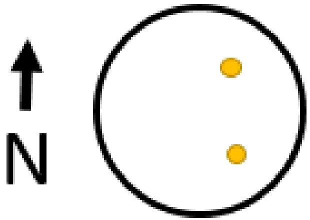
Summary Statistics

	Mean	Min	Max	Range (m)	SD
Elevation (m)	302.77	302.6	303.48	0.88	0.16
Slope (degrees)	20.6	0.47	89.21	88.74	17.05
Aspect (degrees)	212.02	4.11	357.17	353.06	93.94

% Vegetation Cover: 5.7



Surface Profile



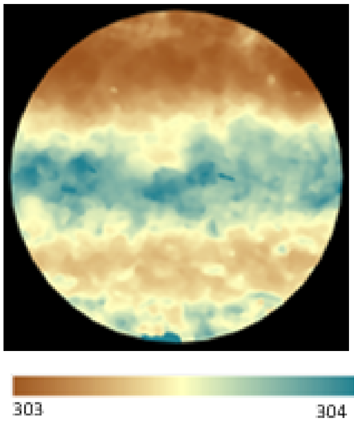
Position of Burrow entrances

Sample C9

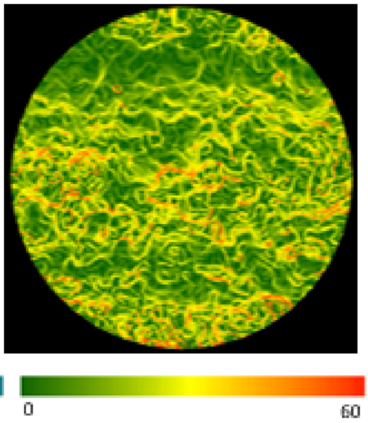
View from W (315°)

View from Nadir (90°)

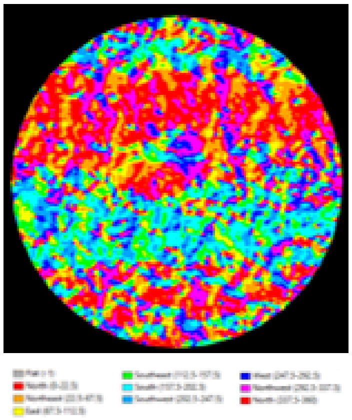
View from E (45°)



Elevation (m)



Slope (Degrees)

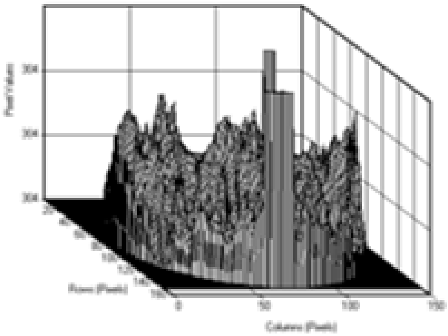


Aspect (Degrees)

Summary Statistics

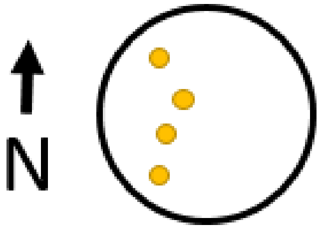
	Mean	Min	Max	Range (m)	SD
Elevation (m)	303.53	303.41	303.68	0.27	0.07
Slope (degrees)	18.97	1.27	57.59	56.32	12.23
Aspect (degrees)	172.72	0	359.15	359.15	115.36

% Vegetation Cover: 26.42



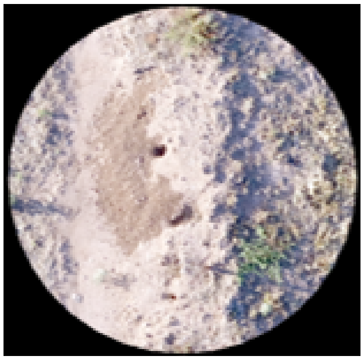
Surface Profile

Sample C10



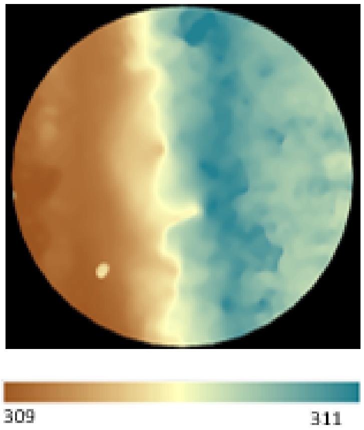
Position of Burrow entrances

View from W (315°)

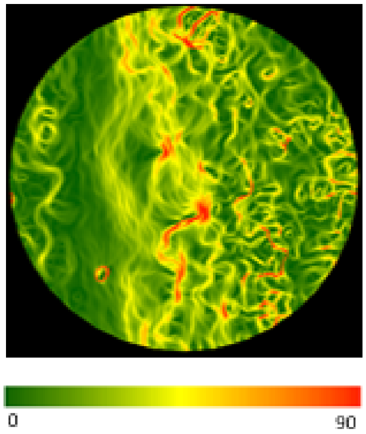


View from Nadir (90°)

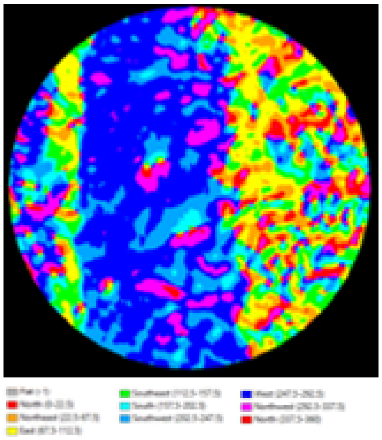
View from E (45°)



Elevation (m)



Slope (Degrees)

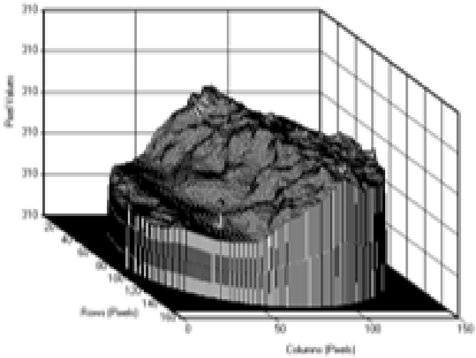


Aspect (Degrees)

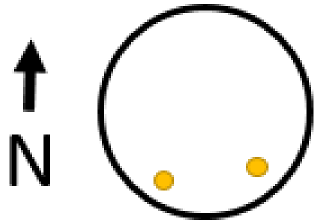
Summary Statistics

	Mean	Min	Max	Range (m)	SD
Elevation (m)	309.95	309.77	310.17	0.4	0.1
Slope (degrees)	14.12	0.22	86.53	86.31	12.39
Aspect (degrees)	193.5	3.7	344.05	340.35	98.6

% Vegetation Cover: 2.71



Surface Profile



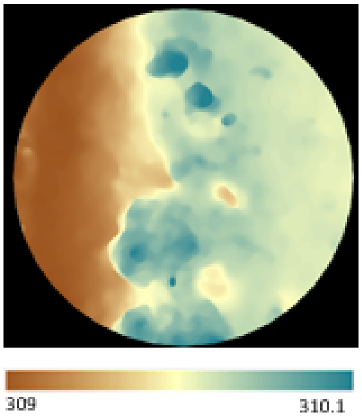
Position of Burrow entrances

Sample C11

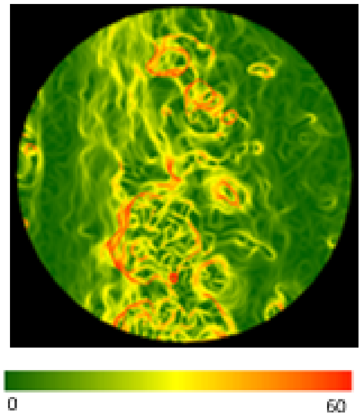
View from W (315°)

View from Nadir (90°)

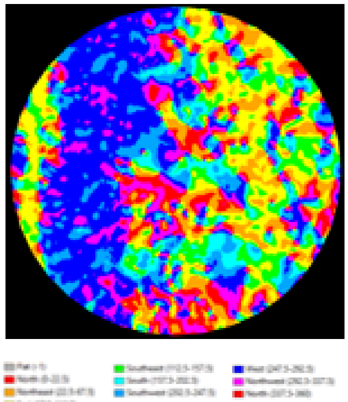
View from E (45°)



Elevation (m)



Slope (Degrees)

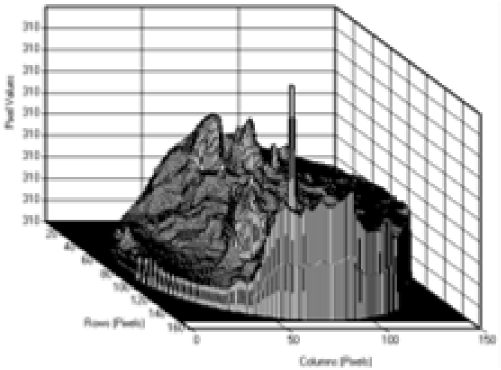


Aspect (Degrees)

Summary Statistics

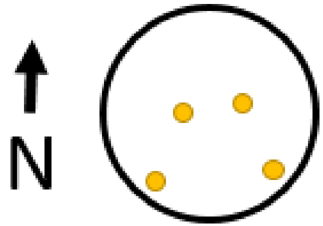
	Mean	Min	Max	Range (m)	SD
Elevation (m)	309.85	309.59	310.02	0.43	0.11
Slope (degrees)	15.32	1.05	56.14	55.09	12.78
Aspect (degrees)	206.03	3.03	357.9	354.87	94.92

% Vegetation Cover: 8.85



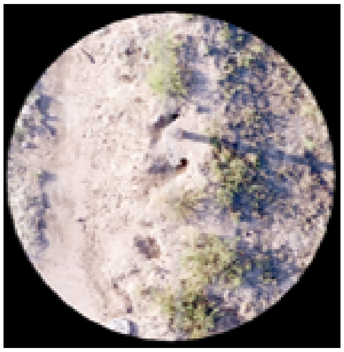
Surface Profile

Sample C12



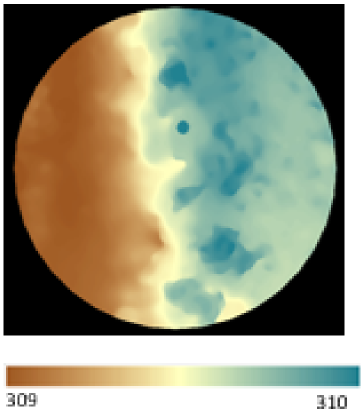
Position of Burrow entrances

View from W (315°)

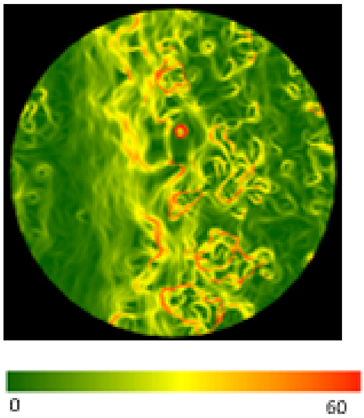


View from Nadir (90°)

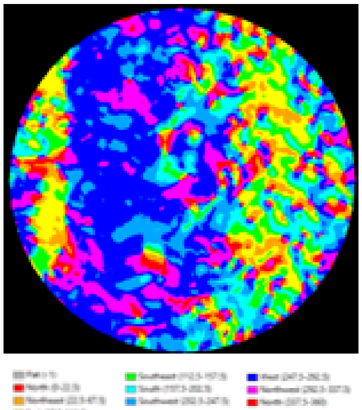
View from E (45°)



Elevation (m)



Slope (Degrees)

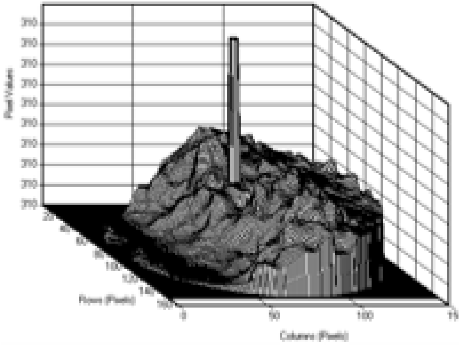


Aspect (Degrees)

Summary Statistics

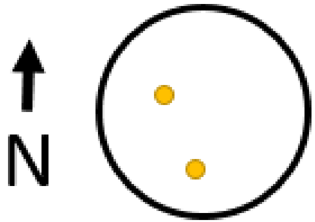
	Mean	Min	Max	Range (m)	SD
Elevation (m)	309.65	309.48	309.9	0.42	0.14
Slope (degrees)	15.66	0.28	59.24	58.96	14.14
Aspect (degrees)	199.21	0	352.04	352.04	91.96

% Vegetation Cover: 2.285

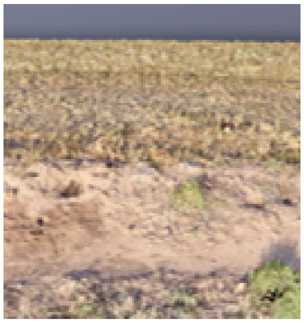


Surface Profile

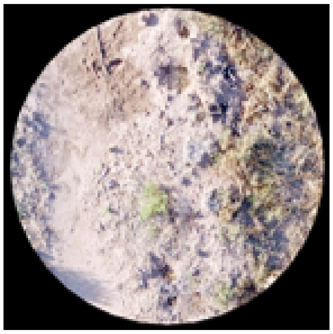
Sample C13



Position of Burrow entrances

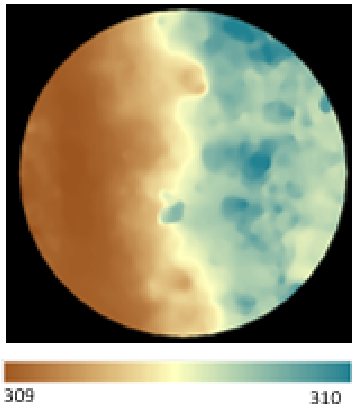


View from W (315°)

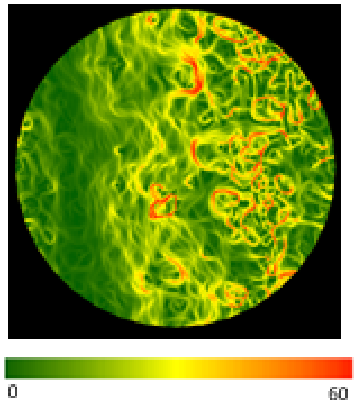


View from Nadir (90°)

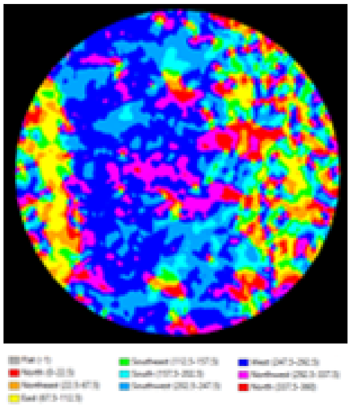
View from E (45°)



Elevation (m)



Slope (Degrees)

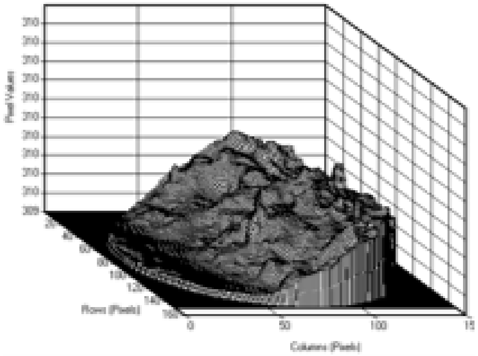


Aspect (Degrees)

Summary Statistics

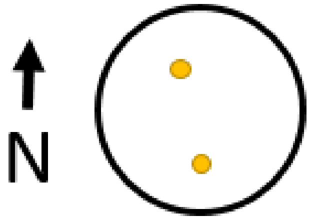
	Mean	Min	Max	Range (m)	SD
Elevation (m)	309.62	309.42	309.84	0.42	0.12
Slope (degrees)	14.44	0.6	52.75	52.15	11.79
Aspect (degrees)	200.1	1.19	358.15	356.96	96.73

% Vegetation Cover: 1.71

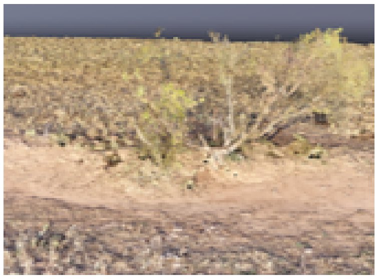


Surface Profile

Sample C14



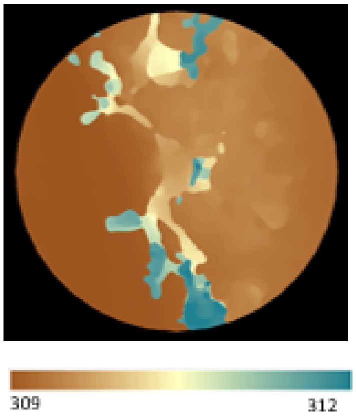
Position of Burrow entrances



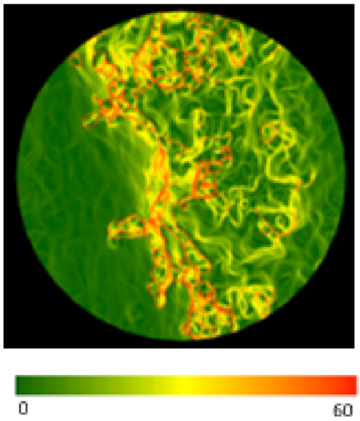
View from W (315°)

View from Nadir (90°)

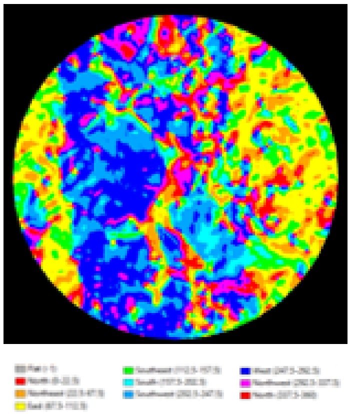
View from E (45°)



Elevation (m)



Slope (Degrees)

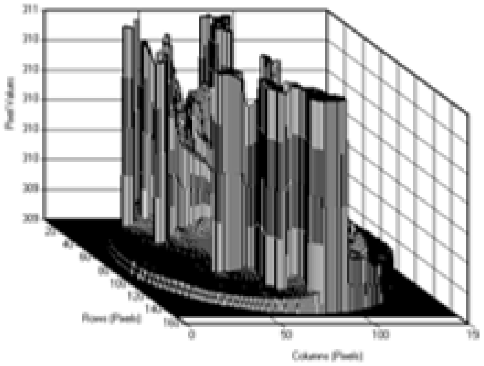


Aspect (Degrees)

Summary Statistics

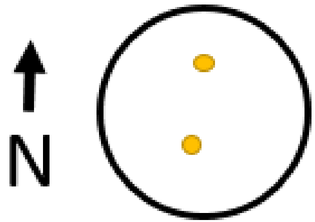
	Mean	Min	Max	Range (m)	SD
Elevation (m)	309.56	309.26	311.11	1.85	0.42
Slope (degrees)	12.75	0.28	53.31	53.03	11.68
Aspect (degrees)	167.4	7.29	349.21	341.92	94.38

% Vegetation Cover: 4.85



Surface Profile

Sample C15

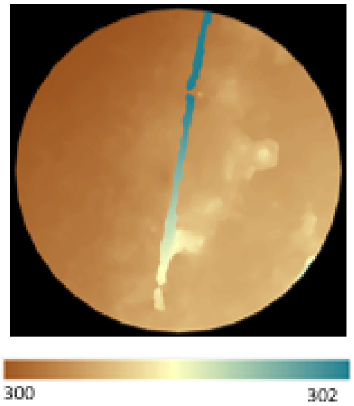


Position of Burrow entrances

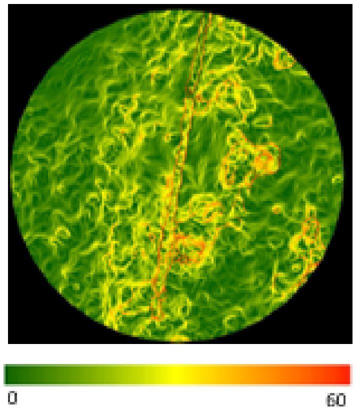
View from W (315°)

View from Nadir (90°)

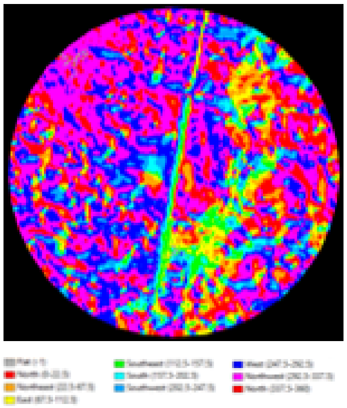
View from E (45°)



Elevation (m)



Slope (Degrees)

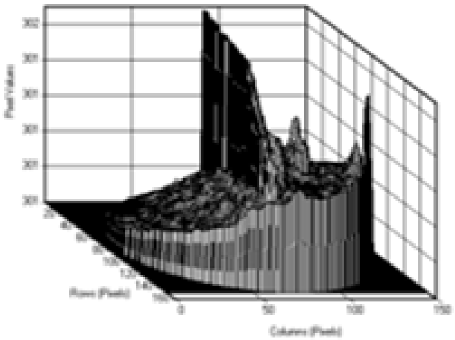


Aspect (Degrees)

Summary Statistics

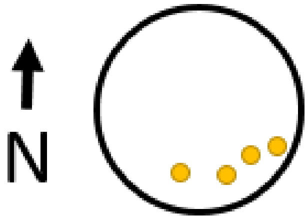
	Mean	Min	Max	Range (m)	SD
Elevation (m)	301.05	300.78	301.41	0.63	0.13
Slope (degrees)	19.39	0.95	57.5	56.55	14.57
Aspect (degrees)	250.7	1.21	358.42	357.21	89.58

% Vegetation Cover: 16.71



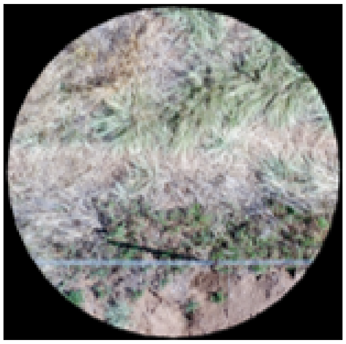
Surface Profile

Sample C16



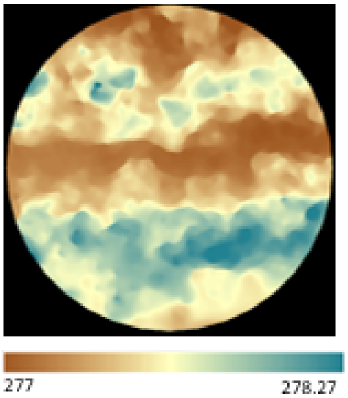
Position of Burrow entrances

View from W (315°)

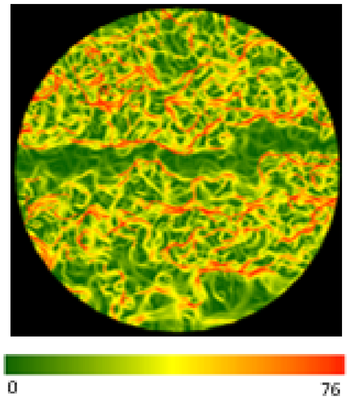


View from Nadir (90°)

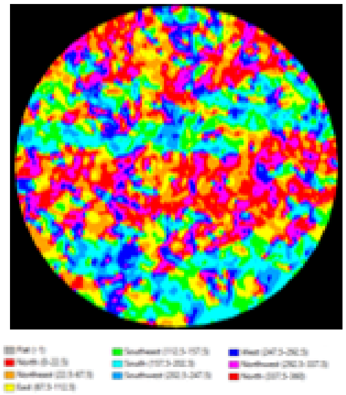
View from E (45°)



Elevation (m)



Slope (Degrees)

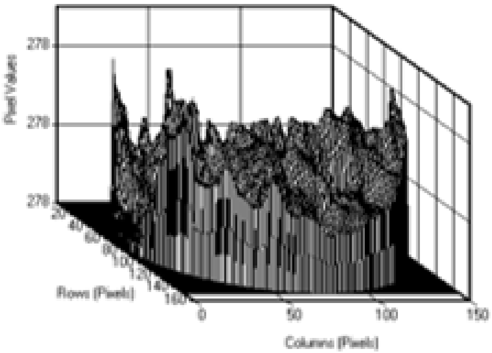


Aspect (Degrees)

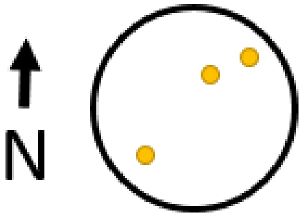
Summary Statistics

	Mean	Min	Max	Range (m)	SD
Elevation (m)	278.09	277.93	278.27	0.34	0.08
Slope (degrees)	26.08	1.43	75.14	73.71	18.73
Aspect (degrees)	193.62	2.34	358.27	355.93	112.07

% Vegetation Cover: 65.28



Surface Profile



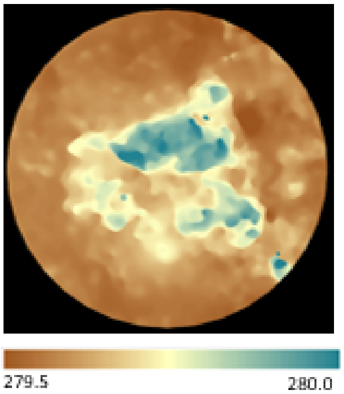
Sample C17

Position of Burrow entrances

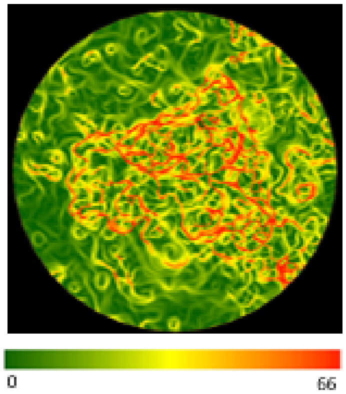
View from W (315°)

View from Nadir (90°)

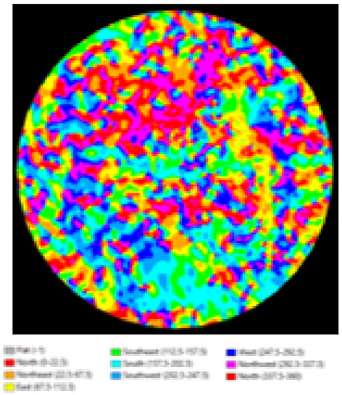
View from E (45°)



Elevation (m)



Slope (Degrees)

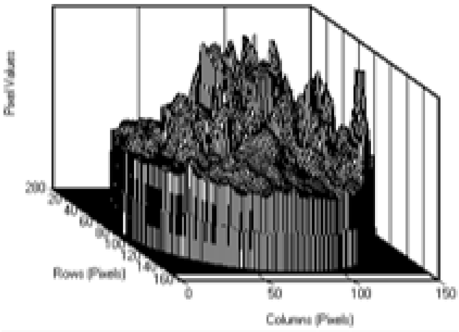


Aspect (Degrees)

Summary Statistics

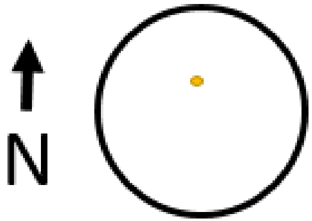
	Mean	Min	Max	Range (m)	SD
Elevation (m)	279.75	279.68	279.95	0.27	0.06
Slope (degrees)	14.84	0.23	65.92	65.69	16.55
Aspect (degrees)	200.3	1.46	358.95	357.49	105.98

% Vegetation Cover: 50.14



Surface Profile

Sample C20

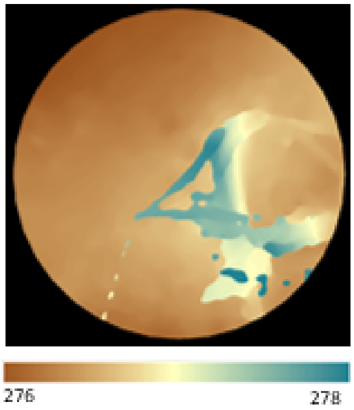


Position of Burrow entrances

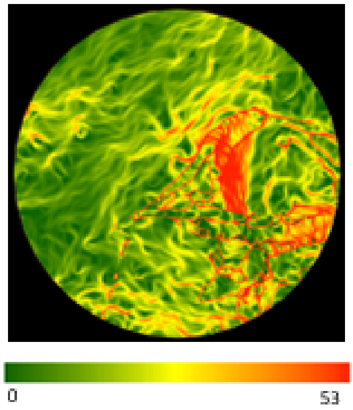
View from W (315°)

View from Nadir (90°)

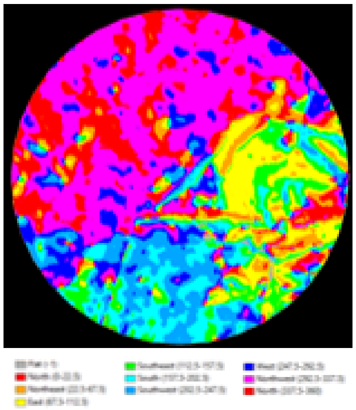
View from E (45°)



Elevation (m)



Slope (Degrees)

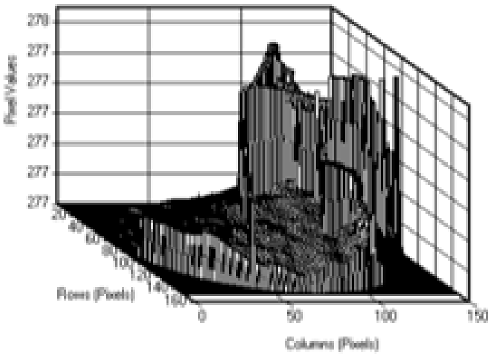


Aspect (Degrees)

Summary Statistics

	Mean	Min	Max	Range (m)	SD
Elevation (m)	276.81	276.52	277.64	1.12	0.19
Slope (degrees)	14.32	0.55	52.62	52.07	9.37
Aspect (degrees)	221.85	9.85	359.29	349.44	99.85

% Vegetation Cover: 39.85



Surface Profile

CHAPTER VII: SURVEY OF TWO LARGE PROPERTIES IN ARCHER COUNTY FOR TEXAS KANGAROO RATS

During summer of 2018 we were provided the opportunity to survey two properties in Archer County, one of undisclosed identity and one owned by Mr. Michael Lewis. Below is a short report of our findings.

<u>Property of Undisclosed Identity</u>.—At this property, we conducted two different sampling regimes in June 2018. In the first we laid down three sets of habitat transects. These are identical to those used for Chapter II. These are each 500-m transects and separated by 100 m to estimate *D. elator* distribution and abundance. Across all of these three sets of transects we caught 1 *Peromyscus leucopus*. In particular, we caught no *D. elator*.

The second sampling regime consisted of placing 43 10-trap transects throughout the property of undisclosed identity in places we felt were the highest probability of obtaining *D. elator*. Again we caught no *D. elator*. We did however catch 3 *Reithrodontomys* sp., 1 *Peromyscus* sp., and 1 *Baiomys taylori*.

<u>Property of Mr. Michael Lewis</u>.—At this property we laid down sets of 500m habitat transects in August at three different sites on the Lewis ranch. We caught 1 *Sigmodon hispidus* and no *D. elator*. We returned this fall to place 45 of the 10-trap transects to complete this survey. We captured 2 *Peromyscus maniculatus*. We did not capture any *D. elator*.

CHAPTER VIII: ESTIMATION OF THE PREDATOR-PREY RELATIONSHIP BETWEEN COYOTES AND TEXAS KANGAROO RATS.

Introduction

Variation among organisms regarding distribution and abundance is the product of interactions between a species and its abiotic and biotic world. Predation is an important density dependent biotic interaction that influences distribution and abundance of prey species and is often overlooked or underappreciated by management and or conservation planners. For example, predation can make the realized niche, and hence distribution, of a species much smaller than the fundamental niche when the presence of predators causes the local extinction of species. Better understanding of the influence of predate-prey dynamics is necessary when attempting effective management and conservation plans.

Little is known regarding predator-prey interaction of *Dipodomys elator*. Although the Texas kangaroo rat likely has a number of predators (Dalquest and Horner 1984) there are no published reports of predation on this species. Coyotes are important predators on many rodent species (Kitchen et al. 1999, Neale and Sacks 2001) and there are numerous accounts of effects of predation on kangaroo rats (Henke and Bryant 1999, Neale and Sacks 2001, Nelson et al. 2007). Herein we report results from examination of a large series of coyote scat samples coming from within the geographic range of the Texas kangaroo rat to estimate frequency of predator prey interactions with coyotes.

Materials and Methods

We obtained from Dr. Robert Martin and Mr. Ken Matocha 590 coyote scats. Scats were collected from 1985 to 2000 and came from the Holcomb Ranch in Hardeman County, Texas. Although there is no published location of this ranch, it was the site of the North and South Grids where Martin (2002) conducted his population work on *D. elator*.

Scats were first washed and then the remaining seeds, stems, bones and other substances such as insect chitin were examined. Uncleaned scats were tied inside hose (L'eggs kneehighs, L'eggs. Winston-Salem NC). Groups of approximately 50 scats were presoaked in warm water for approximately 30 minutes and then placed in a washing machine (Kenmore XXX) and washed (no detergent) based on a heavy soil washing cycle in cold water with 1,2 cup generic laundry detergent with two rinses. Rinsed scats were then placed into paper sacs and then into a drying oven at 50^{O}C degrees for 24 hours.

After washing and drying, scats were examined for remains of Texas kangaroo rats. Each scat was carefully disassembled and any bones or other remains, in particular mandibles and teeth were further examined to determine if they came from *D. elator*. Bones and teeth were compared to those from a reference collection of mammal species occurring Hardeman County assembled at Texas Tech University.

Results

From the 590 scats coming from the Holcomb ranch, we found no remains of D. elator. Coyotes likely consume Texas kangaroo rats but frequency is very low and below the ability to detect given 590 scats. Coyotes likely only rarely prey on Texas kangaroo rats at the Holcomb ranch.

Discussion

Based on the 590 samples we obtained, coyotes likely only rarely prey on Texas kangaroo rats at the Holcomb ranch. Despite this finding, other kinds of predators such as snakes, meso-carnivorous mammals likely do. For example, when together, kit foxes consume more kangaroo rats and coyotes more rabbits in central California. Moreover, badgers, foxes snakes and owls are important predators on kangaroo rats in Texas (Schmidly and Bradley 2016). Two of the most common predators that occur within the geographic range of *D. elator* are coyotes and owls. Because neither of these (coyotes: our study, owls: Stangl et al. 2005) kinds of predators likely do not commonly prey on Texas kangaroo rats, predation pressure on this species is likely light. Based on these findings control or manipulation of coyote populations within the geographic range of *D. elator* likely would not have a large direct and positive effect on Texas kangaroo rats.

Literature Cited

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Kitchen, A. M., E. Gese and E. R. Schauster. 1999. Resource partitioning between coyotes and swift foxes: space, time and diet. Canadian Journal of Zoology 77: 1645-1656.

Neale, J. C. C. and B. N. Sacks. 2001. Resource utilization and interspecific relations of sympatric bobcats and coyotes. Oikos 94: 236-249.

Nelson, J. L., B. L. Cypher, C. D. Bjurlin and S. Creel. 2007. Effects of habitat on competition between kit foxes and coyotes. Journal of Wildlife Management 714: 1467-1475.

Stangl, F. B., Shipley, M. M., Goetze, J. R. and Jones, C. 2005. Comments on the predator-prey relationship of the Texas kangaroo rat (Dipodomys elator) and barn owl (Tyto alba). American Midland Naturalist 153: 135-141.

CHAPTER IX: MANAGEMENT RECOMMENDATIONS FOR THE TEXAS KANGAROO RAT

Results from our research have revealed a number of important insights regarding ecology of *D. elator*. Based on these findings, we highlight a number of potential management strategies that we believe may be effective for conserving this species moving forward.

1) Management implications of one or two genetic populations.
2) First, our road surveys support prior work that suggested that Texas kangaroo rats can be active on unpaved county roads at night, or at the very least use field habitats immediately adjacent to these roads. This, combined with evidence that *D. elator* may not be occupying all suitable habitat across the region, highlights the potential importance of targeting county roads in management plans. Specifically, road grading may provide suitable habitat due to the creation of raised, bare ground that could promote burrow creation and occupancy by this species. Furthermore, continued maintenance of roadsides, such as clearing or reducing vegetation cover in the interstitial areas between the roads and adjacent fields, may facilitate access to unpaved county roads and thereby promote movement of individuals between patches of suitable habitat.
3) Although we encountered large amounts of variation in the amount of vegetation cover across our sites, we did encounter *D. elator* at sites with less forb (e.g., western ragweed; *Ambrosia psilostachya*) and shrub (e.g., honey mesquite; *Prosopis glandulosa*) cover. Restoration practices that open up more bare ground, such as prescribed burns or increased grazing pressure, may help improve habitat quality and potentially facilitate re-establishment of *D. elator* in areas where it was previously documented but where we did not encounter it during our own surveys. In particular, Copper Breaks State Park has seen a

dramatic increase in ground cover over the last two decades. A resident population used to exist at Copper Breaks State Park (Martin 2002) that has subsequently disappeared (Goetze et al. 2015, our study). Copper Breaks State Park should be managed, with fire or grazing or both, to open up ground cover to make it more habitable for Texas kangaroo rats. The Texas Parks and Wildlife Department should consider translocating a group of Texas Kangaroo rats to Copper Breaks State Park, only after ground cover reductions, but from a population as adjacent as possible and not from the captive colony jointly managed by the Texas Parks and Wildlife department and the Fort Worth Zoo.

4) Results from multiple sections of our research and that of others (Roberts and Packard 1973), Martin and Matocha 1991) highlight the importance of soil type as a determinant of where *D. elator* is able to persist. As such, we recommend that any management practices (e.g., prescribed fire) should be targeted for areas with suitable soil. While our results suggest some flexibility of Texas kangaroo rats to use a range of loamy soils, there still appears to be a specific range of soils across which the species can persist. Many sandy areas exist within the geographic range of the Texas kangaroo rat and we suggest that management plans be focused on areas of loamy and not sandy soils.

5) The four major large-scale surveys of Texas Kangaroo Rats (Jones et al. 1988, Martin 2002, Nelson et al. 2011, our study) taken together demonstrate considerable distributional dynamics of this species through time. Each of these four studies have demonstrated a different distributional hotspot (area with many occupied sites) during these four sampling periods. Differences suggest that the distribution of Texas kangaroo rats is dynamic and that any sort of management plans too need to be dynamic. Much more suitable habitat exists that is not occupied across the distribution of *D. elator*. Dynamics suggest that

historical sites, contemporary sites and those that are suitable yet unoccupied might have fairly equal conservation value. Management plans should address wider areas than just those that are currently occupied by Texas kangaroo rats.

6) Given the ubiquity of private land across the region, and the fact that we did not encounter *D. elator* at any of the three state-owned properties in the region, more effort should be directed towards working with private landowners to identify mutually beneficial agreements that help conserve or create suitable habitat for *D. elator*. Specifically, our distribution models suggest that areas within Cottle, Wilbarger, and Wichita counties are currently important locations in terms of available suitable habitat, and special efforts should therefore be made to reach out to landowners in these areas in an attempt to conserve this habitat.

Literature Cited

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Jones, C., Bogan, M. A., and Mount, L. M. 1988. Status of the Texas kangaroo rat (*Dipodomys elator*). Texas Journal of Science 40: 249-258.

Martin, R.E. 2002. Status and long term survival of the Texas kangaroo rat, *Dipodomys elator*. Sect. 6, Project 70. Unpublished report prepared for the Texas Parks and Wildlife, Austin, Texas.

Nelson, A.D., Goetze, J.R., Henderson, S., and Scoggins, B. 2011. Status Survey for the Texas kangaroo rat (*Dipodomys elator*). Technical Report. Texas Parks and Wildlife Dept.

CHAPTER X: FUTURE RESEARCH NEEDS

1) **More Surveys of Private Land.—**A unique characteristic of the state of Texas is the vast amount of private land. Less than 4 percent of land in Texas is public meaning that survey opportunities are extremely limited and status assessments are biased to what is known on the very small percentage of public land. Obtaining permission to survey for rare species such as D. elator is difficult and frustrated by public perceptions of the negative ramifications to finding such species on private property. Indeed, we feel that we had relatively good success obtaining access to private land in this study, although we only obtained access to 19 unique pieces of private property. A better understanding of distribution and abundance of the Texas kangaroo rat is imperative to answering one of the most basic questions regarding conservation of this species—How common and how abundant is this species across its geographic distribution. Through our activities in north central Texas, a number of landowners have warmed up to the idea of allowing surveys on their private property. It is important to seize the moment and to take advantage of improved support for surveys of Texas Kangaroo rats across the private sector.
2) **Determination of Diet.—**One of the most basic characteristics of the ecological niche of a species is kinds and variety of things that it eats. Very little is known of the diets of *D. elator* (See Chapter 1 on preexisting information on the Texas kangaroo rat). One reason is the difficulty of determining diets of elusive species, especially those that fully masticate their food. Nonetheless, modern advances in molecular biology allow for genetic screening of items in the feces of organisms. Such genetic screens can accurately identify what species of plants are important in the diets of Texas kangaroo rats. Understanding the distribution, abundance and conservation status of the resources used

by *D. elator* will go a long way to understanding whether current or future food limitation impinge on the distribution and abundance of Texas kangaroo rats.

3) **Examination of Efficacy of Manipulating County Road Management Practices to Benefit *D. elator*.—**One important finding of this and prior research is documentation of the high frequency by which Texas kangaroo rats use and live in (i.e. construct their burrows) the right-of-ways of unpaved county roads. These roads represent the vast majority of public land available to Texas kangaroo rats across their geographic distribution. *Dipodomys elator* undoubtedly responds to conditions on these unpaved county roads. Nonetheless, which management practices of the roads ((i.e., how and with what frequency are they graded positively affect distribution and abundance of Texas kangaroo rats is unknown and should be explored.

4) **Examination of Efficacy of Prescribed Burning as a Management Tool for D. elator.—**The Texas kangaroo rat is a disturbance adapted species that prefers habitat that is opened up by external agents such as grazing, trampling of habitat or fire. One of the most commonly used tools for range management is fire. Fire thins dead and live vegetative material, opens up resources for pioneer plants to use and can greatly alter the landscape. Frequent fire can maintain rangeland in state (i.e. of plant species composition) that is unattainable in its absence. Moreover, a particular managed fire regime can maintain a particular species composition of plants that is most beneficial to a target foraging species. Nothing is knows as to the response of Texas kangaroo rats to fire or whether management through fire can be a useful strategy to manage populations of Texas kangaroo rats.

5) **Evaluation of Efficacy of Management of "Umbrella Game Species" as a Management Tool for *D. elator*.—**An important economic base in north central Texas comes from hunting for game species on private land. Often land owners manage range so as to enhance habitat for game species (over cattle) so as to enhance the economic benefit of hunting on their land. Many species of game animals occur over the geographic distribution of the Texas kangaroo rats. Some of the most important from an economic perspective are white-tail deer, quail, dove, turkey and ducks. Many of these require different management prescriptions so s to enhance habitat for that particular species. Few private land owners will likely particularly manage for Texas kangaroo rats. Nonetheless, research demonstrating that management for an economically important game species also benefits the Texas kangaroo rats could greatly enhance the likelihood of improving habitat for this species. Moreover, demonstrating such a relationships could facilitate a mutually beneficial incentive plan that could add economic value to private land while promoting habitat for Texas kangaroo rats.

6) **Better understanding of the statistical challenges of abundance-limitations and its effects of understanding distribution and abundance of rare species.—**Two important limitations affect the distribution and abundance of organisms. The first, dispersal limitation, affects the ability of members of a species to populate everywhere because they do not have the dispersal abilities to reach all sites. Dispersal limitation likely affects all species occurring naturally in nature. A second limitation is abundance limitation that particularly affects rare species. Abundance limitation occurs when there simply are not enough individuals to colonize all sites. As a result of these two forms of limitation, for many species there are a relatively large number of suitable yet unoccupied

sites. This poses an important challenge when trying to estimate habitat preferences based on patterns of presence and absence. As a result of abundance-limitation affecting rare species, many suitable sites are not inhabited and this increases statistical noise and makes it difficult to characterize habitat preferences. Most if not all analyses used to examine habitat preferences assume that individuals are at equilibrium and that the total number of individuals is determined by the amount of suitable habitat. This likely is not the case for Texas kangaroo rats. As comparison or our results with other historical surveys indicates, *D. elator* is very dynamic across its geographic distribution, disappearing from areas it was found in previously and popping up in other places where it has never been documented. This suggests that at any one time there are a number of suitable sites that are not being occupied, potentially due to abundance limitation. *Dipodomys elator* likely is not at equilibrium across the landscape and its total abundance likely is not determined by the total amount of suitable habitat. This likely explains why our and other habitat studies describe only weak patterns of habitat affinities. Other statistical methods need to be developed that do not assume equilibrium and a direct relationship between amount of suitable habitat and total abundance if we are to better understand habitat affinities of rare species such as the Texas kangaroo rat.

7) **Remote Sensing**

Our study found that using RGB imagery at <5cm resolution and collected from airborne platforms was able to characterize the features needed for proper identification of active burrows. In addition, Digital Elevation Models collected from airborne platforms were able to provide information on slope and aspect patterns that clearly define the boundaries of circular, linear or irregular mounds in the landscape. Based on our assessment, we can recommend the following for assessing the distribution and abundance of *D. elator* in Texas using remote sensing technologies: 1) use currently available RGB imagery and Digital Elevation Models (from TNRIS, at 10-m pixel resolution) to derive suitability maps for *D. elator* burrows for all counties with *D. elator* presence in Texas, and 2) using landscape scale or county level models, define the areas identified with high suitability for *D. elator* burrows in each county as the sampling area for high resolution imagery <5cm pixel resolution. Then employ either unmanned aerial systems (UAS) or traditional plane-mounted cameras to collect high resolution imagery and digital surface models.