

**Native mice in a novel ecosystem: Morphology, behavior, and sociology predict the role
of prairie deer mice in agriculture**

by

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DEDICATION

This work is dedicated to my wife Kimberly, who has persevered alongside me in this effort, to my daughter Evelyn, whose energy kept me going, and to my unborn child, who helped catalyze my personal transformation.

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CHAPTER 1. INTRODUCTION

This dissertation examines ecological weed control by the prairie deer mouse (*Peromyscus maniculatus bairdii*). It is designed to understand the history, contemporary challenges, and future prospects for the control of weeds with the aid of small mammal seed predation.

1.1 Corn-Soybean Agriculture

Corn-soybean agriculture, the annual rotation of corn planted as a monoculture and then soybean planted as a monoculture, in the Midwestern US involves intensive use of chemical and mechanical management to reduce weeds and maintain soil fertility. Some consequences of this management strategy are the loss of biodiversity (number of species), increased nutrient (nitrogen, phosphorous, and organic matter) loss from fields, and the origin of herbicide tolerant weeds (weeds which are able to grow and produce seed when sprayed with chemicals intended to kill them). However, new strategies provide a means to ameliorate some of these negative effects and promote services that benefit society. One such strategy is multifunctional agriculture, which promotes the management of non-crop services, such as clean water, in conjunction with the production of commodities such as corn and soybean.

1.2 Wildlife in Agriculture

While most common wildlife in the Midwest may interact with corn-soybean agriculture at some point during the year, no vertebrate is as abundant and persistent in this system as the prairie deer mouse. It has long been known that small mammals are common

residents of corn-soybean agriculture, and that a large proportion of their diet consists of waste grain (corn and soybean) and weed seeds (Houtcooper 1978). It has also been clear that prairie deer mice are the most abundant and potentially valuable small mammal in these intensive agricultural systems (Whitaker 1966, Clark and Young 1986, Cardina et al. 1996, Williams et al. 2009). However, these studies have found conflicting results regarding the diet and weed control efficacy of this species. The following suite of studies is designed to address the origins, ongoing conflicts, and potential future efficacy of deer mouse management for the control of weed populations.

1.3 Dissertation Organization

1.3.1 Evolution of Jaw Morphology

It is clear from a multitude of studies that prairie deer mice are well-adapted to the corn-soybean rotations of the Midwestern United States, as they successfully reproduce, persist through winters, and maintain high population levels in most years. What is less clear is by what mechanisms they have successfully utilized this novel habitat. *P. m. bairdii* is a generalist and omnivore, which provides opportunity for the small mammal to use novel and highly nutritious food items such as waste grain. While deer mice were likely competent at processing large seeds historically, the large amount of waste grain and its importance for overwinter survival may have provided a strong natural selection force on the mice.

If this natural selection force were present, it is likely that the morphology of mouse skeletal structures used for mastication would have evolved significantly since the implementation of intensive corn-soybean agriculture. In order to test this, we compared the

morphology of masticatory structures between specimens before 1910 and from 2012-2013, to establish any evolutionary changes in the structures.

1.3.2 Weed Seed Preferences

The second chapter of this study examines seed preferences of prairie deer mice. We focus on two economically important weed seeds, foxtail and velvetleaf. While previous studies have found no preference for the seed types (Williams et al. 2009), foraging theory predicts a strong preference between these seeds. Therefore, I sought to understand if the method of assessing seed preference and the arrangement of seeds within the method influence the outcomes of such studies. Also, the results are based on more natural conditions of mixed seed availability and declining rates of return. Thus, the results better reflect foraging behavior of the species.

1.3.3 Fecal Isotopes

It is difficult to directly evaluate the diet of small mammals over a long period of time and also have fine scale resolution. Therefore, I evaluated the potential of using fecal material, collected readily in many studies, to determine trophic ecology in corn-soybean agriculture. In this chapter, I describe three methods of fecal material collection, the quality of dietary resolution, and the applicability of different methods of collection.

1.3.4 Foraging in Fear

It is well-documented that animals forage according to a perceived risk of predation. For example, extensive studies have been done to evaluate the foraging behavior of mice on moonlit (risky) nights and relative to cover. However, the erosion of farmland presents a novel risk to prairie deer mice. The light soil, which is being exposed by erosion and the loss of dark topsoil is combined with harvesting of nearly all biomass. The light soil contrasts

with a dark fur color of deer mice, enhancing conspicuousness, and producing an environment that may be riskier than the darker soils and thick vegetation that once covered much of the Midwestern US. Thus, this chapter assesses whether prairie deer mice are aware of this increased risk, and forage less intensively in these areas.

1.3.5 Multifunctional Agriculture

Given the negative effects of agriculture on environmental sustainability, as well as the negative effects of intensive agriculture on the future of agriculture, many are calling for a focus on managing not just commodity production, but other services from farm fields as well. This management strategy focuses on the production of non-commodity goods and services and utilizes ecological tools for the management of the system. This type of management promotes the use of native species, such as the prairie deer mouse, to aid in the control of pests, such as weeds and insects. However, in such an intensive agricultural system, such as the corn-soybean agriculture of the Midwestern US, it is not understood if the farmers and extension agents of the region are amenable to such ideas. Therefore this chapter is designed to evaluate the perspectives of farmers, private agricultural consultants, and public extension agents relative to multifunctional agriculture. If the appropriate amount of support exists, there is a greater potential for the implementation of the other findings in this study.

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CHAPTER 2. RAPID MORPHOLOGICAL CHANGE IN THE MASTICATORY STRUCTURES OF AN IMPORTANT ECOSYSTEM SERVICE PROVIDER

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John W. Doudna and Brent J. Danielson

ABSTRACT

Humans have altered the biotic and abiotic environmental conditions of most organisms. In some cases, such as intensive agriculture, an organism's entire ecosystem is converted to novel conditions. Thus, it is striking that some species continue to thrive under such conditions. The prairie deer mouse (*Peromyscus maniculatus bairdii*) is an example of such an organism, and so we sought to understand what role evolutionary adaptation played in the success of this species, with particular interest in adaptations to novel foods. In order to understand the evolutionary history of this species' masticatory structures, we examined the maxilla, zygomatic plate, and mandible of historic specimens collected prior to 1910 to specimens collected after 2010. We found that mandibles, zygomatic plates, and maxilla have all changed significantly since 1910 ($p < 0.01$), and that morphological development has shifted (intercepts: upper jaw: - 0.23 vs. -0.50, $p = 0.048$; lower jaw: -0.28 vs. -0.39, $p < 0.001$). We present compelling evidence that these differences are due to natural selection as a response to a novel and ubiquitous food source, waste grain (corn, *Zea mays* and soybean, *Glycine max*).

2.1 Introduction

Rapid changes to environmental conditions such as climate and landscape have become the normal conditions under which contemporary species must survive and reproduce. Therefore, rapid microevolutionary changes may be critical to the survival of species in an anthropogenic world. Rates of morphological adaptation of vertebrates, once thought to be incommensurate with ecological time scales, transpire quickly in some species under novel ecosystem pressures (Berry 1964, Williams and Moore 1989, Pergams and Ashley 1999, Reznick and Ghalambor 2001, Vander Wal et al. 2013). Surprisingly, few studies have examined morphological changes due to landscape change (Palkovacs et al. 2012, but see Desrochers 2010). Intensive agriculture provides a natural experiment of rapid and extensive modifications to food and habitats in ways that are almost certain to have large implications for natural selection. This rapid change provides an opportunity to study the rate at which a species can change to cope with a dramatically new environment.

Peromyscus maniculatus bairdii is an example of a species that has experienced drastic shifts in its environmental conditions over a short time period. *P.m. bairdii* are the prairie form of the common deer mouse, and are constrained to grassland landscapes throughout the Midwestern USA. Humans have converted more than 80% of this habitat to agriculture (Samson et al. 2004). Following this rapid conversion (less than 200 years), *P.m. bairdii* has become the most common resident vertebrate of corn-soybean rotations (Clark and Young 1986, and this study). In addition, there are gradients of intensity from greater than 70% of the land in corn-soybean rotation, to areas of less than a third of the landscape in corn-soybean rotations (National Agricultural Statistics Service, 2013).

Part of the explanation for the success of *P.m. bairdii* in this new system may be the species' tolerance of a novel diet of native and non-native insects and non-native weed and a preference for crop seeds (Whitaker 1966, Clark and Young 1986, Westerman et al. 2008, Williams et al. 2009). While the non-native insects and weed seeds may be analogous to native species, corn and soybean are completely novel food items introduced by humans. In addition, the large quantity of this high-quality waste grain makes it the most important winter source of calories in agricultural fields (Foster et al. 2010 and Vickery et al. 1994). Dried corn and soybean are hard seeds that are more than an order of magnitude larger than other common prairie seeds (corn: 0.3 g; soybean: 0.12 g; wild sunflower: 0.007g (Alexander et al. 2001)), and this size difference may exert significant pressure on the natural selection of masticatory structure morphology.

In fact, we have found that the mice strongly prefer waste grain (corn and soybean) relative to any other seed type we tested (unpublished data). In order for the mice to process the relatively large but nutritious seeds, they may benefit from a new masticatory morphology specialized for the task. Myers et al. (1996) have shown that the skull morphology of deer mice is only slightly plastic in its response to food hardness, while other studies have found larger plastic responses in the mandibles of mice (Renaud and Auffray 2010). We also know that gape and bite force are related, and that the morphology of the skull can be an important factor in the relationship of these two parameters (Williams et al. 2009, Davis et al. 2010, Piras et al. 2013). Due to this relationship, we expect that mandible morphology may be strongly correlated to feeding efficiency in the species.

Rodent morphological evolution studies are numerous (e.g., Boell and Tautz 2011, Hoekstra et al. 2006, Mullen and Hoekstra 2008, Pergams and Lacy 2008). For example, a

closely related species *Peromyscus polionotus* has been a key example in evolution of coat color (Mullen and Hoekstra 2008). Researchers have found that strong selection by visual predators have likely led to coat color variations of subspecies. This approach has detected a top-down form of natural selection, with predation acting as a force of evolutionary change. Fewer studies have examined evolutionary changes of natural small-mammal populations due to bottom-up regulation from changes in food sources. However, Goheen et al. (2003) did find morphological variation between locations for squirrels that had recently experienced a range expansion, and suggested cranial morphology changes could be associated with feeding efficiency on novel food items.

Given previous findings that masticatory structures are intimately linked to food quality, and that rapid changes in food quality can drive significant microevolutionary patterns (Philips and Shine 2004, Renaud and Auffray 2010), *P.m. bairdii* could be an example of such contemporary evolution (*sensu* Stockwell et al. 2003). In order to determine the role of human-modified landscapes in this species' evolution, we test the prediction that since 1910 the zygomatic plate and masseter region would increase in size relative to the whole specimen, the coronoid process would change in size and shape, and that the condyle of the mandible would be larger on some specimens due to plastic responses to food hardness. We also test the prediction of location-specific changes in morphology associated with the intensity of corn-soybean production.

2.2 Methods

2.2.1 Digitization

In order to test the hypotheses, the skull morphologies of historic (1870-1910) and contemporary (2012-2013) specimens of *Peromyscus maniculatus bairdii* were compared. A collection of historic specimens was created, starting with a search of the online Global Biodiversity Information Facility database (gbif.org) for museum collections of *P. m. bairdii* skulls from before 1910. In order to generate direct comparisons of historic and contemporary species, the possible specimens were narrowed based on regional concentrations of historical specimens. This research found concentrated collections in northeastern Illinois, central and south-central Iowa, northeastern Kansas (2 locations), southwestern Minnesota, and northeastern North Dakota (Figure 1 and Table 1). Specimens were shipped from the National Museum of Natural History (Washington, D.C.), and we visited the Field Museum of Natural History (Chicago, IL), University of Kansas Biodiversity Research Center (Lawrence, KS), and University of Iowa Museum of Natural History (Iowa City, IA). The vertebrate collection from the Museum of Natural History at Harvard was unavailable during our sampling period, due to renovations.

Based on historic specimens, six concentrations of specimens were identified that would allow for direct comparison to contemporary specimens. Contemporary specimens were collected from each of the locations identified from this process. Mice were snap trapped, using Museum Specials (Woodstream Corporation, Lititz, Pennsylvania) within corn and soybean fields at each location, following recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of Iowa State University

(Permit Number: 4-12-7335-W). In parallel with the historical specimens, specimens were collected from six locations: Ames IA, Ottawa KS, Manhattan KS, Emerado ND, Waseca MN, and Shabbona IL (Fig. 1 and Table 1). At each location, up to 40 adult *P. m. bairdii* were collected. Thus, snap traps were set for 1-3 nights (checking traps each morning), depending on trap efficiency at each location, between July and September 2012 in Iowa, Kansas, North Dakota, and Minnesota. Traps were set for 1-3 nights in July 2013 again in Minnesota and in Illinois. Skulls were removed and placed in a dermestid beetle colony until clean. In order to determine the changes in shape of *P. m. bairdii* jaws, data were collected from upper and lower jaw structures associated with masticatory muscle attachments. To this end, photographs were taken of the left, lateral perspective of skulls and mandibles separately for all specimens. A few exceptions occurred when historic specimens could not be disarticulated, so photographs were taken with skulls and mandibles attached. Occasionally, photographs of the right side of the specimen were taken, when the left side was too damaged for analysis. A setup of a digital camera with a macro lens was used, set at approximately 0.5m from the specimen, and a mm ruler was oriented along the long axis of the specimen and camera lens. A Canon EOS XT with an 8.0 MP sensor with a 100mm macro lens (EF = 1:2.8) was used for all photography. After all pictures had been collected, maxilla-zygomatic plate and mandible landmarks were digitized for all specimens. Landmarks followed McPhee (2004) and Myers et al (1996), but were modified for this species and question (Figure 2). Nine landmarks and 7 semilandmarks were digitized on the maxilla-zygomatic plate (MZP), and 10 landmarks and 7 semilandmarks were digitized on the mandible (MAN). Landmarks are a combination of Type 1 and Type 2 landmarks (points of intersection of structures and points of maximum or minimum curvature). Semilandmarks are landmarks along a curve that

are moved along that curve during the analysis. All specimens were digitized in tpsDig2 (vers. 2.17). In order to test for errors associated with order of photography, 25% of the specimens were re-photographed. No evidence was found that specimens from the original and follow-up test were different ($MS = 0.00247$, $p = 0.22$). Ten percent of specimens were re-digitized and a significant effect of practice time was found on digitization of MZP, but not MAN. Therefore, all MZP were re-digitized to remove experience bias.

2.2.2 Statistical Methods

This study uses modern geometric morphometrics to evaluate size and shape changes separately. In order to do this, digitized points of each specimen are scaled to a common size, the centroid of each specimen moved to the origin (0,0), and rotated to minimize differences among all specimens. The result is a set of coordinates for each specimen that retains only variation that is due to shape differences. Size is removed, but recorded in the process for other analyses, such as allometry and changes in specimen size with time.

Modern geometric morphometric analyses were conducted using *geomorph* (Adams and Otárola-Castillo, vers. 1.1-3) in R (vers. 3.0.2). This analysis uses a generalized Procrustes analysis (GPA: Gower 1975, Rohlf and Slice 1990). The process removes rotation and position of digitized specimens, leaving only metrics of shape (Zelditch 2004). During the process, semilandmarks are slid along a curve (Bookstein 1991:376-382) to minimize differences among specimens. Following these steps, the analyses are conducted on the rotated, scaled, and slid landmarks of all specimens. In this process, the centroid size (Csize) of each specimen is determined, and specimens are scaled to a Csize of 1 for all further processing. Based on the Csizes, static allometry was examined, which is a change in shape

correlated with growth in size but not through developmental stages. A common allometric component (CAC), which is a standardized measure of shape of a single specimen relative to the mean shape of all specimens, was also calculated (Mitteroecker et al. 2004). The multivariate shape matrices were then regressed on Csize to determine if there is a significant trend in shape change with size, using linear regression and comparing slopes with ANCOVA (Forsman 1996). Visual inspection of the regression residuals revealed no patterns.

When size and rotation have been removed, the landmarks of specimens are aligned to minimize differences across all landmarks, and projected into tangent space. This process removes curvature associated with other procedures in morphometric analysis. The x,y coordinates in tangent space can be compared as matrices of coordinates. In order to determine if significant differences exist among groups, the effects of period (before 1910 or after 2010), geographic location, centroid size, percent corn-soybean in landscape, and interactions were assessed as predictors of morphology. This analysis produces Procrustes distances, and the sum of squares is evaluated against a Procrustes ANOVA, which is analogous to a permutational MANOVA (Goodall 1991, Anderson 2001), and permuted 999 times in this study. Following this, thin plate spline deformation grids qualitatively describe the changes in shape between historic and contemporary specimens. In this presentation, non-parallel lines in a deformation grid indicate a change in shape. Trajectories of changes were also calculated for each location. Specifically, the distance and direction of a trajectory are calculated in the 2D space of axis 1 and 2 of a principal components analysis. Since axes 1 and 2 represent the maximum variation explained in the multivariate data, the distance between 2 points in this 2D space represents an estimate of overall difference. The trajectory

of change between 1910 and 2012 was calculated from the reference (historic) to the target (contemporary) average specimen at each location. These trajectories were then evaluated against one another to determine significant differences in size or direction of change (Collyer and Adams 2007; Adams and Collyer 2007; Adams and Collyer 2009).

In sum, we analyzed 150 historical and 160 contemporary specimens (Table 1). The first two axes of MZP and MAN explained 38 and 40 percent of the variation in the data sets. Greater than 95% of the variation was explained within the first 17 axes of both datasets (out of 32 and 34 axes).

2.3 Results

Along PC axis 1, both maxilla-zygomatic plates (MZP) and mandibles (MAN) broaden, primarily dorsoventrally (Figure 4). Mouse MZP and MAN morphology are significantly different between historical and contemporary specimens across the Midwestern US and within each location (Table 2). We also found significant static allometry of size and shape for MAN and MZP across all specimens (Table 2) (Figure 4). In addition to size, period is a significant predictor of the allometric regression intercepts after accounting for location (Table 2 and Figure 5). Further investigation of these patterns revealed that allometric slopes (interactions of period and size) of contemporary specimens were shallower than historic specimens (0.08 vs. 0.19 and 0.09 vs. 0.13; Figure 5), with a significant effect of period on slope for MZP (Table 2). In contrast, slopes of allometry were parallel for MAN, supporting a consistent difference due to time period for all sizes. In total, contemporary and historic MZP grew along different shape trajectories (illustrated by different and intersecting slopes), while MAN grew along the same trajectory, but through different regions of morphospace (illustrated by non-intersecting slopes of different elevations).

Not only did time periods have different allometric growth patterns, contemporary masticatory structures are also significantly larger than historic structures. MZP size increased significantly from 14.73 (0.92) to 15.03 (0.89) ($MSE = 7.42$, $F_{1,299} = 10.15$, $p = 0.002$). The average size of MAN also increased significantly from 17.56 (0.89, 1SD) to 17.74 (0.87) ($MSE = 2.825$, $F_{1,299} = 4.10$, $p = 0.04$) (Figure 6). Location was also a significant predictor of Csize for MZP and MAN, but there were no significant interactions between the predictors (location: $MSE = 5.63$, $F_{5,299} = 7.70$, $p < 0.001$ and $MSE = 5.84$, $F_{5,299} = 8.48$, $p < 0.001$; interaction effects: $p = 0.13$ and 0.09) (Figure 6).

Since morphology varied significantly by time and location, we investigated how these changes occur in morphospace. Trajectories of evolutionary change show that MZP have experienced little directional change along the two major PC axes (Figure 7). In contrast, mandible morphology has clearly experienced a directional change across the Midwestern US, as specimens are separated in morphospace by time on PC I (which explains the maximum variation in the data). In contrast, MAN have more occurrences of significantly different trajectory orientations by location than do MZP (Table 3), suggesting different directions of change. MZP trajectories are actually more frequently variable than MAN, though. Only Ottawa, KS MAN had a significantly larger trajectory of change than other locations.

In order to better understand the role of intensive agriculture on jaw morphology, we removed location from the analysis, and replaced it with the continuous variable, percent corn-soybean. After accounting for variation due to Csize, there was a significant interaction between period and percent corn-soy land cover in a county on MAN morphology, but the simple effect of percent corn-soy was insignificant (Table 2 and Fig. 8). Percent corn-soy

was also a significant predictor for contemporary and historical specimen MZP morphology (Table 2 and Fig. 8). However there was a significant interaction effect of the two as well (Table 2). Similarly, when we evaluated the effect of corn-soybean and time period on Csize, we found both were significant predictors for MAN and MZP (all $p < 0.01$), while the interaction effects were non-significant (both $p > 0.2$) (Figure 8). Size of both structures increased with time, but decreased with amount of corn-soybean agriculture in the landscape.

Thin plate spline deformation grids show that there were patterns to change in mandible and maxilla morphology since 1910 (Fig. 9). In general, the masseter and condyle had the most consistent expansion (both anterioposteriorly and dorsoventrally) in the MAN. This was more striking in the specimens from regions of high corn-soybean agriculture. The coronoid process is also rotated anteriorly in landscapes of high percentage corn-soybean, but maintains the historical posterior directionality in areas of low percentage corn-soybean agriculture. The maxilla and zygomatic plate show less distinction among sites. However, there appears to be a broadening (both anterioposteriorly and dorsoventrally) of the zygomatic plate or the portion of the maxilla lying directly above the molar row. The coronoid process, masseter, condyle, and zygomatic plate are all attachment regions for masticatory muscles, supporting a shift in diet from softer and smaller food items to a reliance on larger, harder food items.

2.4 Conclusions

We found significant differences in masticatory structure morphology that was explained by both location and time, and we found ample evidence that this is due predominantly to evolutionary change, rather than plasticity. Multiple studies have found that

rodent mandibles are phenotypically plastic, and that morphology changes significantly without evolution when diet is altered. The changes are typically an overall broadening with exposure to hard food types (Volkman 2009, Renaud and Auffray 2010). Skull morphology is less plastic in similar studies (Myers et al. 1996). For example, Myers et al. (1996) found that *Peromyscus maniculatus* raised on a hard diet in the lab had smaller lateral centroid size, a broader zygomatic plate, movement in the incisor insertion position, and a foramen that this study excluded. All of these morphological changes, except the foramen position, were also significantly influenced by family membership, typically to a greater degree than what was explained by food hardness. Volkman (2009) found similar results in the test of food hardness on mandible morphology. She reared deer mice from a few females, and found significantly larger centroid size, taller mandibles with a wider condyle when the mice were fed harder foods throughout development. However, in this controlled laboratory experiment, diet explained just 7.2% of the morphological variation, while relatedness (young from the same mother) explained 41.2%. Our study found morphological changes that qualitatively mirror the changes seen in lab studies with increased food hardness. Renaud and Auffray (2010) also examined plasticity in domestic mouse (*Mus musculus domesticus*) mandibles, by raising mice on foods of different consistencies. The major morphological changes they describe are movement in the incisor alveolus, uplift of the molar region, and change in the angular process. However, they found no change in size due to food types. Their overall changes illustrate a similar broadening of the posterior half of the mandible, as in our study. They conclude that there is a strong correlation of plastic and late ontogenetic changes in shape seen in the wild. They suggest that plasticity and ontogenetic changes provide similar adaptations to food types and availability, which would also be predicted from our study.

Despite the fact that our morphological changes are analogous to those found in assessments of plasticity, the large amount of variation explained by time, despite the diversity of familial relationships, variation in trapping time and year, ameliorates the directional effects of diet on plasticity. Since *P. m. bairdii* are omnivores, and utilize seeds and insects throughout the spring and summer months, their diets would have varied, and likely would have consisted of little waste grain (Clark and Young 1986). The changes we found also match skeletal structures involved in masticatory muscle attachment, and by extension are associated with increased bite force (Baverstock et al. 2013). For example, the enlarged maxillary zygomatic process, maxilla, condyloid process, and coronoid process are attachment points for the superficial and deep masseter muscles as well as the zygomatico-mandibularis muscle (Baverstock et al. 2013). Williams et al. (2009) confirmed that jaw-muscle anatomy was predictive of bite force in small rodents. They found that bite force is optimized at 40-50% gape, and that specialized skeletal morphology interacts with musculature to promote strong bites on other species. Additionally, we were able to show that the amount of corn-soybean in the landscape now was a significant predictor of masticatory structure shape. Maxillae-zygomatic plates were significantly influenced during both periods, historic and contemporary, which undermines the value of corn-soybean as a driver of the observed morphology in maxillae-zygomatic plates. However, while contemporary mandibles were significantly associated with corn-soybean percentage in the present day, historic mandibles were not, and there was no interaction of the two parameters. Similar to other studies that have found larger change in jaw morphology with food hardness, the amount of corn-soybean in the landscape seems to have a more intense impact on mandible morphology evolution in the species.

Adding to the evidence that evolutionary change is the principal factor in the morphological variation we observed, is allometric heterochrony, the evolution of ontogeny, which we found in masticatory structures (Klingenberg 1998). The different values for mandible, across sizes indicate that even young adult mice, which are unlikely to have relied heavily on waste grain, have different morphologies than historic specimens. This interpretation is supported by Clark and Young (1986) who found that in late spring, mice in corn fields relied predominantly on insects as food. This form of allometry associated with food and adaptation to differing foods has been detected before. For example, Lopez et al. (2013) found that two species of snakes exhibit differing allometric intercepts, likely associated with different diets, similar to our finding in mandible allometry. Magnhagen and Heibo (2001) found similar results in pike from different lakes, again associated with differing diets. Magnhagen and Heibo found significant differences in the allometric slopes among lakes as well, similar to our finding in the zygomatic plate and maxilla. Interestingly, Egset et al (2012) were unable to generate a change in allometric slope with artificial selection, though they were able to change the elevation of the slope in just 3 selection episodes. In our study, contemporary zygomatic plate-maxilla had significantly shallower slopes and mandibles were shallower, though not significantly. This indicates that there is less morphological variation in adults of different sizes than was present historically.

Rapid morphological changes similar to those found in our study have been recorded in laboratory and natural populations experiencing intense natural selection pressures. This includes the example of Brown and Brown (1998) who found significant morphological change in swallow populations after a single storm event. Brown and Brown (2013) found similar results due to the natural selection of car strikes on swallow populations, with strong

selection pressure on wing length, and by extension flight maneuverability. In contrast, McPhee (2004) found that 35 generations of relaxed selection resulted in oldfield mice distinct from wild-caught or founder mice, and no clear patterns of evolution. Similarly, Grant and Grant (2002) found unpredictable evolution in Darwin's Finches over 30 years. The shapes of the finches' beaks changed, but the rate and directionality of the change varied dramatically.

Species are adapting to rapid human-caused environmental change. This study supports this sort of rapid change. These rapid adaptations allow for the persistence of species in human-dominated landscapes, but they may represent a shift in ecosystem roles. For instance, our study provides insight into the changing role of an ecosystem service provider. *P. m. bairdii* has adapted, despite omnivory and generalization, to newly available food, indicating a strong selection pressure toward a single type of food. In order to protect valuable services from species such as *P. m. bairdii*, we should take careful note of morphological changes associated with the ecosystem services provided (in this case, seed destruction). Based on these patterns of change, we may be able to identify future changes or limits to rapid evolution. For example, if this population had been smaller or less diverse, rapid changes may not have been possible (Vander Wal et al. 2013). Generalists are thought to often overcome the challenges of novel environments through pre-adaptation, plasticity, and flexibility (Sih et al. 2011). Thus, generalists often thrive where specialists struggle; our study provides an additional piece of evidence to explain this resiliency: adaptation to more efficiently use novel resources.

2.5 REFERENCES

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Table 1. Summary of specimen collection sites. Historical location is an estimate from museum records and contemporary locations are the coordinates of the sampled field. Percent corn and soybean in 2012 is based on National Agricultural Statistics Service numbers (nass.usda.gov). The number of acres of land planted to corn or soybean was divided by the recorded size of each county in which sampling occurred.

	Location		% Corn & Soybean 2012
	Before 1910	2012-2013	
Illinois (N=40)	Cook and Lake County	41.83913,-88.865365	81
Iowa (N=53)	Knoxville and Central Iowa	41.990805, -93.685187	76
Minnesota (N=41)	Fort Snelling	44.070972,-93.525711	75
North Dakota (N=53)	Northeast North Dakota	47.953432,-97.434925	33
Ottawa, KS (N=75)	Lawrence	38.537739,-95.245275	21
Manhattan, KS (N=48)	Onaga	39.213044,-96.595392	14

Table 2. Summary of predictors of jaw morphology. Location, period, size, and interactions were modeled as predictors of mandible and maxillae-zygomatic plate morphology, using a Procrustes distance metric and resampling techniques. Then the same analysis was done with the percent of corn-soybean agriculture in the landscape (as in Table 1) replacing location. Values are mean square errors explained by each parameter, based on all 310 specimens as described in Table 1. Significance codes are as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, . $p < 0.10$.

Mandibles	
Parameters	MS Value
Location	0.004***
Period	0.027***
Location x Period	0.005***
Size	0.013 ***
Period x Size	0.0019 (p=0.44)
Corn-Soybean Cover	0.004.
Corn-Soybean Cover x Period	0.006**
Maxillae-Zygomatic Plates	
Parameters	MS Value
Location	0.009***
Period	0.011**
Location x Period	0.009***
Size	0.018 ***
Period x Size	0.008 **
Corn-Soybean Cover	0.026***
Corn-Soybean Cover x Period	0.020***

Table 3. Differences in evolutionary trajectories by location. Significant values are in bold. A significant p-value indicates that the trajectories of the two location are significantly different in size or direction of change. Size of change represents the magnitude of morphological change between historic and contemporary specimens, while the orientation represents the direction of morphological change in PC space. Both are based on the first two PC axes resulting from geometric morphometric analysis. All sample sizes are the same as in previous tables and figures, based on the following: EMND – Emerado, North Dakota (contemporary) and northeastern ND (historic); IOWA – Ames, IA (contemporary) and central IA (historic); MAKKA – Manhattan, KS (both); NILL – Shabbona, IL (contemporary) and northeastern IL (historic); OTKA – Ottawa, KS (contemporary) and Lawrence and Fort Leavenworth (historic); WAMN – Waseca, MN (contemporary) and south-central MN (historic).

		Maxillae-Zygomatic Plates						Mandibles					
		EMND	IOWA	MAKA	NILL	OTKA	WAMN	EMND	IOWA	MAKA	NILL	OTKA	WAMN
Size	EMND												
	IOWA	0.021						0.005					
	MAKA	0.017	0.005					0.001	0.006				
	NILL	0.002	0.019	0.014				0.008	0.004	0.009			
	OTKA	0.013	0.008	0.003	0.011			0.018	0.014	0.020	0.010		
	WAMN	0.013	0.009	0.004	0.010	0.001		0.004	0.001	0.005	0.004	0.015	
Orientation	EMND												
	IOWA	110.4						51.8					
	MAKA	41.7	105.0					67.8	54.6				
	NILL	141.9	64.9	120.8				82.9	69.0	86.2			
	OTKA	59.1	88.64	71.0	112.2			62.0	68.5	75.2	62.3		
	WAMN	86.0	84.2	76.0	71.3	91.0		68.6	54.3	80.1	56.8	45.1	

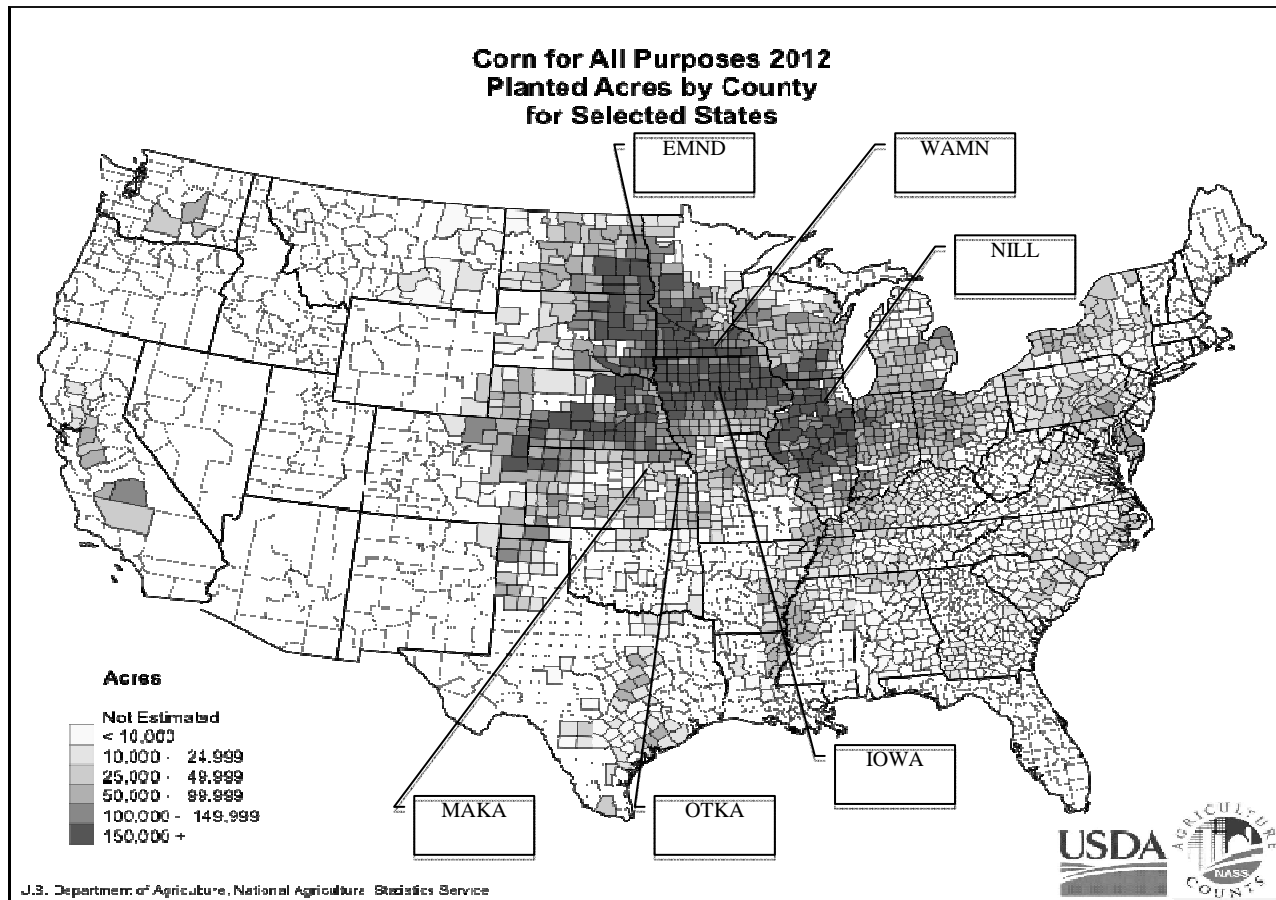


Figure 1. Locations of *Peromyscus maniculatus bairdii* trapping. We sampled up to 40 adult *P. m. bairdii* from each location designated on the map. These were used because concentrations of historical specimens from before 1910 were available from the region. The map illustrates the spatial distribution of corn-soybean agricultural intensity in the Midwestern US. Three of our locations (NILL, IOWA, and WAMN) are in regions of intensive corn-soybean agriculture, while the other three (MAKA, OTKA, and EMND) are in agricultural regions that produce much less corn and soybean. All contemporary specimens were collected from corn-soybean fields. Sample sizes are as in Table 1.



Figure 2. Landmarks (circles) and semilandmarks (triangles) digitized onto each specimen.

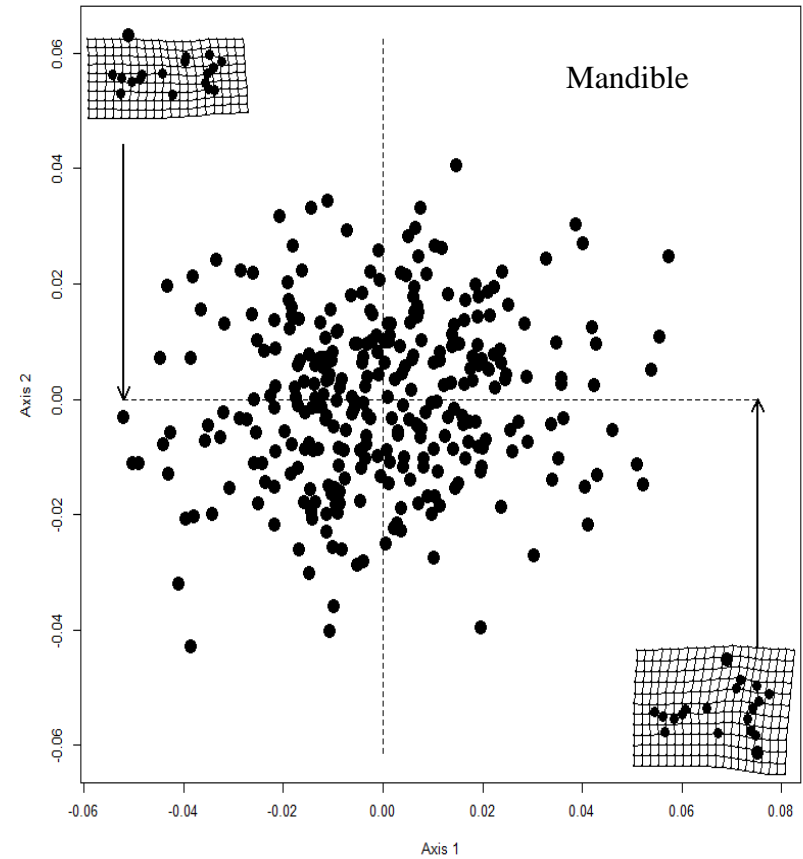
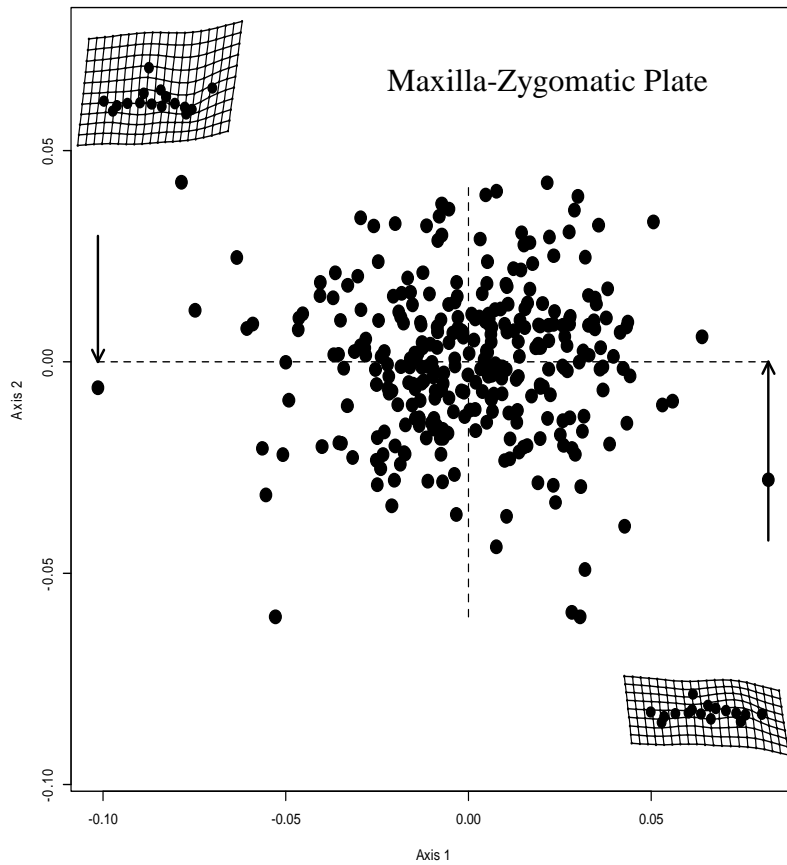


Figure 3. Tangent space for maxillae-zygomatic plates (left) and mandibles (right). PC axes 1 and 2 explain the maximum amount of variation in the data (maxillae-zygomatic plates: 27 and 13%, mandibles: 25 and 13%). The thin plate spline deformation grids in each corner represent the largest difference among all specimens, by indicating what each structure looks like at the high and low ends of PC axis 1, as described in the methods.

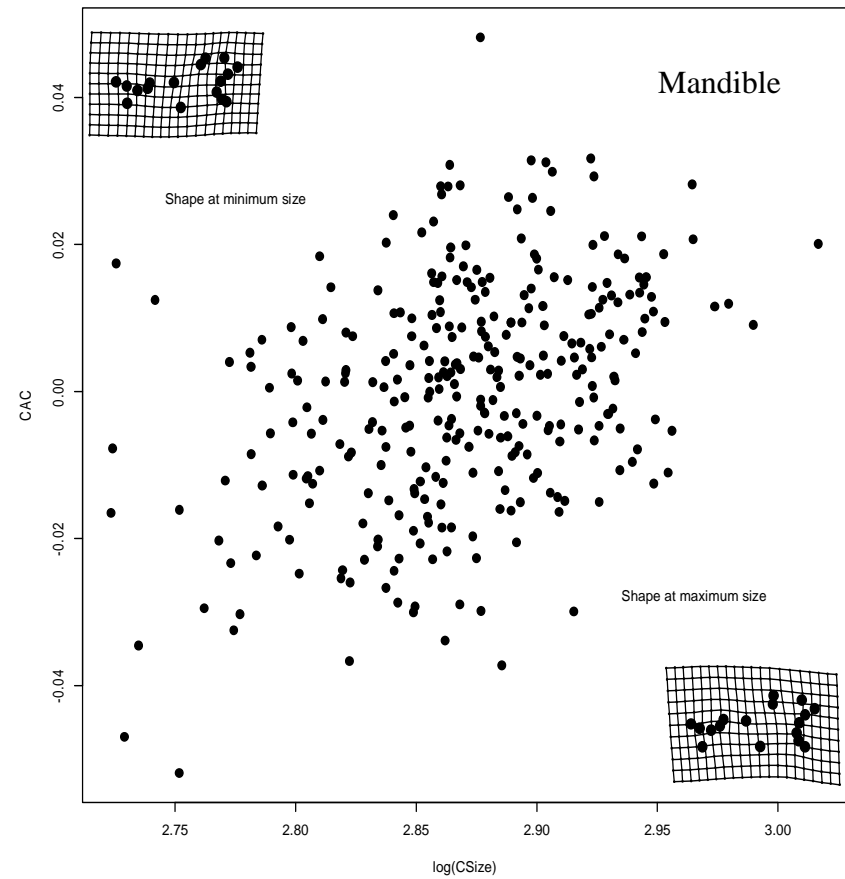
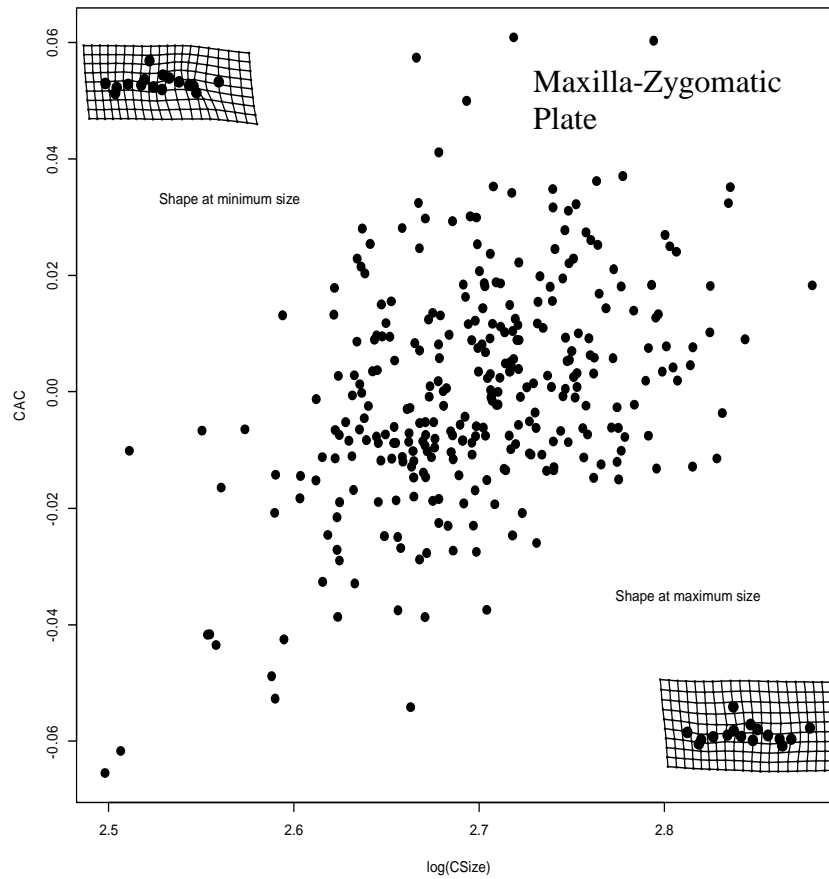


Figure 4. Allometry of maxillae-zygomatic plates and mandibles. Data are generated by plotting the log of the centroid size and common allometric component of each specimen. Black circles are all specimens (N=310) from museum collections and 2012-2013 trapping sessions. Thin plate spline deformation grids in corners represent maximum morphological change based on size change.

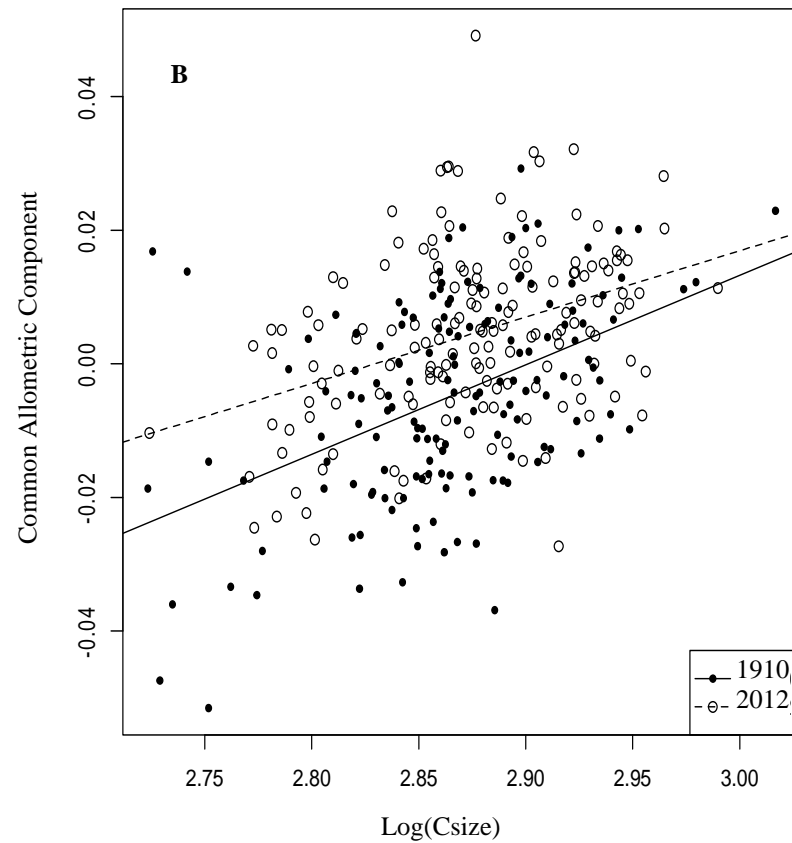
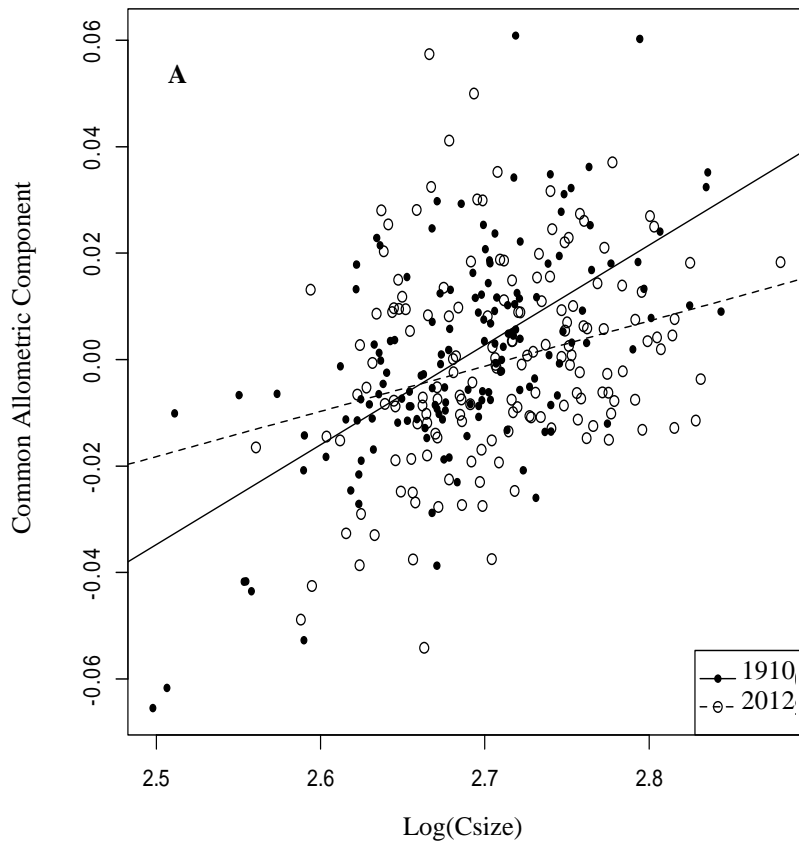


Figure 5. Allometry of maxilla-zygomatic plate (A) and mandible (B) by time period. Prediction lines represent a regression of shape values within time period, based on 150 historic and 160 contemporary specimens. Allometry tests show significant effects of size and year on shape for both structures. Interaction of size:period is significant for maxillae, but not mandibles. Regression lines are historic maxillae: $\text{CAC} = 0.1876 \cdot \text{Csize} - 0.5037$; contemporary maxillae: $\text{CAC} = 0.08492 \cdot \text{Csize} - 0.23$; historic mandibles: $\text{CAC} = 0.134 \cdot \text{Csize} - 0.3886$; contemporary mandibles: $\text{CAC} = 0.1005 \cdot \text{Csize} - 0.284$.

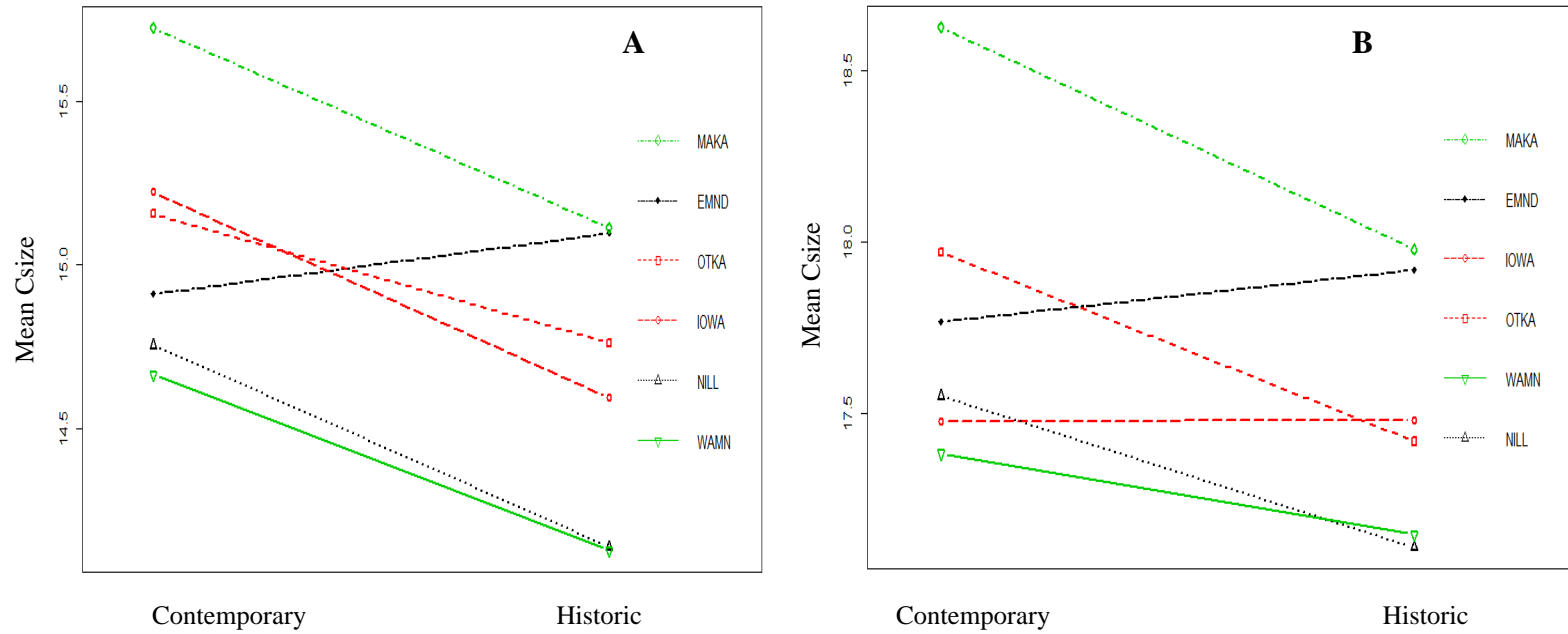


Figure 6. Size differences in mouse maxillae-zygomatic plates (A) and mandibles (B). All data depict average centroid size, which is calculated using all landmarks included in the morphometric analysis. Figures depict mandible and maxilla size differences by location and year. Site acronyms are as in table 3. Site-specific sample sizes are as follows: EMND(31/22;contemporary/historic), IOWA(28/25), MAKa(16/32), NILL(24/16), OTKA(35/40), WAMN(26/15). Historic specimens were collected prior to 1910, and contemporary specimens were collected in 2012 and 2013.

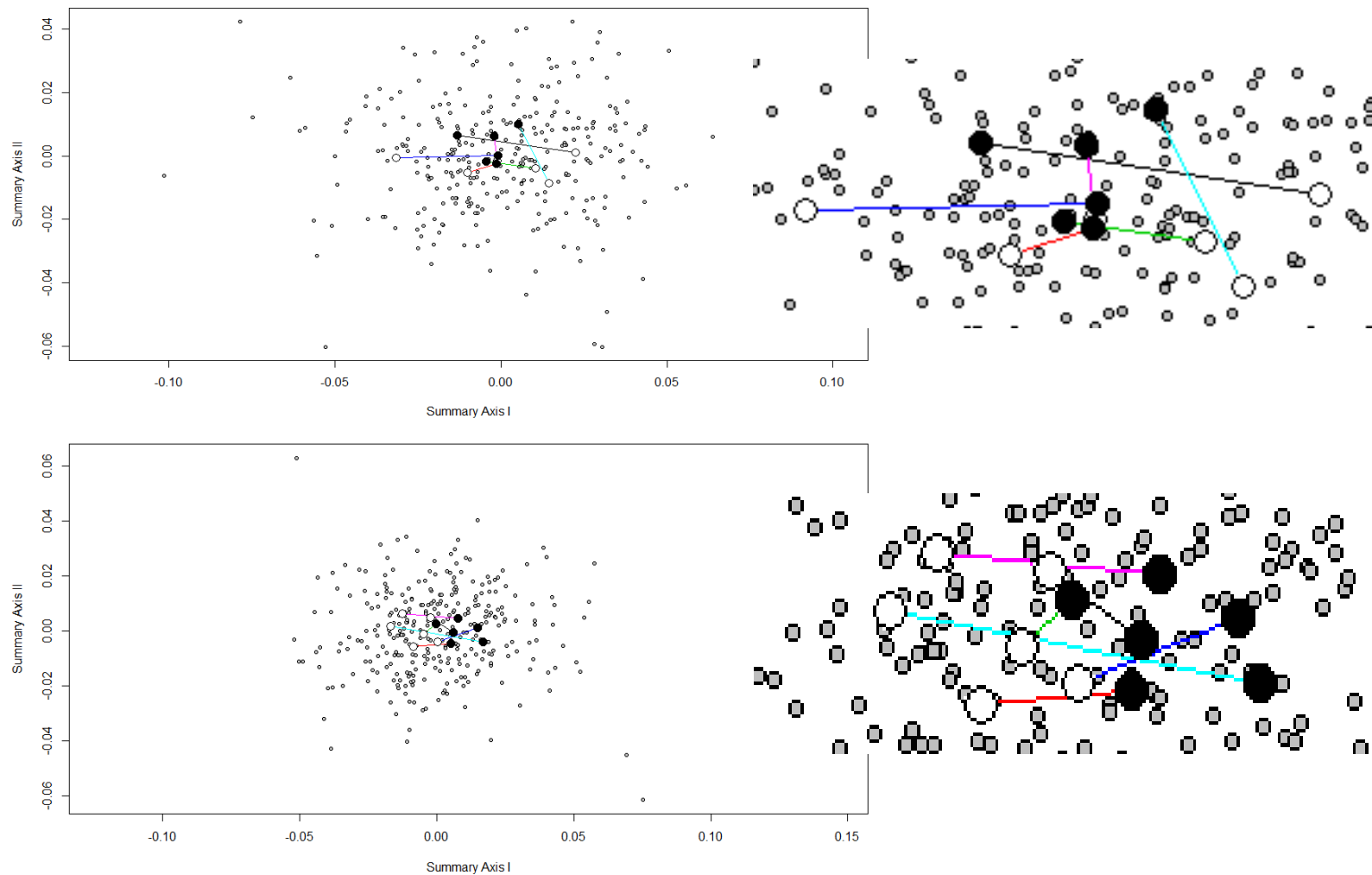


Figure 7. Trajectories of shape change in maxillae-zygomatic plates (top) and mandibles (bottom) by location. Circles are historic (black) and contemporary (white) location averages. Lines connect the historic and contemporary average specimen, and are color-coded by location (black = EMND, red = IOWA, green = MAKA, blue = NILL, cyan = OTKA, and magenta = WAMN). See Table 3 for significant differences among locations for trajectory size and orientation.

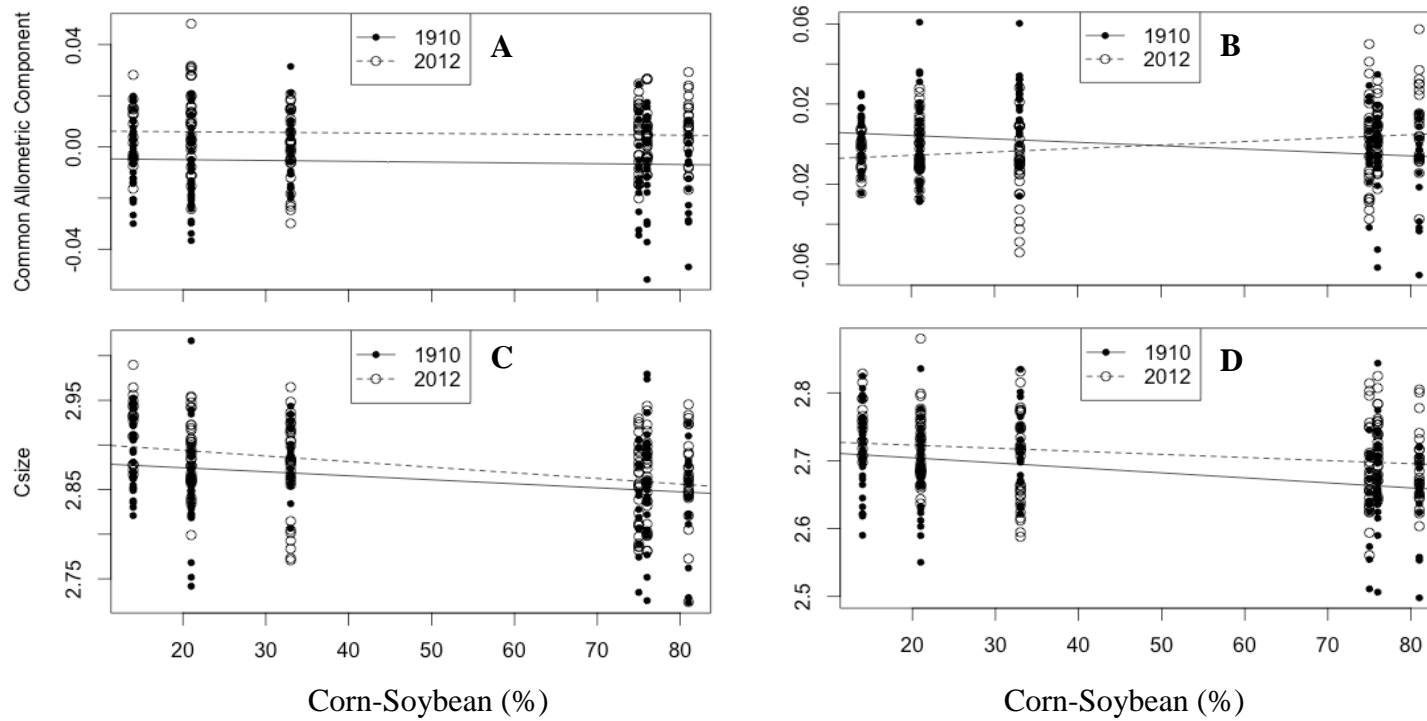


Figure 8. Relationships of percent corn-soybean in the landscape to specimen size and shape. Mandible shape (A) varies with time but not with intensity of corn-soybean agriculture, while Maxilla-Zygomatic Plate shape (B) varies with time, intensity of corn-soybean agriculture, and the interaction of the two. Mandible size (C) is greater now than historically, but smaller in locations that now have greater corn-soybean agriculture (though this was true historically as well). Maxilla-Zygomatic Plate size (D) also varies with time and corn-soybean cover, but lacks any interaction between the two parameters. Values are based on specimens as described in Table 1, including all specimens in the study.

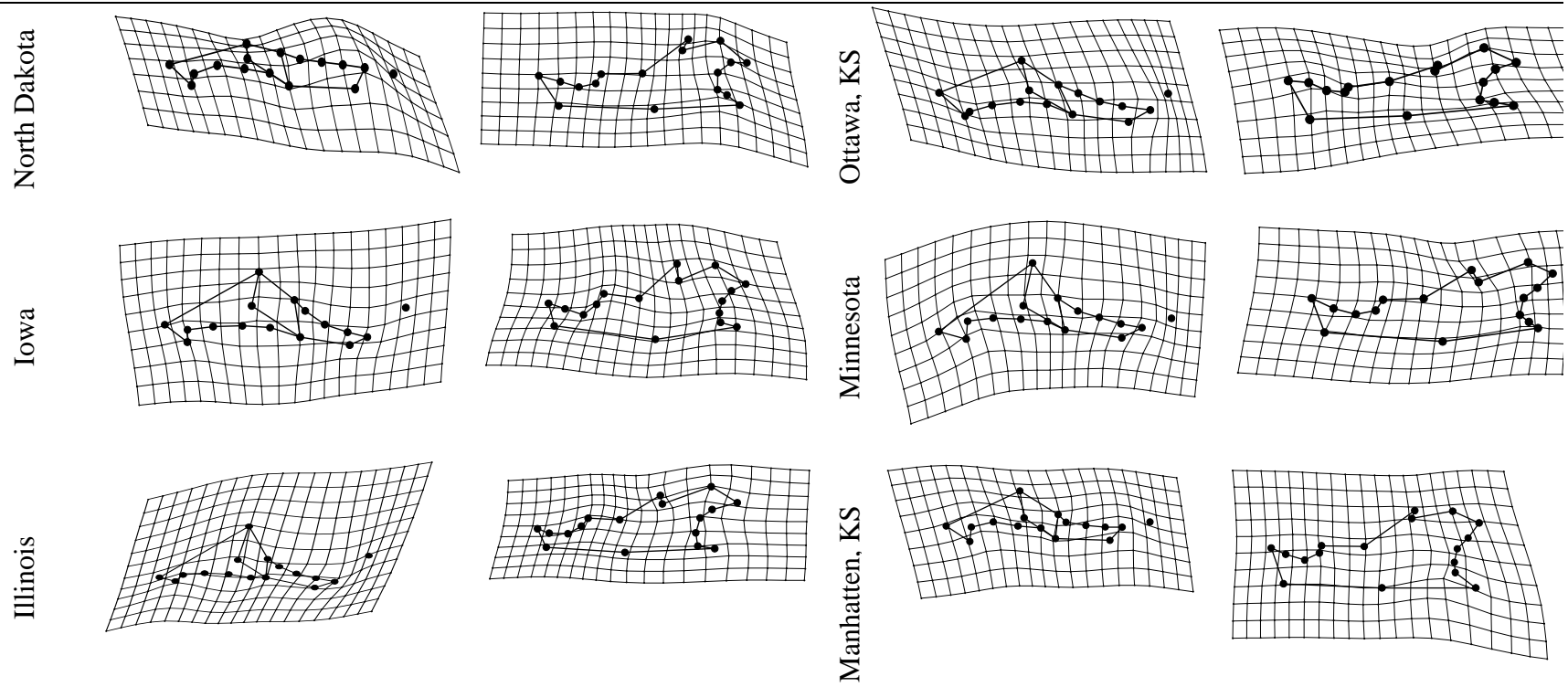


Figure 9. Location-specific changes in *Peromyscus maniculatus bairdii* jaw shape. Thin plate spline (TPS) deformation grids illustrate the changes in shape that have occurred in each location between approximately 1910 and 2012. TPS grids represent a hypothetical change in shape, based on required bending energies associated with making changes to a 2-dimensional object using the least amount of force. If an object is compared to itself, all lines would be parallel or perpendicular to all other lines, and all grid squares would be of equal size. These TPS grids are shown at 3x magnification to clarify changes. Parallel lines represent no change between objects, and all non-parallel lines represent a change in shape. Landmarks are as in Figure 2 and specimens are as described in Table 1.

CHAPTER 3. WEED SEED PREFERENCES AND THE SEASONALITY OF CACHING BY *PEROMYSCUS MANICULATUS BAIRDII* (PRAIRIE DEER MOUSE) WITH DECLINING RATES OF RETURN

A paper to be submitted to *Weed Science*

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ABSTRACT

Herbicide tolerance poses a serious risk to weed control efficacy. In contrast, a high rate of seed removal by vertebrate and invertebrate animals continues unfettered. This study is designed to better understand the foraging decisions of an important weed seed predator in Midwestern US row-crop agriculture, the prairie deer mouse. We presented mice with velvetleaf (*Abutilon theophrasti*) and giant foxtail (*Setaria faberi*) seeds in equal amounts, but in varying densities. We found that mice had a significant preference for giant foxtail seeds regardless of how seeds were presented (foxtail: 12.02 ± 0.56 g vs. velvetleaf: 5.34 ± 0.41 g). We also found that mice preferred to store velvetleaf seeds for later consumption (0.28 ± 0.10 g of foxtail and 0.92 ± 0.28 g of velvetleaf; $\beta = 0.30$, $SE = 0.15$, $P < 0.07$). On average, mice cached. These results are in keeping with those of other studies of caching rodents.

3.1 Introduction

Small mammals are significant seed predators in global agriculture (Cardina et al 1996, USA; Booman et al 2009, Argentina; Marino et al 2005, Netherlands; Harrison and Schmoll 2003, USA; Fischer et al. 2011, Germany). In fact, over-winter studies in Iowa found up to 92% of seeds were lost to predators, and live-trapping surveys indicated that prairie deer mice (*Peromyscus maniculatus bairdii*) were the dominant rodent seed predators

(Williams et al. 2009). However, their study found no preference between foxtail and velvetleaf seeds, despite large disparities in their morphological and life history traits. Determining seed preference from this study is problematic though, as the researchers did not use declining rates of nutritional return, nor did they examine caching behavior explicitly. If caching behavior removes significantly more seeds than a mouse can consume, this behavior could enhance the efficacy of weed control.

Seed preferences by mice may enhance the seed survival of less-preferred seeds by allowing them to escape predation. In agricultural settings, deer mice preferentially consume corn and soybean, likely due to the high nutritional value of the waste grain (Vickery et al 1994). Similarly, weed seeds are likely to be subject to different rates of predation by mice. Deer mice in agricultural settings are not known to have a preference between grass and forb seeds, despite the potential impact preference can have on weed population control (Williams et al. 2009, Westerman et al. 2008). However, there has been some evidence that mice typically prefer larger seeds in agricultural settings (Brust and House 1988, Mittelbach and Gross 1984).

In addition to high levels of seed consumption, prairie deer mice also cache significant amounts of seeds in burrows and scatter hoards (Vander Wall and Longland 1999). There are few details of this foraging behavior in agricultural settings (but see Houtcooper 1971, Howard and Evans 1961). Houtcooper (1971) found only a few instances of caching, and seeds were mostly foxtail. Howard and Evans (1961) found that deer mice stored ragweed, clover, grass, and acorn seeds in grasslands surrounded by woodland. They also review other studies that describe a variety of seed preferences from different systems. None of these reviewed studies observed burrows in corn-soybean rotations. This gap in

understanding the foraging ecology of deer mice poses a significant barrier to predicting the population dynamics of weeds.

In this study, we evaluate 1) the preference for two representative and economically important seed types, 2) caching behavior for both weeds, and 3) how different densities affect seed consumption and caching. We hypothesized that mice would 1) prefer to eat foxtail seeds due to the ease with which they can be processed, 2) preferentially cache velvetleaf which has a thicker seed coat and longer seedbank residency time, 3a) cache more seeds when seeds are in higher density, and 3b) not change the amount of food they eat with different densities of weed seeds.

3.2 Materials and Methods

This study was conducted in Ames, IA (Enclosures: 41° 59' N, -93° 40' W; Dairy Field: 41° 58' N, -93° 38' W; Section 19: 41° 59' N, -93° 41' W) utilizing mice and seeds from local farms in Story County, IA. The study species, the prairie deer mouse, is the only abundant small mammal found in corn and soybean fields in this region. The two weed seeds, giant foxtail and velvetleaf, have been found to be readily consumed by prairie deer mice. These weed species are economically important throughout the Midwestern U.S. (Bridges and Baumann 1992), but they differ greatly with regard to germination behavior, growth habit, fecundity, seed longevity and size, and other morphological, physiological, and phenological characteristics (Warwick and Black 1986; Buhler and Hartzler 2001; Dekker 2003) (Table 1). The velvetleaf used in this study had been grown and harvested for another study, and then stored in a seed facility for 1-2 years. The foxtail used in this study was

collected from an infestation on a local farm. It was separated from harvested grain, and used within 1 year.

We designed the first study to assess whether mice preferred either of the economically important weed seeds, when presented in a giving-up-density (GUD) design. The intent of a GUD is to ensure that there is a declining rate of return within a single foraging patch. As an animal consumes food within a GUD patch, it becomes harder to find the next food item. This ensures that the foraging behavior mimics natural limitations of finding food, as the animal removes food items. Ultimately, the amount of food remaining in a patch reaches an asymptotic value, representing a lack of further significant foraging in the patch. This value is indicative of the amount of food available in other patches, as an animal is assumed to quit foraging in a patch when other patches are more profitable (Brown 2007). In order to evaluate preferences, we placed 1 gallon buckets with 1 liter of sand and approximately 3g of seed in pairs of a foxtail bucket and a velvetleaf bucket. We placed 4 pairs of buckets at the corners of a 20 m square. We designed the study to provide a single foraging mouse, or burrow of multiple mice, access to all four corners. A prairie deer mouse's home range has been recorded to be 40m in radius (Blair 1940), though in highly productive crop fields, we tend to find more than a single mouse in a 40m radius (unpublished data). Buckets were left in the field for approximately 1 week, between December 2008 and March 2009. Each field had 5 grids, separated by at least 50m, and we used three fields (Dairy, East Section 19, and West Section 19 Hereafter: A, B, and C), with all grids from separate fields separated by more than 1km. Results were evaluated by grid, since this may represent the average behavior of a single animal. Statistical analyses treated

grids as a single experimental unit, and observations on that grid were treated as repeated measures of the grid.

We designed the second study to test whether foraging behavior on these two economically important seed types was altered by the presentation and density of seeds. In order to address these simultaneously, we presented mice with both seed types and at a constant total weight of seeds, but in different arrangements and in different amounts of substrate. All experimentation was conducted in four 3 m by 3 m enclosures in central Iowa, such that we could test 4 mice consecutively. Each enclosure room consisted of ~0.3 m depth of dirt over concrete, and ~1 m of aluminum flashing around the edge to keep mice from escaping. Hardware cloth and fiberglass walls were an additional 1m tall, and the roof was sheet metal. Within the enclosure, mice were free to move about, and had access to water, and 1 or 2 foraging buckets (Table 2). We supplied mice with wooden boxes and cotton to act as artificial burrows. Mice were able to burrow in the dirt floor as well, and may have made small surface caches or even used other burrows to store food. In the second phase of the study in 2013, we placed mice in 3m x 0.3m runways covered with 1.25 cm opening hardware cloth, restricting movement, burrowing, and caching behavior. These runways were inside the same enclosures as the previous phase of the study.

To test preferences when seeds were in variable densities and arrangements, we collected adult mice from nearby corn and soybean fields using Sherman live traps, and we immediately moved them into enclosures. Individual mice were kept in captivity for no longer than 14 days. The first phase of the study occurred between February and June 2011, and the second phase in August to November 2013. We followed ASM guidelines (Sikes et al. 2011) for live-capturing, transferring, and keeping mice. Treatments in the enclosures

consisted of 2 levels of seed density within a bucket (21g or 42g per liter) and seeds were mixed or kept separate (Table 1). At the beginning of an experimental trial, each treatment was present in one of the enclosures, and the order of the treatments was randomized to prevent legacy effects. All mice were exposed to all 4 treatments. Since mice were all presented with a different treatment upon entering the enclosures, they were not given time to acclimate. In the follow-up runway experiment, mice were given 1-2 nights to acclimate to the experimental setup, with abundant millet. Only two treatments were present in this phase: 21 and 42 g of seed per liter of sand, mixed.

Foraging buckets consisted of 3.8 l round plastic containers. Buckets had either 0.5 or 1.0 l of play sand sifted to 1 mm size or smaller granules. We mixed velvetleaf and foxtail seeds, from sources previously described, into the sand according to Table 1. We sifted seeds from the sand on the third day, and applied a random treatment to that enclosure by mixing in new seeds. We mixed seeds at approximately mid-day to reduce disturbance of foraging activities. In order to collect regular caching data, we opened artificial burrows and removed cached seeds on the same day. We baked seeds at 190C for 20-30 minutes to destroy potentially harmful organisms. We sifted seeds again to further remove sand. We then processed the sample in a seed-separating device and manually removed loose seed coats and fecal material from the sample and weighed the remaining seeds. We analyzed these results in JMP Pro (10.0.0, SAS Institute 2012). We first analyzed the difference in seed preference, constraining for animal identity using standard least squares mixed effects regression models. We then used the same to test the effects of seed density, mixture, and sex on this tendency to eat or cache one seed or another more intensively. We also evaluated the tendency to cache seeds as a result of gender, season, and seed type using contingency tables.

3.3 Results and Discussion

In a recent commentary, Service (2013) explains that herbicide tolerance is outpacing herbicide development, and he indicates that farmers “have little to fall back on.” Our study along with others suggests that this is not the case (Cardina et al. 1996, Davis and Liebman 2003, Harrison and Schmoll 2003, Westerman et al. 2005, Williams et al. 2009). In fact, four major factors suggest that mice are consistently important for weed seed consumption in Midwestern agroecosystems, especially during cold periods of the year. First, the prairie deer mouse does not hibernate (Getz and Brighty 1986), unlike insect species, which exhibit little or no activity during periods of below-freezing temperatures (Denlinger and Lee 1998). Second, they have high metabolisms and high levels of activity that require them to take advantage of concentrated energy sources, such as seeds (Degen et al. 1998). Third, because insect larvae and weed seedlings are largely absent during cold months, seeds become a primary resource for rodents. Finally, in addition to large amounts of predictable seed consumption, mice cache large quantities of seeds to reduce heterogeneity in food availability. This prediction that mice and other rodents should be important weed seed predators during cold months is consistent with data reported by Harrison and Schmoll (2003), who found that rodents (principally *Peromyscus* spp.) consumed about 1000 giant ragweed seeds/m² between November and February, and that their seed consumption rate was lower at other times of year.

Our first study found that mice had a significantly lower foxtail GUD (2.57 ± 0.02 g) relative to velvetleaf (2.89 ± 0.02 g) ($\beta = 0.16$, $SE = 0.02$, $P < 0.001$), showing that when seeds become increasingly hard to locate with consumption, mice preferred to consume foxtail seeds. This pattern was consistent throughout the sampling period (Figure 1). The

model of GUD values treats each block of 8 buckets as a location, and this location is treated as a random variable, and considers GUD values repeated measures of that location. Since buckets are paired at corners, and all corners are accessible by a single mouse or a group of mice from a single burrow, GUD values may represent the behavior of mice in patches of mixed foxtail and velvetleaf seeds. In other words, mice perceive the two buckets of seeds as a single foraging patch. As further evidence, preliminary studies found when seeds were presented to mice in buckets separated by 20 m, neither seed was consumed to a greater degree (velvetleaf GUD = 2.64 ± 0.04 g and foxtail GUD = 2.58 ± 0.02 g, $n=36$ buckets from July to October). This was likely due to buckets being perceived as separate patches, and seeds were being consumed to a level that matched the background availability of food.

The second phase of our study of feeding and caching preference included 11 mice during the 4-treatment study, and 12 more mice during the 2-treatment follow-up study. We had approximately equal numbers of males and females. On average, mice ate 17.3 grams of seed over three nights; of which 12.02 ± 0.56 (1SE) grams were foxtail and 5.34 ± 0.41 grams were velvetleaf (Figure 2). Males and females consumed approximately equal amounts of seed. In the second phase of the study, mice also consumed significantly more foxtail than velvetleaf by weight ($\beta = 3.69$, $SE = 0.70$, $P < 0.001$, Figure 2). The preference for consuming smaller seeds with short seed bank persistence and caching larger seeds with longer seed bank persistence is consistent with previous research and foraging theory (Smallwood et al. 2001, Brodin and Clark 2007, Chang et al. 2009, Wang and Chen 2009, Xiao et al. 2013). The ease of processing the seed likely motivates the consumption of the grass seed, which other studies have found (Zhang and Zhang 2008). As previously mentioned, other studies have failed to detect a strong preference for foxtail over velvetleaf

using open trays (Williams et al. 2009). However, this study had no declining rate of return for seeds. Our study simulates searching for food throughout a home range, making our study a more accurate depiction of choices made under increasingly difficult conditions.

We also analyzed if the density or arrangement of seed species had any effect on seed preference. The intent was to determine if stands of mixed weeds, which shed seeds as a mix, will be treated differently than seeds shed in more monospecific patches, and whether mice would respond functionally to high densities of seeds. We could find no evidence that mice altered their foraging behavior due to the presentation of seeds (density or mixture) (Figure 3).

In contrast to feeding, mice cached significantly more velvetleaf than foxtail by weight ($\beta = 0.30$, $SE = 0.15$, $P < 0.07$, Range: 0-3.3 g of foxtail and 0-10.0 g of velvetleaf; Figure 2). On average, mice cached 0.28 ± 0.10 g of foxtail and 0.92 ± 0.28 g of velvetleaf (Figure 2). Males cached 0.81 ± 0.31 grams of seed on average per night (foxtail = 0.40 ± 0.19 and velvetleaf = 1.21 ± 0.59), and females cached 0.73 ± 0.26 grams of seed per night (foxtail = 0.35 ± 0.19 and velvetleaf = 1.10 ± 0.48). The long seed bank time of velvetleaf likely motivates the preference for caching the forb seed (Smallwood et al 2001 and Xiao et al. 2013). Larder hoarding of seeds in burrows is well documented, but has not been evaluated for this mouse in agricultural settings. Based on this study and its consistency with other studies, we can predict that mice will eat small, short seedbank seeds and cache larger, longer seedbank seeds.

We did also find complex interactions for seed caching. When we evaluated the tendency of mice to cache as a binomial outcome, caching behavior varied with season (spring: 86%, summer: 47%, fall: 12.5%). Foxtail was cached significantly more frequently

than velvetleaf in the spring, when 86% of the samples had a cache (95 and 76% respectively, $n = 42$, $\chi^2 = 3.36$, $P = 0.07$), but not during the other times. Additionally, in the summer when 47% of the samples had a cache, females cached significantly more frequently than males (75% and 19% respectively, $n = 32$, $\chi^2 = 10.80$, $P = 0.001$), but no differences were detected at other times of the year. Finally, in the fall when only 12.5% of the samples had a cache, velvetleaf was cached significantly more frequently than foxtail (25 and 0% respectively, $n = 40$, $\chi^2 = 7.65$, $P = 0.006$), though this distinction was not evident in other seasons. We also analyzed caching as a function of density and mixture of seeds. Just as in the assessment of eating preference, we found that none of these factors influenced the preference of seed type (Figure 3).

This study suggests that prairie deer mice treat economically important grass and forb seeds distinctly. However, there is also strong evidence that mice are not deterred from eating velvetleaf seeds. For example, Lobo et al. (2013) found when deer mice encounter seeds with secondary compounds that deter consumption, they actively avoid consuming the seed, even when it is in high abundance. Our study found consistent behavior between the seeds, but no evidence of such an active avoidance of seeds. Due to the consistent behavior and lack of seed deterrence we found in this study, responses to seed shed events should be robust to other conditions. When combined with the territoriality of the mice, as well as the known home ranges in agricultural settings, we can make predictions about how much seed mice will be able to consume in the fall. Since we found no evidence that mice consumed more seeds when seeds were more easily obtained, we can presume that individually mice may not have an impact on a dense stand of weeds, but population level studies are needed to determine if mice will respond numerically to the increased value of a food patch.

Alternatively, controlling other food sources, such as waste grain, may be beneficial to weed seed predation. The large range in amount of seeds cached, especially velvetleaf, should be further evaluated to determine how important caching is to weed regulation and whether caching can be increased. This study ultimately provides important information about the services provided by the prairie deer mouse, a native mammal that currently dominates the 42 million ha of corn and soybean fields across the great plains of North America (NASS statistics and personal observation from trapping in Illinois, Iowa, Kansas, Minnesota, and North Dakota). Given that this species has the potential to be economically very important, work should continue to clarify this species' role in commodity production and as an alternative to herbicide-based weed control.

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Table 1. Seed properties of giant foxtail (*Setaria faberi*) and velvetleaf (*Abutilon theophrasti*). Fecundity and seedbank survival data were adapted from Westerman (2005) and Davis (2003). Seed coat and size were evaluated and estimated in this study.

Seed	Fecundity/plant	Seedbank Survival (per year)	Seed Coat	Size
Giant Foxtail	~1000 seeds	~50%	Thin, papery	170 mg/ 100 seeds
Velvetleaf	~200 seeds	~75%	Thick, leathery	1037 mg/ 100 seeds

Table 2. Treatments used to study mouse responses to seed arrangement. Buckets were approximately 4 liters in size, with a single hole for mice to enter the bucket and forage. Mixed and separate refers to whether the foxtail and velvetleaf were encountered simultaneously by the mouse in each bucket (mixed), or if they were kept in separate foraging buckets (separate).

Treatment	Buckets	Total liters of Sand	Seed Density/liter	Mixed/Separate
A	2	1	42	Separate
B	1	1	42	Mixed
C	2	2	21	Separate
D	2	2	21	Mixed

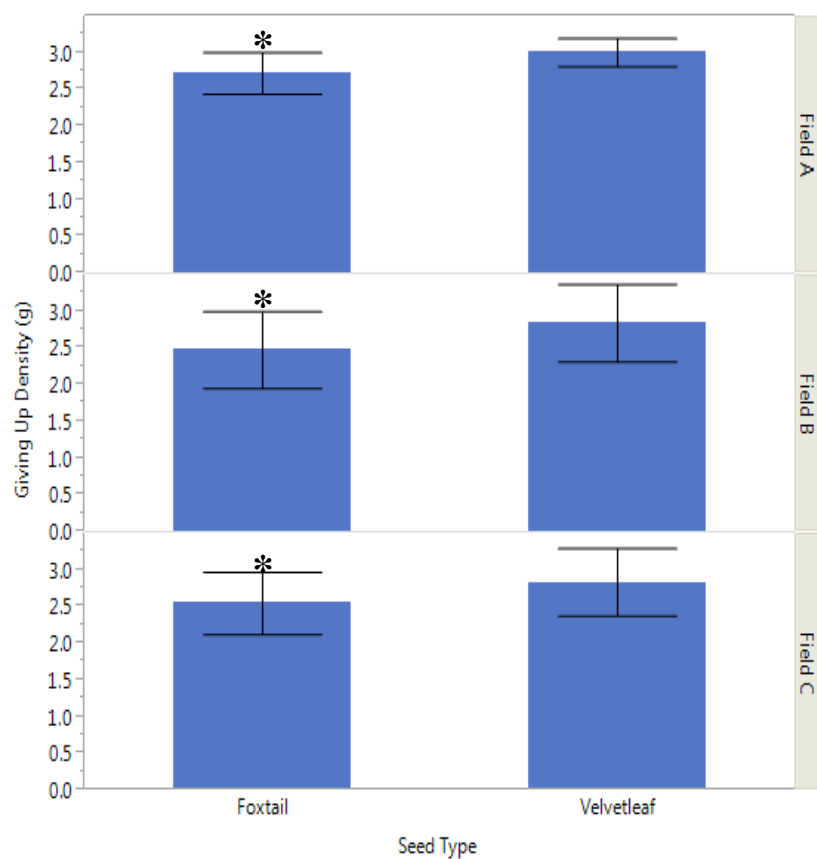


Figure 1. Giving up density by field. Bars are averages and error bars are standard error of GUDs in each of the 3 fields. A total of 96 buckets (48 of each seed) were sampled approximately weekly from December 2008 to April 2009. An asterisk denotes significantly lower GUD values within a field.

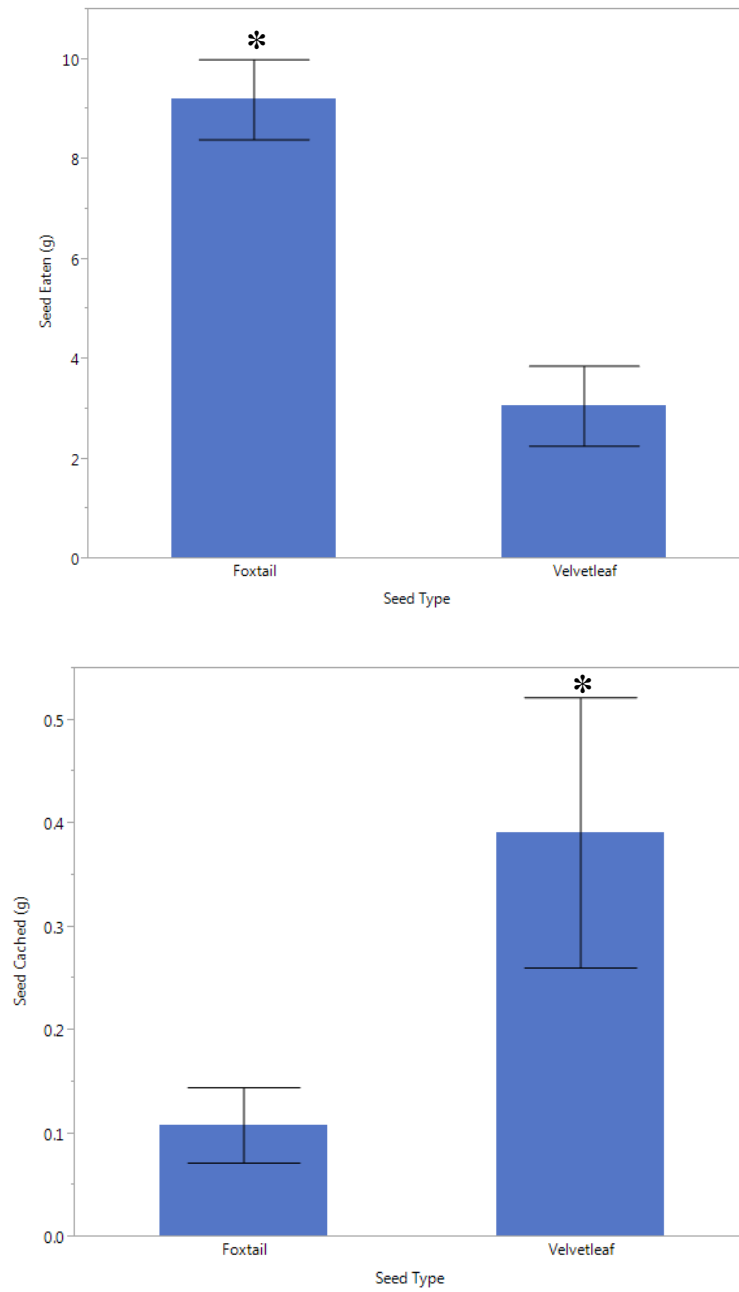


Figure 2. Mean amount of seed eaten (top) and cached in an artificial burrow (bottom) over a 3-day period. Box and whisker plots show the median and interquartile range of seeds eaten or cached by prairie deer mice ($n=23$ mice). Mice were contained in an experimental enclosure, and offered only the two seed types mixed into either 1 or 2 liters of sand as described in Table 2. An asterisk denotes significantly higher values.

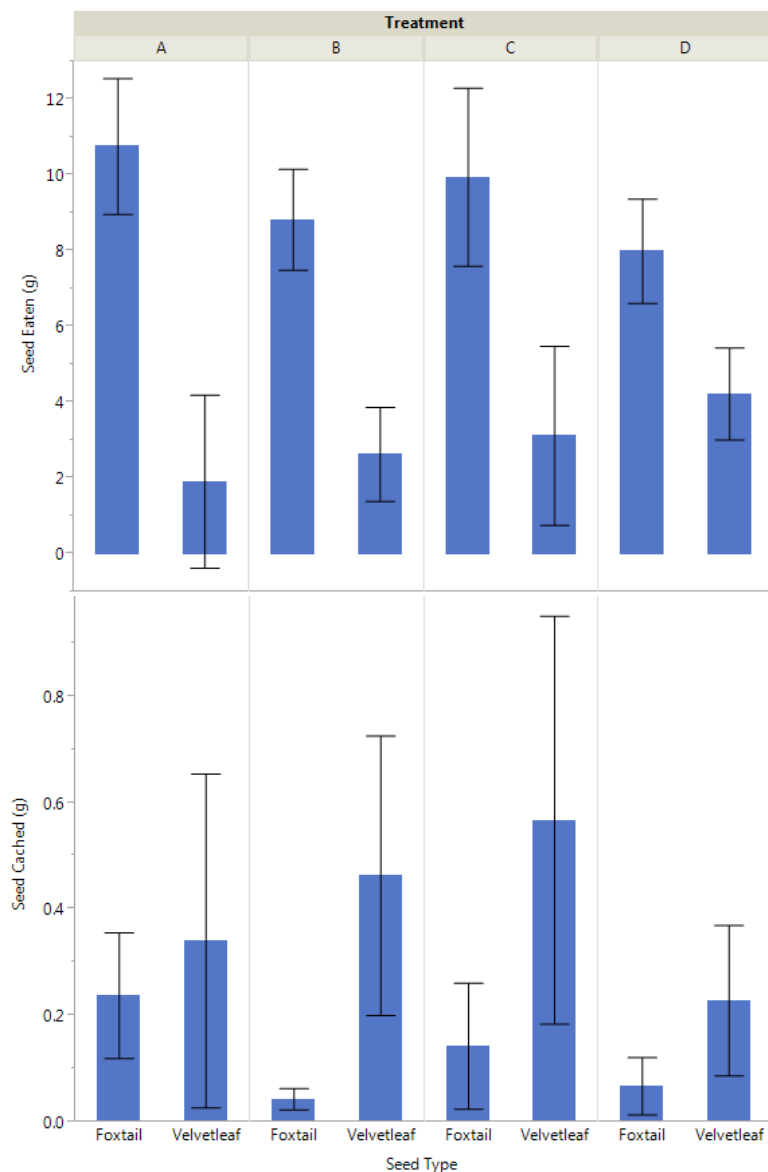


Figure 3. Mean amount of seed eaten and cached. Data are from 23 mice allowed 3 nights to forage in GUD buckets. Treatments were: A – 1 liter of sand divided between 2 buckets, with seeds in separate buckets (each seed @ 42g/l, each bucket @ 42g/l); B – 1 liter of sand in 1 bucket, seeds mixed (bucket at 42g/l, each seed @ 21g/l); C – 2 liters of sand in 2 buckets, seeds separate (each bucket at 21g/l, each seed at 21g/l); D – 2 liters of sand in 2 buckets, seeds mixed (each bucket at 21g/l, each seed at 10.5g/l). No treatments had significantly different values.

CHAPTER 4. THE USE OF SMALL MAMMAL FECAL MATERIAL FOR DIETARY ANALYSES BY ISOTOPE ANALYSIS

A paper submitted to the *Journal of Mammalogy*

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ABSTRACT

Small mammals are important predators in many agricultural systems, and through their diet they can help to regulate pest insect and weed populations. This study is designed to test whether field-collected fecal material is an effective means of detailing the foraging ecology of small mammals in simple agricultural settings. We conducted three studies to evaluate the efficacy of this technique: field-collected fecal material from unknown animals, fecal material from known animals in an enclosure with known diets, and fecal material from known animals in the field during a shift from non-reproduction to reproduction. Some significant patterns in isotope values included a significant decrease in $\delta^{15}\text{N}$ due to nursing (4.57 ± 0.19 vs 3.28 ± 0.47 , $P = 0.02$), significant shifts in ^{13}C and ^{15}N amounts relative to spring thaw ($\delta^{13}\text{C}$: -13.34 vs. -10.72 , $P = 0.01$, $\delta^{15}\text{N}$: 0.92 vs. 4.09 , $P = 0.03$), and a significant correlation between the relative amounts of two seed types and $\delta^{13}\text{C}$ (slope = 5.46 , $SE = 1.82$, $P < 0.01$). The use of this technique will help to elucidate foraging patterns of this economically important species. This is also the first example we could find that used field-collected fecal material of small mammals to evaluate stable isotopes.

4.1 Introduction

In a recent special edition of *Journal of Mammalogy* (2012), Ben-David and Flaherty point out that estimating diet quickly from stable isotopes is problematic, especially when

species consume diets in different isoscapes (distributions of isotope value combinations). They emphasize that more tests of quick-turnover isotopes in natural systems are necessary to evaluate the efficacy of stable isotopes in trophic ecology. Because the trophic ecology of *Peromyscus maniculatus bairdii* enhances agricultural weed population control, making it economically important (Westerman et al. 2005; Westerman et al. 2008; Williams et al. 2009), it makes a prime study species for this sort of test. Fortunately, researchers have already described stable isotope fractionation and turnover times for this species fed commercial food items in the lab (Hwang et al. 2007 and Miller et al. 2008), allowing the comparison of controlled diets and environments to free-ranging animals under normal ranges of environmental conditions.

Fecal material is common evidence of the short-term diet, as it contains several indicators of trophic ecology: DNA, food remains, and isotopes (Dickman and Huang 1988; Piggott and Taylor 2003; Salvarina et al 2013), and has a fast turnover rate (gut passage time). However, the ability to detect and evaluate genetic markers limits DNA analysis (Murray et al. 2011). In addition, visual analysis of fecal material is problematic, especially when trying to evaluate proportions of items in a diet, or when there is variable digestibility (Dickman and Huang 1988). In contrast, Salvarina et al. (2013) found, under controlled conditions, that bat fecal isotopes are indicative of trophic shifts within a few hours, illustrating that fecal isotopes are indicative of recent trophic ecology. However, we were unable to locate any studies that evaluate the stable isotopes of field-collected fecal material from small mammals.

To test the efficacy of stable isotopes from field-collected fecal material, we evaluated whether stable isotopes in fecal material indicate the composition of a weed-seed

diet, and whether field-collected samples have consistent trophic patterns. We also evaluated whether the reproductive status of females alters isotope values. We hypothesized that 1) diets of mixed seeds will mimic patterns from lab studies, 2) winter-spring diet shifts will be detected in $\delta^{15}\text{N}$ levels, 3) corn and soybean in the diet will be detected in $\delta^{13}\text{C}$ levels, and 4) reproductive status of females will alter $\delta^{15}\text{N}$.

4.2 Materials and Methods

4.2.1 Fecal Material Collection and Processing

There were three phases to our study of fecal isotope values in *Peromyscus maniculatus bairdii*. In the first phase of the study, we used field-caught mice to evaluate the fractionation of ^{13}C and ^{15}N . Fractionation occurs when the relative amount of a stable isotope (e.g. ^{14}N and ^{15}N) changes, due to metabolic processes. In this phase, a single mouse was the source of fecal material, and the diet was controlled. However, it utilized weed seeds commonly found in fields as the sole food source. We were thus able to evaluate the isotope values of the diet and fecal material of the same individual to determine fractionation rates. In the second phase, we evaluated the fecal material left behind from a previous study of foraging behavior. In this phase, we processed samples from giving-up-density buckets (3.8 l plastic pails with 1 l of sand and ~3 g of weed seeds). We used the fecal material that remained in the stored samples. In the third phase of the study, we collected material from individual female mice in a harvested soybean field. The mice were tagged and could be followed through time. We focused on later winter and early spring to detect patterns associated with reproductive states. Mice were nesting in wooden boxes designed to mimic natural burrows and had access to background rates of soybean and supplemental corn in

most cases. Mice in these boxes nested and cached seeds similar to observations of natural burrows.

In the first phase of the study, we evaluated the effectiveness of fecal isotopes in detecting the diet of *P. m. bairdii* when consuming naturally available foods (mesocosm study). In order to establish this study, we used Sherman live traps to collect *P. m. bairdii* from corn and soybean fields in Ames, IA from February to March in 2010. Each of four mice were moved into a 3 m x 3 m enclosure, specifically into an artificial nest box. We repeatedly collected fecal material from each mouse during the study, and 17 samples of fecal material were analyzed. All samples were collected after mice had been in the enclosure for more than 3 days. The internal dimensions of the wooden nest boxes were 12cm x 12cm x 10cm, had a removable lid, and a single opening with 2.5cm corrugated tubing to mimic a burrow tunnel. In each box we added 2 cotton nestlets (PharmaServ, #NE3600). In each 3m enclosure, we provided the mouse with water and a supply of food that contained equal amounts of giant foxtail and velvetleaf seeds mixed into sand trays. Sand trays were giving-up-density trays with 1l of sand and ~42g of seed total (twice as much as mice were known to use in three nights). Mice foraged in these trays for 1 and 3 nights. Due to the time of year and the ambient temperatures, mice had no access to animal food, and enclosures were inspected regularly for surface caches and new burrows.

In order to evaluate the fractionation of diet to fecal isotopes and determine the consistency of results, we collected precise data on diet composition and sampled fecal material from individual mice. We weighed seeds before mixing them into the sand. After the mice foraged for 3 nights, we sifted seeds from the sand with a 1mm sieve. We also collected seeds from the artificial burrows. From this combination of uneaten seeds, we could precisely

evaluate the diet of the mice. On the same day we collected seeds, we collected fecal material from the foraging tray. We used this fecal material for the isotope analysis (details below).

All of the fecal material returned to the lab was either frozen and later heated or immediately heated to 190C for 20 minutes to destroy pathogens. After we heated and dried the seeds, we used a seed separator to remove loose seed shells, and then we processed samples with forceps to remove additional fecal material and other foreign objects. We immediately weighed the seeds. For fecal analysis, we collected single fecal droppings from each mouse enclosure for a single date, and this was the sample. We also analyzed the isotope composition of subsamples of both seed types. We selected these randomly from the source seed bags.

In the second phase of the study, we evaluated whether fecal material that was unintentionally collected in a previous study would provide insight into trophic ecology of free-ranging mice. In order to evaluate this available data, we collected fecal material from giving-up-density tray samples that came from cornfields in central Iowa. We conducted the foraging study in 2008, but we separated the fecal material from the samples in 2011. The original study design was blocked, with 4 sample points at the corners of a 15m square. At each corner were 2 buckets with 1 liter of sand and 3g of foxtail or velvetleaf. We conducted the study from January to March 2008. There were 4 squares per field and 3 fields. Field A was a silaged field (with corn cobs and stalks removed from the field in early fall) that had approximately annual additions of manure as a main source of nitrogen addition. It also received synthetic fertilizers periodically. In fields B and C, synthetic fertilizers are the main source of nitrogen addition and the fields are managed in a corn-soybean rotation. For fecal analysis, we collected a single fecal dropping from each seed sample in a field for a given

date. We combined all fecal material from a field for a single date. This combined material was the sample.

In the final phase of the study, we evaluated the diet and isotope fractionation of female mice. In order to evaluate reproductive status, we collected fecal material directly from female mice in late winter to early spring 2012. This collection was facilitated by using artificial nest boxes we placed in a harvested soybean field in the fall of 2011. This experimental setup was part of another project to evaluate the effects of food addition on mouse reproduction and movement (unpublished). Pairs of buried nest boxes were arranged in transects, 20m apart, and 4 burrow pairs in a transect. We installed 5 transects, for a total of 20 burrows. As part of the other study, we spread corn around half of the burrows in a 20 m radius in the late fall of 2011. During the sampling period in 2012, we visited nest boxes periodically and when we encountered a female, we recorded weight, nipple size, perforation, sperm plug, or pregnancy. For fecal analysis, we collected 2-3 fecal droppings directly from each female mouse. We also collected corn and soybean samples from each transect during the sampling period. We analyzed these seeds for isotope composition and compared the values to the fecal isotopes.

In all studies, we returned samples to the lab, dried and sterilized the sample in an oven at 190C for 20 minutes, and then homogenized the sample for subsampling using a mortar and pestle. We extracted a single subsample of ~0.5 mg from each pulverized sample, added this subsample to a tin cup, and analyzed the subsample in an isotope analyzer. We stored these subsamples in the tin capsule in a lab desiccator until isotope analysis.

4.2.2 Isotope Analysis

Ratios of stable heavy to light isotopes of nitrogen and carbon can be used to detect structure of food webs, diet shifts, and anthropogenic impacts (See Dalerum and Angerbjorn 2005; Layman et al 2007; and Crawford 2008 for reviews). Specifically, mammals tend to excrete depleted levels of heavy nitrogen isotopes (^{15}N) in urea relative to the proportion in their diet (Vanderklift and Ponsard 2003). This physiological fractionation causes an enrichment (increase relative to food) of whole body and fecal heavy isotopes by approximately 3-4‰ compared to the diet (Gaebler et al. 1966; DeNiro and Epstein 1981; Minagawa and Wada 1984; Owens 1987). This relationship can vary across taxa, but values are relatively similar for closely related organisms in similar environmental conditions (Vanderklift and Ponsard 2003 and Crawford 2008).

One cause of variation in isotope fractionation is digestion. For example, different digestive systems (e.g. hindgut versus foregut fermentation) result in different levels of heavy isotope enrichment (Sponheimer et al 2003). In addition, food sources have variable decomposition in the digestive tract (e.g. soft-bodied versus hard-bodied invertebrates), which can affect isotope ratios, and thus field studies are necessary to determine if this source of variation overwhelms the dietary signal (Crawford 2008). Unlike nitrogen isotopes, carbon isotopes ($^{12}\text{C}/^{13}\text{C}$) do not tend to fractionate between diet and many body tissues or fecal material (DeNiro and Epstein 1978). However, due to the kinetic isotope effects of different photosynthesis pathways, C3 plants (e.g. soybean and velvetleaf) are relatively depleted in ^{13}C compared to C4 plants (e.g. corn and foxtail), providing strong signals of diet.

In order to determine whether these patterns are detectable in field fecal isotopes, we processed subsamples via a Finnigan MAT Delta Plus XL mass spectrometer in continuous

flow mode connected to a Costech Elemental Analyzer at Iowa State University (Department of Geological and Atmospheric Sciences). Reference standards (Ammonium Sulfate [IAEA-N-1]; Ammonium Sulfate [IAEA-N-2]; Caffeine [IAEA-600], Sucrose [IAEA-CH-6; RM 8542], and Acetanilide [laboratory standard]) were used for isotope corrections, and to assign the data to the appropriate isotope scale (12 total standards for the field study, 12 for the mesocosm study, and 23 for the maternal study). The combined uncertainty (analytical uncertainty and average correction factor) for $\delta^{13}\text{C}$ is ± 0.21 , 0.80 , and 0.06‰ (VPDB) and $\delta^{15}\text{N}$ is ± 0.06 , 0.20 , and 0.17‰ (Air), respectively for the three studies as above.

Heavy isotopes are typically found in low abundance (^{14}N , 99.64%; ^{15}N , 0.36%) (Audi et al 2003), so their values are reported as parts per thousand (permil or ‰) (Fry 2006). The typical equation used for reporting stable isotope values is:

$$\delta(\text{Heavy Isotope}) = ((R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}) * 1000$$

For nitrogen and carbon:

$$\delta^{15}\text{N} = ((R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}) * 1000$$

Where $R = ^{15}\text{N}/^{14}\text{N}$ of the sample and the standard.

$$\delta^{13}\text{C} = ((R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}) * 1000$$

Where $R = ^{13}\text{C}/^{12}\text{C}$ of the sample and the standard.

4.2.3 Statistical Methods

All analyses were completed in JMP Pro 10.0.2 (SAS, 2012). We used standard least squares analysis to evaluate the effects of time and multiple reproductive condition states on isotope composition. We used one-way analysis of variance to evaluate before and after thaw, as well as bivariate reproductive condition. When repeated measures were analyzed,

we assigned the identity of the mouse as a random variable to first remove variation due to identity.

4.3 Results

All samples had sufficient carbon and nitrogen for isotope composition analysis. Thus, we were able to evaluate late winter diet in 2009 and 2012, while using three collection techniques. In the mesocosm study, we were able to assay 4 mice on known diets of velvetleaf and foxtail. In the field study, we analyzed 22 subsamples from 3 corn fields in late winter 2009. Finally, in late winter 2012 we used fecal material collected directly from female mice in soybean fields supplemented with corn. Using this final technique, we analyzed data from 33 mice over 4 weeks (2 before spring thaw and 2 after spring thaw).

4.3.1 Mesocosm Study

For the mesocosm study, the entire range of isotope values for the $\delta^{15}\text{N}$ of fecal material was higher than the average values for foxtail and velvetleaf (Table 1). Since the composition of diets was known in this phase of the study, we were able to determine that the average fractionation of $\delta^{15}\text{N}$ was enrichment by 1.91 ‰ ($SD = 0.55$), but the fecal values of $\delta^{15}\text{N}$ were not significantly changed by seed diet composition ($P > 0.1$). The latter result was expected, as the $\delta^{15}\text{N}$ values of foxtail and velvetleaf were not especially different (Table 1). The range of $\delta^{13}\text{C}$ values overlapped with the average values of the weed seeds, but exceeded them as well (Table 1). The average fractionation for $\delta^{13}\text{C}$ was $-2.37(2.53)$, and the ratio of foxtail:velvetleaf was a significant predictor of fecal $\delta^{13}\text{C}$ (slope = 5.46, $SE = 1.82$, $P < 0.01$, Figure 1). We analyzed fractionation of isotope ratios in two ways. When we treated each subsample as independent, fractionation rates were significantly different between 1 and 3 nights ($\delta^{15}\text{N} = 1.68(0.36)$ after 1 night and $2.24(0.64)$ after 3 nights and $\delta^{13}\text{C} = -0.50(2.4)$

after 1 night and 1.54(1.8) after 3 nights: $F_{1,15} = 5.46$ and 4.96 , $P = 0.03$ and 0.04 , respectively). Combined with the fact that $\delta^{15}\text{N}$ results from 3 nights more closely matches values found in other studies, 3 nights is a significantly better sample period. However, when we corrected for repeated measures within a single individual, the differences were no longer significant ($F_{1,3} = 4.37$ and 4.05 , $P = 0.12$ and 0.09), possibly due to a very small sample size of animals.

4.3.2 Field Study

The fecal material that we collected from the cornfield foraging study had a range of isotope values that exceeded the average value for corn from the field, as we expected (Table 1). The range of values for $\delta^{13}\text{C}$ included the average value for corn. $\delta^{13}\text{C}$ values declined through time ($R^2 = 0.48$, $\beta = -0.09$, $SE = 0.02$, $t = 4.15$, $P < 0.001$) and $\delta^{13}\text{C}$ values were significantly lower after spring thaw than before spring thaw (-13.34 vs. -10.72 , $F_{1,24} = 7.28$, $P = 0.0126$), indicating a shift toward C3-based foods. However, $\delta^{15}\text{N}$ values did not change through time ($R^2 = 0.01$, $\beta = -0.006$, $SE = 0.01$, $t = 0.38$, $P = 0.7$) and values were not significantly different after thaw than before (6.73 vs. 6.61 , $F_{1,24} = 0.039$, $P = 0.85$), indicating a stable trophic diet across this time period. The $\delta^{15}\text{N}$ values of the field A, however, were significantly lower than the other two fields combined (4.87 vs. 7.35 , $F_{1,24} = 25.47$, $P < 0.0001$) (Figure 3), perhaps due to a different fertilizer regime. The ranges of $\delta^{13}\text{C}$ values were consistent across all three fields.

4.3.3 Maternal Study

The range of fecal values for the maternal study indicated that females ranged from a complete reliance on soybean to a complete reliance on corn (Table 1 and Figure 4). $\delta^{15}\text{N}$ was significantly higher after thaw (4.92 vs. 4.09 , $F_{1,47} = 4.72$, $P = 0.0349$) (Figure 5). $\delta^{13}\text{C}$

was not significantly different after thaw than before (-23.47 vs. -27.82 , $F_{1,47} = 0.49$, $P = 0.49$). In addition, mice with nipples rated as currently nursing had a significantly lower $\delta^{15}\text{N}$ value (3.28 ± 0.47 vs 4.57 ± 0.19 , $F_{1,41} = 6.17$, $p=0.0172$) while controlling for identity and time period (Figure 6). In contrast, perforation, sperm plug, and pregnancy were not significant predictors of isotope values.

4.4 Discussion

Previous work on fecal isotopes from mice has detected reliability in summarizing diets (Hwang et al. 2007), but no mouse fecal studies have been done on free-ranging animals or using a natural diet. In our study, we found that fecal isotope values of *Peromyscus maniculatus bairdii* reflect their diets when fed common weed seeds normally available in corn-soybean agriculture (Figure 1). This finding builds on previous work that shows fecal material reflects diet in an array of animals in the wild (Sponheimer et al. 2003; Painter et al. 2009; Codron et al. 2011), as well as laboratory studies of small mammals (Hwang et al. 2007; Salvarino et al. 2013). In addition, fecal isotope values of prairie deer mice matched predictions from these previous studies; $\delta^{13}\text{C}$ was not fractionated from diet, and $\delta^{15}\text{N}$ was enriched by approximately 2‰ in fecal material relative to diet.

We also found ecologically relevant isotope value patterns in free-ranging mice (Figure 2). Across three fields, mice had consistent patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value shifts. As Ben-David (1997) illustrated, generalist predators (martens) switch with the availability of food items, and this is detectable in isotope patterns. We also found that mice relied on C4-based foods (C4 plants and herbivores of C4 plants) throughout the study period, matching expectations for diet (Figure 3). In addition, we were able to detect unique nitrogen sources

on fields [manure (low $\delta^{15}\text{N}$) vs. synthetic fertilizers (high $\delta^{15}\text{N}$)] (Figure 3). The most apparent and potentially useful pattern detected, though was the increase in consumption of primary consumer tissue near spring thaw, and a decrease thereafter (Figure 2). Since spring thaw represents an opportunity for the emergence of insects and the opportunity to forage for them (e.g. pupae) in the soil, the availability of animal protein should increase at this time. Other studies have shown that *P.m. bairdii* have a preference for insects when they are available, relative to the seeds that they subsist on through the winter (Whitaker 1966; Clark and Young 1986; Flick, unpublished data). Another possible explanation for this short-term shift in isotope value is cannibalism of mouse carcasses in experimental nest boxes during late winter, which we have detected (unpublished data).

We detected smaller shifts in diet during the maternal study (Table 1) than during the free-range study. The small but significant enrichment in $\delta^{15}\text{N}$ and no change in $\delta^{13}\text{C}$ (Figure 4) are best explained by the large amount of corn available for consumption (placed by us for another project). Even with the overwhelming amount of corn consumed, mice significantly increased their consumption of animal tissue following spring thaw (Figure 5). In contrast, we found no evidence that mice in different reproductive states were consuming different diets. This is despite the high energy requirements for lactating females, which increase until one week before weaning, and the strong correlation between litter size and kilocalorie consumption (Stebbins 1977). In addition, maturity and reproductive activity were not associated with altered isotope values. Successful reproduction and nursing of offspring however, did cause a 1.3 ‰ decrease in $\delta^{15}\text{N}$ of fecal material (Figure 6). This is supported by limited other studies that suggest that milk preferentially receives ^{15}N isotopes, reducing the values in other bodily tissues (Kurle 2002). However, it is in contrast to Ben-David et al

(2012), who found that pregnancy and milk production did not produce any more fractionation of isotopes than those predicted by body mass change. The best explanation for this difference may be that they were studying muscle and red blood cells. Rates of incorporation into different tissues, compared to the excretion of non-incorporated isotopes, could explain this difference (Rio et al. 2012), as could differential turnover times.

Despite strong effects of timing and nursing, and the tendency for reproduction to increase toward summer (but not in our samples), we found no evidence for an interaction between the two effects. Nursing had a larger effect than time, but both were significant when included simultaneously in the model. While we found no interaction effects in our study, it is reasonable to think that in studies where nursing is not taken into consideration, significant depression of $\delta^{15}\text{N}$ values may occur, and shifts in diet may go undetected. For example, in our free ranging mice the lack of increase in $\delta^{15}\text{N}$ after spring thaw may result from a high percentage of nursing mice. In systems supplemented with food, much as is accomplished by the waste grain in crop fields, deer mice will reproduce more frequently and even during the winter (Taitt 1981). Further isotope research in this system should carefully account for this.

The combined results indicate that both direct and indirect sampling of fecal material is effective in detecting ecologically relevant trophic behavior in our system. While Ben-David et al. (2012) show evidence that the trophic ecology of complex diets is difficult to evaluate, simpler systems, such as agroecosystems, provide a unique opportunity to test the efficacy of stable isotope techniques. This paradigm of limited choices or switching behavior has been useful in isotope studies of other tissues (Ben-David et al. 1997 and Bodey et al. 2010). However, it has had limited utility with fecal analysis (but see laboratory studies of

Salvarino et al. 2013). This study shows that future research can utilize fecal material collected through non-invasive methods to detect broad patterns in this species' trophic agroecology. It should also be broadly applicable in other simplified systems with economically important small mammals.

4.5 REFERENCES

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Table 1. *Isotopic values of food and fecal material.* The reported values for each sample are the difference in relative abundance of the heavy stable isotope ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) between the sample and an international standard.

Experiment	Sample	$\delta^{13}\text{C}$ (VPDB)		$\delta^{15}\text{N}$ (Air)	
		Average ($\pm 1\text{SE}$)	Range	Average ($\pm 1\text{SE}$)	Range
Mesocosm study: Wild mice in captivity	<i>Velvetleaf</i>	-26.19 (0.56)	-30.80 to -11.5	3.27 (0.37)	0.00 to 4.20
	<i>Foxtail</i>	-12.52 (0.27)	-15.00 to -11.54	3.15 (0.18)	0.00 to 6.56
	<i>Feces</i>	-14.04 (0.61)	-19.11 to -7.82	5.17 (0.15)	4.36 to 6.94
Field study: Wild mice sampled advantageously	<i>Feces</i>	-12.23 (0.54)	-16.63 to -7.99	6.68 (0.31)	3.99 to 8.80
Maternity study: Wild females sampled directly	<i>Corn</i>	-11.51 (0.23)	-12.27 to -10.64	6.08 (1.61)	-0.67 to 11.42
	<i>Soybean</i>	-27.35 (0.25)	-28.09 to -26.45	-0.28 (0.26)	-1.11 to 0.80
	<i>Feces</i>	-23.27 (0.54)	-26.92 to -15.48	4.61 (0.24)	2.27 to 7.85

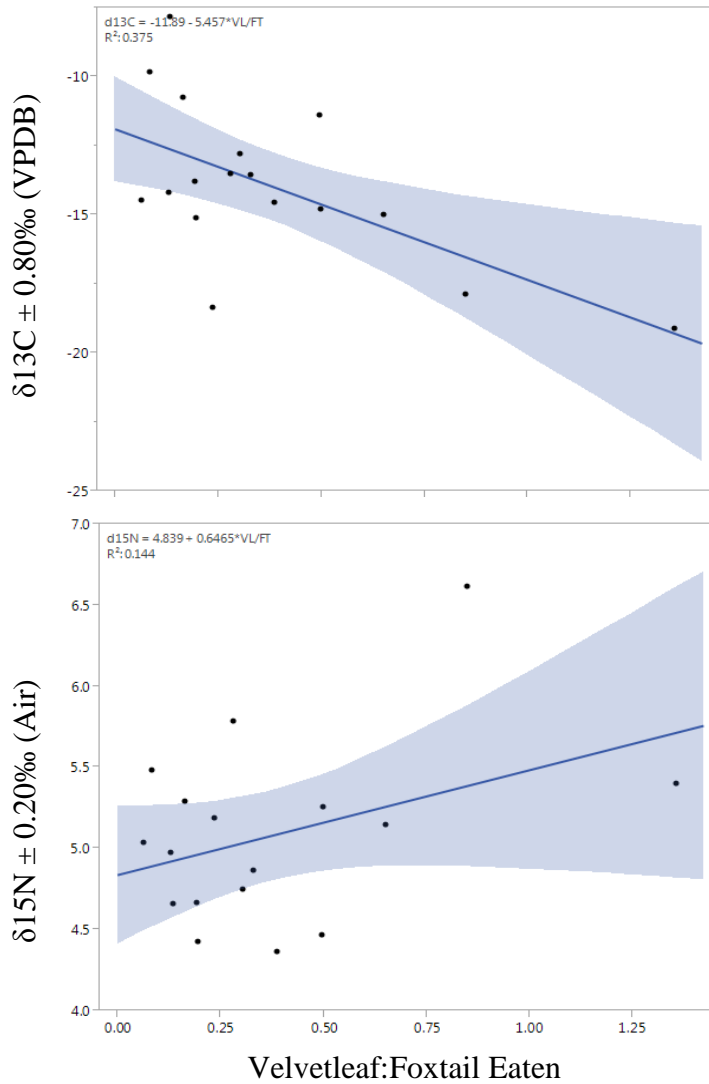


Figure 1. The effect of *Peromyscus maniculatus bairdii* weed seed diet composition on fecal isotope values. Graphs represent the least squares fit line of $\delta^{13}\text{C}$ (top) and $\delta^{15}\text{N}$ (bottom) as a result of diet composition (velvetleaf seed mass:foxtail seed mass). Shaded regions are the 95% confidence interval for each fitted line. Results are from 4 *Peromyscus maniculatus bairdii* captured in central Iowa, and kept on a controlled diet of the two weed seeds. Fecal material was collected repeatedly for these 4 mice, and the 17 data points represent repeated samples from these 4 individuals.

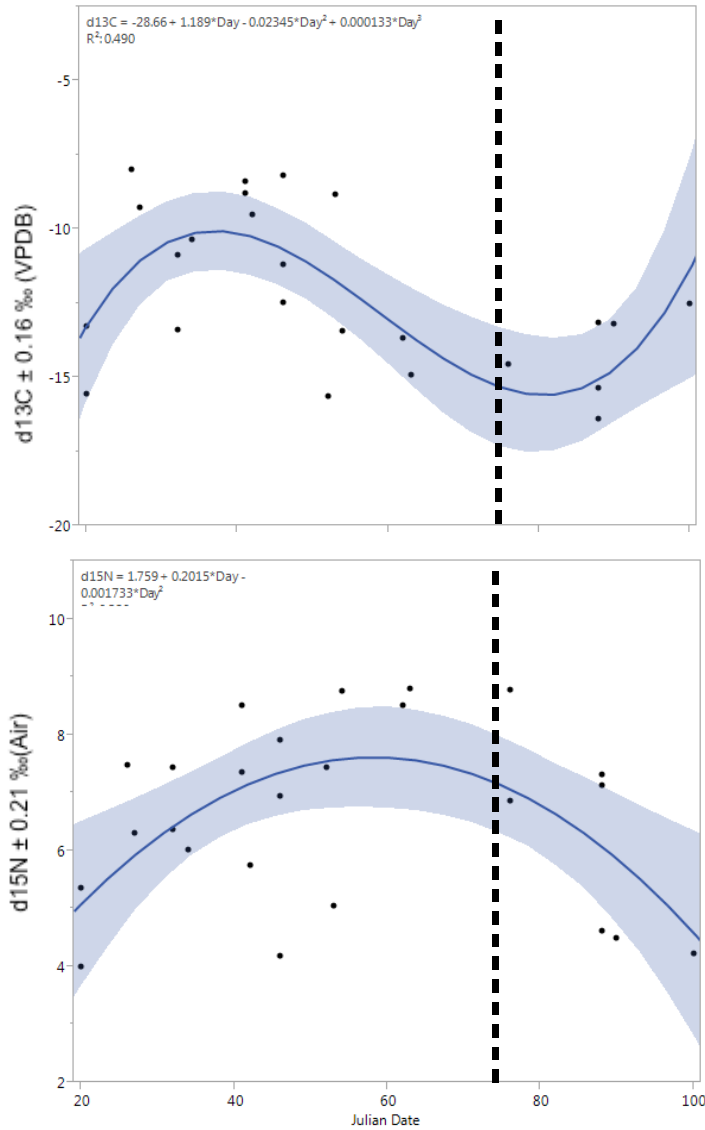


Figure 2. Trophic patterns in *Peromyscus maniculatus bairdii* diet through time. The isotopic values are generated from fecal material collected between January 1st and April 1st 2009 (N = 22). All samples are from corn fields in central Iowa. The day of the year predicts $\delta^{13}\text{C}$ (top) and $\delta^{15}\text{N}$ (bottom) values of feces. The fit line is the quadratic function of the effect of day on isotopic values, and the shaded region is the 95% confidence of the fit line. The $\delta^{13}\text{C}$ cubic line is significant for all three parameters ($P=0.001$, 0.039 , and 0.0039), while the $\delta^{15}\text{N}$ quadratic line is significant for day^2 ($P=0.004$), but not for day ($P=0.24$). Lines shown had the lowest AICc values of up to 3rd order polynomials. This expectation, of complex shifts in diet is in line with a increasing reliance on certain foods as spring nears, and a sudden change after spring thaw. Data were generated from up to 120 buckets per sampling period (3 fields, 5 grids/field, 8 buckets/grid). Actual sample points varied, due to losses of buckets and lack of foraging in some grids. The material from a field was homogenized into a single sample from each sampling date. The fit line represents the fit across all three fields.

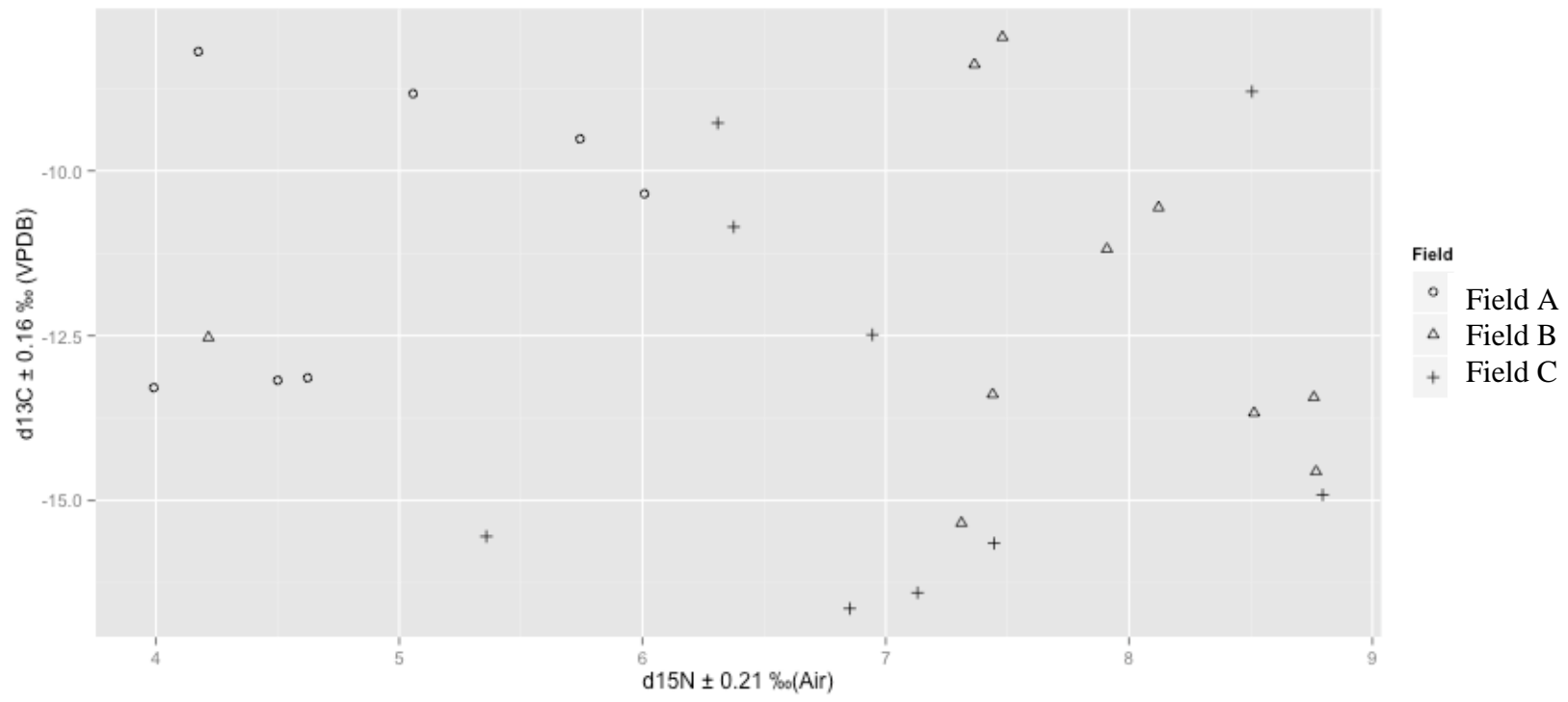


Figure 3. Trophic space of *Peromyscus maniculatus bairdii* in 3 corn fields in central Iowa. Values are generated as described in Figure 2.

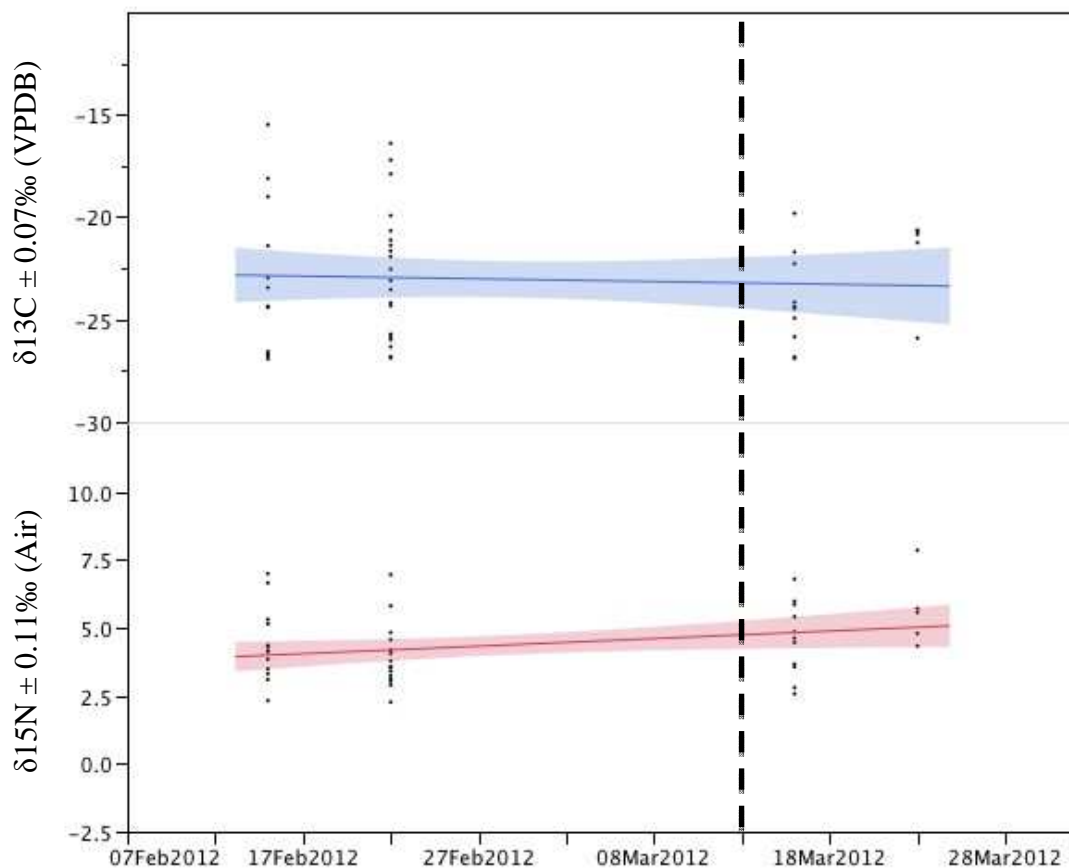


Figure 4. Temporal patterns of stable isotopes for female *Peromyscus maniculatus bairdii* in central Iowa. The fit line is a 1st order approximation of the effect of time of year on isotopic values, and the shaded area is a 95% confidence interval around that estimate. Fecal material was collected directly from 33 female mice in artificial burrows. Dashed line represents approximate spring thaw event.

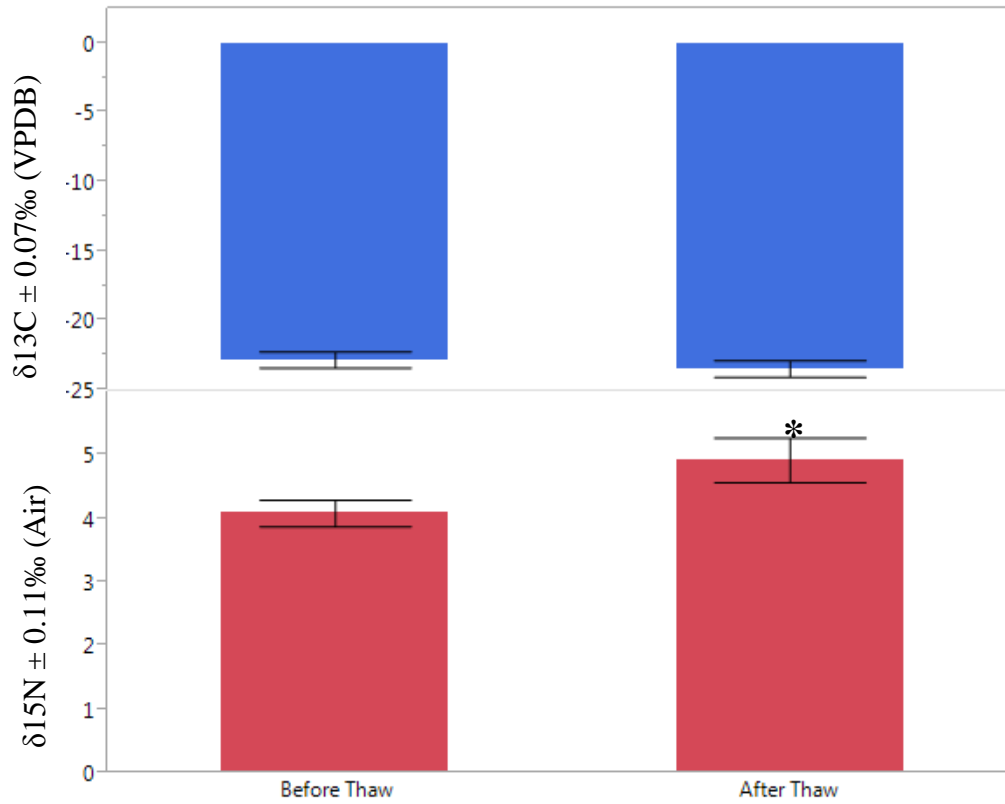


Figure 5. Comparison of stable isotopes before and after spring thaw. Values are from fecal material of 33 female *Peromyscus maniculatus bairdii* as described in Figure 6. Bars are means with error bars representing 1 standard error. An asterisk denotes a significantly higher value.

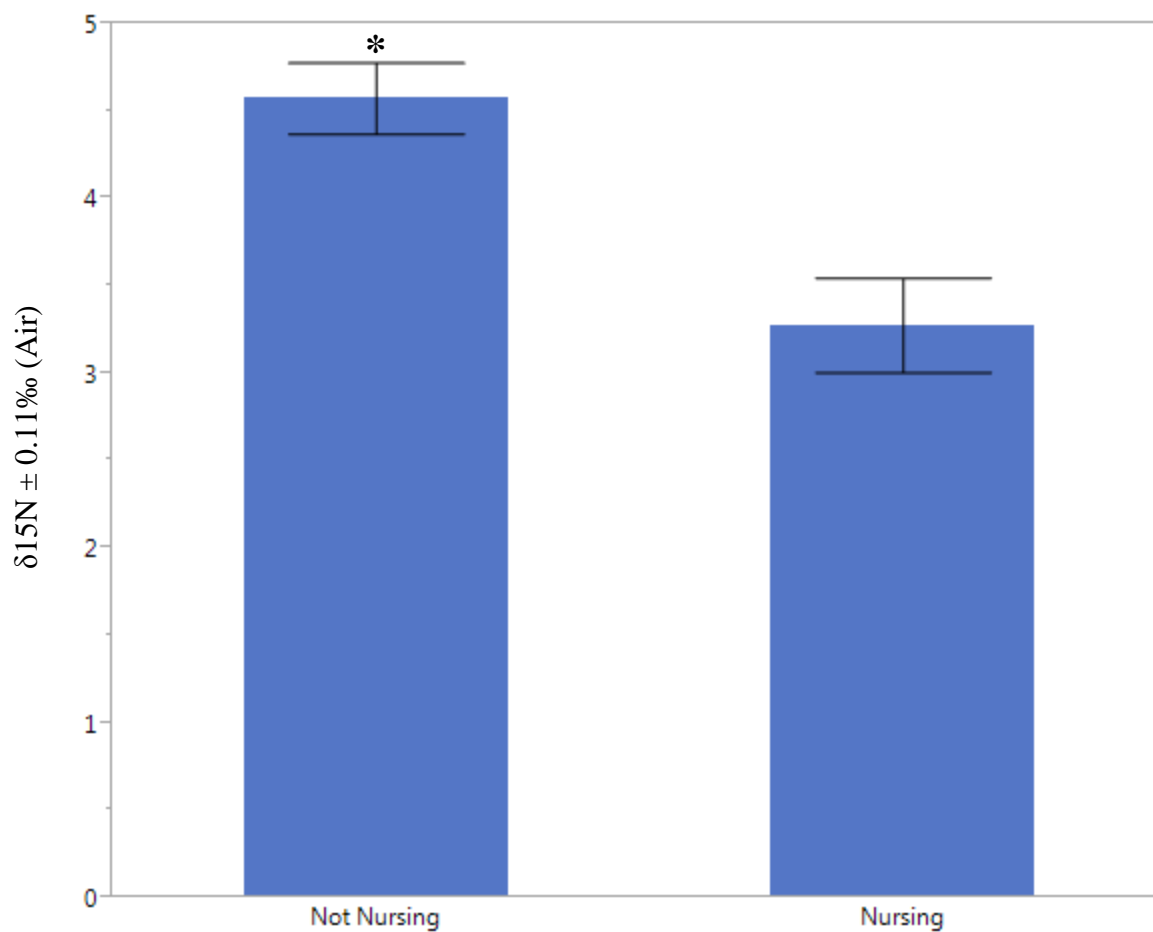


Figure 6. Comparison of stable isotope values based on nursing. Values are from fecal material of 33 female *Peromyscus maniculatus bairdii* as described in Figure 6. Females were considered to be nursing if their nipples were large, or they were observed to be nursing young. Bars are means with error bars representing 1 standard error. An asterisk denotes a significantly higher value.

CHAPTER 5. SUBSTRATE MATCHING: PRAIRIE DEER MICE (*PEROMYSCUS MANICULATUS BAIRDII*) INCREASE FORAGING INTENSITY IN HABITATS THAT ENHANCE THEIR CRYPSIS

A paper to be submitted to *Behavioral Ecology*

John W. Doudna and Brent J. Danielson

ABSTRACT

Many species are surviving in novel ecosystems that in some ways mimic their historical habitat, but also have significantly altered disturbance regimes. For example, the prairie deer mouse has successfully transitioned from a perennial tallgrass prairie biome to an annual row crop agriculture system, which has little to no vegetative cover for six or more months each year. In this system, mice are at increased risk of predation, especially by visual predators. In addition, recent changes in soil quality, especially high amounts of erosion, have lightened soil color, and resulted in a mismatch for the dark brown coat color of the species. We sought to determine if the mice respond to a lack of crypsis when foraging for common weed seed species. If mice forage less intensively on light substrates, this has the potential to reduce the species' potential to regulate weeds. Indeed, we found that mice forage significantly less on light substrates than on dark substrates, matching expectations of crypsis recognition by the mouse (difference: $1.96 \text{ g} \pm 0.28$; $t \text{ Ratio} = 6.42$, $P < 0.001$). The impact of substrate color did not interact with a simulation of moonlight. The simulation of moonlight had a smaller and insignificant effect compared to substrate color. Studies of foraging small mammals should account for this substrate color issue, and studies of weed regulation should also take into account natural heterogeneity in soil color.

5.1 Introduction

Row-crop agriculture represents a novel ecosystem (*sensu* Hobbs et al. 2006), with high disturbance and simplified communities that allow for the study of behavioral adaptations. *Peromyscus maniculatus bairdii* (prairie deer mice) forage in these agricultural landscapes, which pose novel predation risks. The corn-soybean fields that represent the majority of the species' contemporary range lack vegetative cover for approximately six months each year. However, this subspecies evolved as a specialist of prairie habitat, which is dominated by dense vegetation throughout the year that drastically limits visibility to aerial predators and making behavioral adaptations to enhance background matching of little value. The two exceptions to this are post-fire and snow-covered ground. After a fire, charcoal and very dark soils would be exposed, and they are appropriately matched by the overall dark coat of the species, while snow cover typically results in subnivean space that mice utilize, so that they are rarely exposed above the snow.

While the vegetation structure has changed dramatically, most predators of small mammals are still present in the Midwest. The prairie biome was the dominant habitat of the Midwestern United States (hereafter: Midwest) for eight thousand years (Anderson 2006) and only in the past 200 years or less have *P.m. bairdii* been exposed to this increased level of risk. In addition, the species is unable to utilize fencerows or field edge vegetation, as they are rarely found in these habitats, being more common in field centers (unpublished data). Thus, it is of interest whether this species has evolved or is still evolving risk management strategies to this new predation regime. Indeed, we have found evidence for evolution in this time frame from other work in our lab, specifically a change in jaw shape due to novel foods (Doudna and Danielson, in review).

Mice can ameliorate predation risks through behavioral responses to environmental cues. For small mammals, indirect cues such as moonlight and cover indicate predation risk and alter foraging behavior (Kaufman and Kaufman 1982, Clarke 1983, Brillhart and Kaufman 1991, Brown et al. 1992, Orrock et al. 2004). However, responses to typical cues may be insufficient in intensive agriculture. This is because intensive agricultural production has exposed lower soil horizons that are lighter in color and may expose mice to increased attacks due to increased conspicuousness (Kaufman 1974). In fact, studies on “beach mice” have found that mice mismatched to their substrate are at much greater risk of predator attacks than camouflaged specimens (Vignieri et al. 2010). One possible mechanism to reduce these risks is that mice have evolved the capacity to, or had a preexisting capacity to avoid substrates that would decrease their crypsis.

This study evaluates whether mice respond to illumination and substrate color by reducing their seed predation rates in patches and on nights that increase their predation risk. We used a semi-natural enclosure experiment to evaluate whether mice would forage less intensively on the lighter substrate due to a perceived increase in predation risk. We also hypothesized that mice would forage less intensively when illumination levels were high, due to a perceived increase in predation risk.

5.2 Methods

We used an enclosed experimental setup with wild-caught mice to evaluate the roles of lighting and substrate on mouse foraging behavior. We conducted this study in Ames, IA (445300E, 4647800N 15T) during fall and winter 2011-2012 and summer and fall 2013. We trapped *Peromyscus maniculatus bairdii* in corn and soybean fields with Sherman live traps

following American Society of Mammalogists guidelines for the use of wild mammals (Sikes et al. 2011). Mice were collected from traps early in the morning and immediately taken to an artificial burrow that contained cotton nesting material. This burrow was connected to an experimental runway by corrugated tubing, approximating a mouse's natural burrow tunnel. The sealed burrow-runway combination was nested in a protected enclosure (see below for details). We repeated the experiment 5 times between September 2011 and March 2012. Three mice escaped during the study, and we were able to include 17 mice in the analysis.

We used experimental runways nested in enclosures to maintain a relatively controlled environment, while exposing mice to natural patterns in light:dark and temperature. We constructed 4-3m x 3m enclosures for studying *P.m. bairdii* foraging behavior and caching (Figure 2). The building had a tin roof and translucent fiberglass panels surrounding the upper half of the building, which provided similar light intensity to an overcast day within the enclosures. We added ~1m of aluminum flashing to the lower portion of each enclosure to prevent mice from escaping the enclosures for a previous study. We placed two equidistant light-emitting diode (LED) lights (from a string of holiday lights) along the center beam of each enclosure in an attempt to simulate moderate moonlight intensity (45-90 mLux). In order to restrict movement to the experimental setup, we constructed a 3m x 0.25m runway within each enclosure. We covered each runway with ~1.25cm opening hardware cloth in a tunnel shape, approximately 0.25m high x 0.25m wide. Visually, the hardware cloth tunnel provided very little obstruction between the interior and exterior (Figure 2). We installed a small access door at each end of the runway to process foraging trays. We covered opposite halves of the runway floor with light (Quikrete® Play Sand) and dark (Black Blast® Blasting Sand) sand. At each end of the runway, we placed a foraging tray with sand that matched the

runway floor sand. We provided mice with water in an automatic dispenser placed in the middle of the runway. Upon exiting the burrow tunnel, a mouse was able to turn left or right toward one of the two foraging trays (described below).

We used paired giving-up-density (GUD) trays within each runway to evaluate risk perception. The foraging trays in each runway consisted of 18 grams of seed mixed in 1 liter of sand. The intent of a GUD is to ensure that there is a declining rate of return within a single foraging patch. As an animal consumes food within a GUD patch, it becomes harder to find the next food item. This ensures that the foraging behavior mimics natural limitations of finding the next food item as food is consumed (Brown 2007).

Trays were placed near the ends of each runway. We pre-sifted sand with a 1mm sieve to standardize particle size. We collected and replaced seeds every 3 days. We randomly ordered 4 treatments: LED lights on with velvetleaf (*Abutilon theophrasti*) seeds (which closely matches the dark substrate), lights off with velvetleaf seeds, lights on with foxtail (*Setaria faberi*) seeds (which closely matches the light substrate), and lights off with foxtail seeds. Velvetleaf is a relatively large (2-3 mm) forb seed with a leathery pericarp from which the seed has to be excised, while foxtail is a smaller (1-2 mm) grass seed with a papery pericarp that can be lifted from the seed. Light levels inside the enclosures simulated new moon, starlit nights (0-10 mLux), and quarter moon, clear nights (45-90 mLux) based on outdoor recordings in a nearby harvested field. All mice experienced each of the four treatment combinations, but in randomized order. Between treatments, we cleaned out burrows and left them open to dry. When mice scattered sand from the tray, it was assumed to contain seeds and we sifted that sand as well. We sifted all sand from foraging trays through a 1mm sieve between mice.

During the late fall of 2012, we placed temperature data loggers in each tray of sand to evaluate any differences in temperature for trays. Results provided no evidence of temperature differences between sand color treatments, indicating no differences in metabolic costs of foraging between the two trays.

We sterilized and standardized seeds in order to measure GUDs and infer consumption of seeds. We baked all collected seeds at 190C for 30 minutes to destroy microorganisms and to standardize moisture content. We cleaned seeds with a sieve, seed separating apparatus, and forceps. This process removed fecal material and empty seed coats. Then we weighed seeds on an electronic balance, and recorded their total weight in grams. Optimal foraging theory predicts that the decision of when to leave a food patch is based on missed opportunity costs (e.g. breeding), metabolic costs (e.g. higher energy expenditure), and perceived predation risk (Brown and Kotler 2007). This study eliminates metabolic and missed opportunity costs as well as equalizing travel time, so that differences in seeds remaining in food trays reveal the perceived cost associated with predation risk (either as spending less time in the patch or spending more time being vigilant to predation). The remaining weight of seeds in each tray is the giving up density (GUD), and the difference in GUD values represents the costs associated with perceived predation risk.

We used mixed-effects models to evaluate the various treatments in the study, while controlling for variation due to individual behavior. Differences in seed weights remaining in trays were analyzed with a standard least-squares regression model with light and seed type as well as all interactions as fixed effects, and mice as a random effect in JMP® Pro 10.0.2 (SAS Institute Inc. 2012). The intercept of the model represents the average difference in

GUDs for each mouse, and the significance test of the intercept evaluates whether the differences between trays are significantly non-zero.

5.3 Results

Mice in the experimental setup responded well to the environment and experimental setup. No mice declined in health or vigor during the study and many mice actually gained weight. A few mice did eventually escape from the wire enclosure into the larger enclosure. Most mice made no efforts to escape that we could detect. Mice also cached seeds in the artificial burrow in similar proportions as seen in our other studies (Flick and Danielson, in review)

Mice consumed both types of seeds under all conditions and in both colors of sand substrate. Differences in GUDs between substrate color were not significantly different when lights were off than when lights were on (1.63 ± 0.39 , 1 SE vs. 2.30 ± 0.71 g, respectively; $\beta = 0.37$, SE = 0.30, $P = 0.24$; Figure 3). Differences in GUDs were also not significantly altered by seed type (1.81 ± 0.54 vs. 2.11 ± 0.56 g; $\beta = 0.20$, SE = 0.28, $P = 0.50$). There was also no significant interaction of either of these treatments ($\beta = 0.18$, SE = 0.27, $P = 0.52$). In order to determine if substrate matching significantly influenced mouse foraging behavior, we tested for a non-zero difference between the two foraging trays. The average difference between light and dark substrate was 1.96 g (± 0.28). GUD values were significantly lower in dark trays (light-dark tray GUDs: Intercept = 1.97 ± 0.31 , t Ratio = 6.42, $P < 0.001$).

5.4 Discussion

P. m. bairdii foraging behavior indicates they perceive a higher risk associated with foraging on lighter colored substrate relative to darker substrate. Visibility of seeds had no impact on foraging rates, as there was no interaction of seed type and substrate color. This finding is consistent with previous research showing that *P. maniculatus* improves foraging efficiency through olfactory cues (Vander Wall 1998). Mice tended to leave more seeds behind when the lights were on, but it was a non-significant difference. Previous studies have consistently found that mice forage more intensively on darker nights (Kotler 1984; Brown et al. 1988; Orrock et al. 2004). Since we could not eliminate all background light, it is possible that the lack of significance in our study could be due to variation in ambient lighting throughout the study. Also, we only mimicked a moderate amount of moonlight, where previous studies have simulated much higher levels or used greater intensities of natural moonlight (Kotler et al. 2001 and Bengsen et al. 2010). Alternatively, it is possible that the effect of light was ameliorated in our study by providing mice with a behavioral escape from the higher predation risk: foraging on a dark substrate.

The preference for foraging on dark substrate matches visual estimates that the mouse is more cryptic on a dark substrate, and supports our hypothesis that mice perceive the risk associated with reduced crypsis. This risk perceived by mice is likely an accurate perception, as Kaufman (1974) found that more conspicuous mice were more likely to be attacked by owls, suggesting an adaptive advantage of foraging on cryptic substrates. Additional evidence using models suggests that substrate matching and the resultant camouflage are important for minimizing predation events of small mammals (Vignieri et al. 2010). Since all mice foraged in both patches, the difference in substrate GUDs indicates that rather than

avoiding non-cryptic patches, the mice may be acting more vigilant during their foraging.

This interpretation is supported by current research, including Kotler et al. (2010) who found desert rodents were able to evaluate current moon phases and their own nutritional state to make complex foraging decisions. Few studies have explicitly examined the role of substrate in perceived predation risk, but Hughes et al. (1995) and Kotler et al. (2001) found that desert gerbils perceive if substrates are suitable for quick escape from predator attacks. Kotler et al. (2001) also found that foraging substrate influenced GUDs, but they attribute this to altered efficiency of foraging. Abu Baker and Brown (2012) found a similar result; two species prefer different foraging substrates due to variable foraging styles. Our study adds substrate matching or camouflage to the list of small mammal decisions associated with substrate.

Since Kettlewell's finding in 1955 that moths recognize appropriate backgrounds on which to land, substrate matching has been recorded in multiple species, especially in association with predation risk. For example, the fleeing behavior of grasshoppers enhances crypsis by selecting cryptic background with a greater frequency than expected by chance (Gillis 1977 and Eterovick and Cortes Figueira 1997). Multiple species of frog have shown the same perceptive behavior, choosing camouflage-enhancing substrate when fleeing a perceived predation risk or choosing a resting site (Morey 1990 and Eterovick et al. 2010). Several species of lizard have shown similar propensity for substrate matching (Gibbons and Lillywhite 1981 and Gillis 1989). However, we could find no studies of mice behaving in a way that enhances crypsis on a substrate. In fact, a study of squirrels found no perception of risk when background color was manipulated, while finding strong antipredator responses to distance to refuge and escape substrate (Thorson et al. 1998).

The behaviors in this study could be a result of rapid adaptation to a novel ecosystem or pre-existing phenotypic plasticity. Supporting rapid adaptation potential, Sih et al. (2011) described species with preexisting adaptations to human alteration of the environment and species with short generation times and suitable genetic variation as species that can persist in human-dominated environments. Which of these two pathways may be contributing more to the success of *P. m. bairdii* is unclear, but the drastic changes from prairie to crop field suggests adaptation rather than pre-adaptation to this environment. In fact, our study falls in line with current research, as examples of rapid evolutionary adaptation are emerging (Møller 2008, Atwell et al. 2012). Often, these studies find behavioral shifts of foraging and exploration that promote the species' survival in highly modified landscapes. In addition, our lab has found evolution of jaw morphology in *P. m. bairdii* (Doudna and Danielson, in review). We found that the species has undergone evolutionary changes in jaw morphology in 100 years, and that there is strong evidence that the intensity of corn-soybean agriculture is the best predictor for these changes. We concluded that the large size and hardness of dry corn and soybean were sufficient selection agents to promote the evolution of jaw morphologies. Similarly, the strong selection pressure induced by increased predation during winter may have promoted behavioral evolution in prairie deer mice.

In addition to an ecologically-interesting result, this study may provide insight into economically important behaviors of *P. m. bairdii*. Prairie deer mice consume seed and waste grain in corn-soybean fields, and they have been found to aid in the regulation of weed populations (Williams et al. 2009). Therefore, if soil color influences where and to what degree the mice forage on seeds, erosion could reduce the weed regulatory role of this species (Sivy et al. 2011). While lighter substrate is often associated with reduced fertility,

chemical inputs provide nutrients for plant growth. Thus, if mice preferentially forage in darker patches of the field and reduce foraging intensity in light patches, it might result in areas of high weed density, adversely affecting crop productivity or increasing costs to commodity producers. Future studies on field conditions and their effects on foraging intensity will need to be combined with plant demography studies to gain a better understanding of potential impacts on commodity production.

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Figure 1. *Soil color heterogeneity*. An example of soil color heterogeneity of Midwestern row crop agricultural fields. Even on farms with little topographic variation, erosion is exposing lighter soils. This heterogeneity may lead to behaviors in mice that reduce their economic value to farmers.



Figure 2. *Experimental enclosure*. Each enclosure (4 total were used) is approximately 3 meters on each side, with ~15cm of soil, and has 1 meter high aluminum flashing around the bottom. It is covered with a weatherproof roof, and is surrounded with dark fiberglass panels to minimize light penetration. Runways are constructed of a wooden plank, wire mesh, and two colors of sand are placed on opposite ends. Runways are connected to wooden burrows by corrugated tubing. A single mouse is placed in each burrow, and is restricted to foraging in the experimental runway. On each end, there is a tray of 1 l of sand that matches the substrate on that side and ~18g of seed. A watering device is in the middle of the runway.

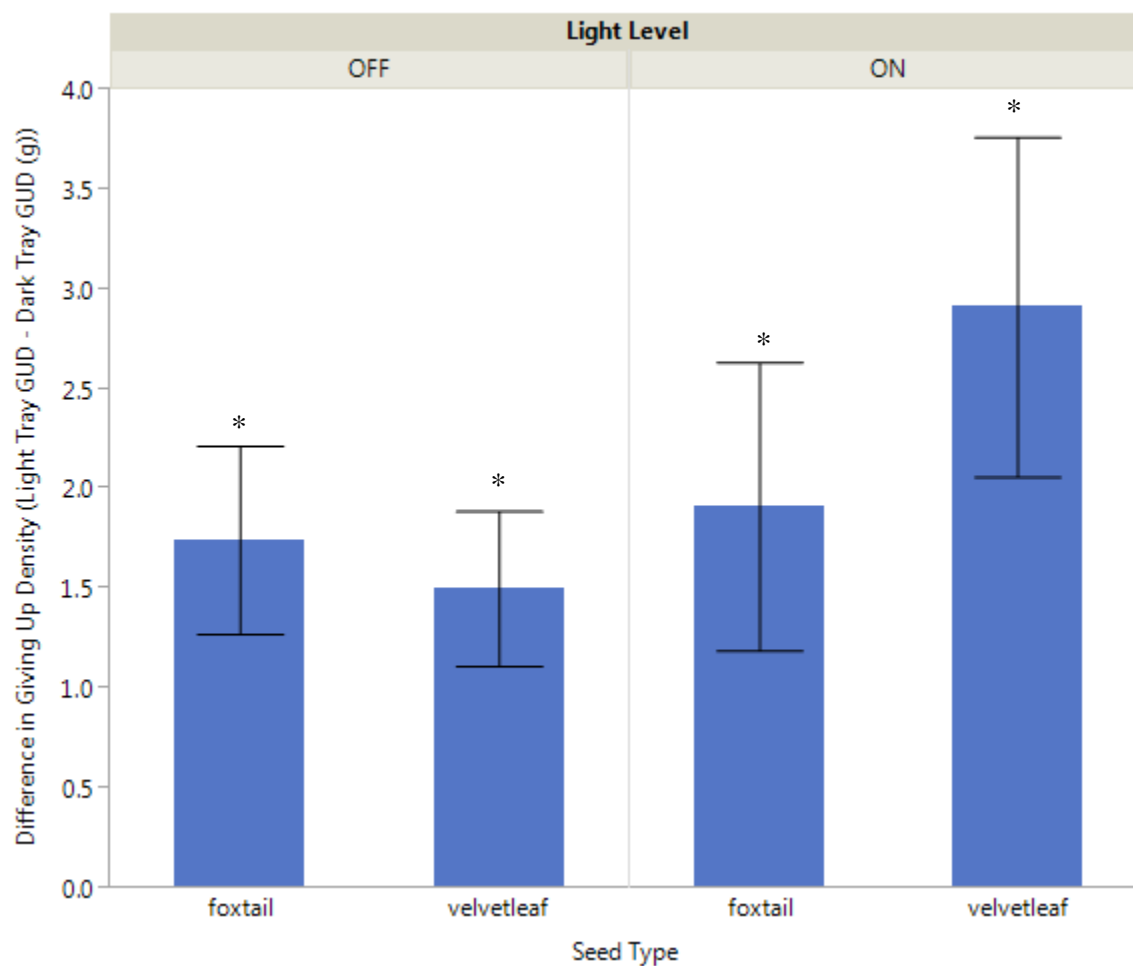


Figure 3. *The effects of substrate color on Peromyscus maniculatus bairdii giving up densities.* Two common weed seed species (velvetleaf – *Abutilon theophrasti* and foxtail – *Setaria faberi*) were tested, and crossed with two light treatments (off: <10 and on: 45-90 mLux). Boxplots display median and quartile differences between light and dark GUD trays (grams of seed remaining), in which mice foraged for three consecutive nights. Data are from 17 mice. All differences are significantly non-zero, and there are no significant differences due to seed type or light level.

CHAPTER 6. PERSPECTIVES OF EXTENSION AGENTS AND FARMERS TOWARD AN ECOSYSTEM SERVICE PARADIGM IN THE UNITED STATES CORN BELT

A paper to be submitted to the *Journal of Agricultural Education and Extension*

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ABSTRACT

Purpose: Our goal was to determine the perspectives of extension agents and farmer clients regarding multifunctional agriculture in the United States Corn Belt. Our secondary intention was to identify least-resistant routes of diffusion for complex conservation innovations.

Design/Methodology/approach: We used surveys to evaluate the perspectives of farmers, crop professionals, and extension agents. The survey was conducted in central Iowa, and included individuals predominantly from Iowa involved in commodity research and production.

Findings: We found indicators that all groups had positive perspectives about multifunctional agriculture in commodity production (summed scores of 18.28 ± 0.47 , with 12.00 as a neutral score), including a positive effect of prairie to cropland (2.56 ± 0.07 , with 2.0 as a response of no effect). Older farmers were more likely to support conservation funds and be familiar with the term “ecosystem service” ($P < 0.001$ and 0.07). Farmers with more farmland were less likely to perceive a positive effect of prairie on adjacent farmland ($P = 0.03$).

Practical Implications: The positive perspectives of farmers support the promotion of multifunctional agriculture. There are similarities in perspectives of the three groups that can support effective communication.

Originality/Value: This is the first study we could find that evaluated perspectives on multifunctional agriculture in Corn Belt commodity production. We could also find no studies that evaluated the perspectives of commodity producers with the same set of questions used to evaluate extension agents in this region.

6.1 Introduction

The agricultural systems found in the US Midwest have socio-technologically evolved to efficiently provide goods and services to humans, such as food, fiber, feed and fuel, at large scales. In addition, agriculture can contribute to non-crop goods and environmental services such as carbon sequestration or increased biodiversity. Collectively these outputs are considered ecosystem services, as they are naturally derived services to humanity (Millennium Ecosystem Assessment 2002). Despite the economic efficiencies apparent in the current conventional (commodity-based) management approaches in the US Midwest, row-crop agriculture is simultaneously associated with multiple negative externalities (e.g., soil erosion and water quality impairment) that are increasingly being experienced at broader scales (Robertson and Swinton 2005; Nassauer 2007). Fortunately, it is possible to manage farm systems jointly for high-yielding, low cost commodities as well as for enhanced environmental services (Secchi et al. 2008; Jordan and Warner 2010; Tomer et al. 2013). In such cases, specific farm management can variously help enhance or otherwise maintain on-farm productivity over time, ameliorate negative on and off farm impacts of farming, or outright cause or mediate the production of environmental goods and services that would not otherwise occur (Swinton et al. 2007; Scherr and McNeely 2008). Such farm management approaches applied at landscape-scales can lead to broadly valued

environmental benefits with minimal if any tradeoffs to the commodity basis of agriculture (Boody et al., 2005; Secchi et al. 2008). The outcome of this type of farm management is agricultural activity that extends beyond its role of producing commodities and serves several other functions such as renewable natural resources management, biodiversity conservation, environmental management with broad contribution to the socio-economic viability of rural areas; this outcome is known as multifunctional agriculture (MFA) (OECD 2003; Boody et al. 2005; Renting et al. 2009).

A challenge for the widespread adoption of MFA management techniques in this region however, is that traditional “change-agent” roles as well as modes of communication between farmers and university or private extension agents may be insufficient, and that negative perspectives of farmers and extension agents may suppress adoption (Jordan 2010). Historically, agricultural extension and education, as per the US Land Grant Cooperative Extension Service tradition, have typically been focused on crop production; as noted by Warner (2008), one of the key responsibilities of publicly funded extension agents is the transfer of production technology developed and tested by Land Grant Experiment Stations. Likewise, private extension professionals such as crop advisors, seed and chemical dealers, and technical service providers tend to focus on farm-level elements of financial and production risk as well as proprietary management interests (e.g., Norsworthy et al. 2007; Rivera and Sulaiman 2009; Riar et al. 2013). From an outreach standpoint, confounding the promotion of MFA is that the outcomes are complex, largely non-market, and to a large extent manifest at landscape scales. Economically, at the farm scale MFA involves management that focuses on environmental management that typically does not lead to short-term financial gain. Rather, it often involves landowners to accept direct and opportunity

costs (Secchi et al. 2008; Atwell et al. 2010). Yet MFA is quite compatible with commodity oriented landscapes (e.g., Coiner et al. 2001; Boody et al. 2005) and has provided the impetus for the production of non-conventional or emerging marketable services (e.g., carbon sequestration, nutrient trading). Nevertheless, public and private extension personnel and programming are and will likely remain key change agents/ tools in a region where commodity based agriculture has become somewhat dependent upon Land Grant and private agricultural research and development (Rivera 2011). Expanding extension education beyond strictly commodity production management will require a broader understanding of agricultural systems, a greater degree of communication on diverse topics, and positive and receptive attitudes regarding the impetus to MFA, approaches to MFA (at farm and landscape scales) and the overall goals of this agricultural system.

Due to this expansion of responsibilities, it is insightful to explore the perspectives of the key stakeholder groups with common metrics. Such evaluation will determine if farmers and extension agents have a positive attitude toward ecosystem services, and whether perspectives among the groups are similar enough to allow effective communication (Rogers 2003). With this information, we can also identify which extensions agents (public or private) may be the most effective in the promotion of MFA so as to better target the results of applied MFA research. This targeting approach will promote more efficient communication strategies for broader stakeholder information dissemination. With more effective promotion of MFA and a broader dissemination of information, MFA acceptance may increase and help maximize non-commodity ecosystem services.

One way to evaluate barriers and opportunities to extension agent and farmer communication is to detect Rogers' (2003) homophilies and heterophilies (e.g., Kock et al.

2010). Rogers (1995) identified heterogeneous perspectives of communicators (heterophily) as a hindrance to effective communication. In contrast, homophily enhances effective communication, due to similar perspectives among communicators. When considering the communication of complex conservation agricultural strategies, the identification of farmer and extension agent perspectives is critical, as it will identify heterophilies and homophilies, and thus promote better communication. Since crop professionals and extension personnel often present conservation innovations to farmers as part of their outreach responsibilities, it will be important to determine the perspectives of these groups, to understand the quality of information farmers are receiving.

In addition, knowledge of farmer perspectives can also enhance the identification of likely “early adopters” of novel conservation strategies as well as those farmers with a general receptiveness to new ideas. Knowler and Bradshaw (2007) reviewed the literature on how farmer perspectives influence adoption of novel conservation techniques. These researchers concluded that farmers feel compelled to adopt conservation strategies when they recognize environmental degradation and are concerned about reducing the negative impacts. Farmers also needed to perceive that the conservation strategy had tangible benefits that they could manage at the scale of their farm. Wossink and Swinton (2007) showed that farmers were willing to provide non-crop ecosystem services when they understood that joint production of non-marketed ecosystem services and crop productivity were possible. More pragmatically it has been noted that some farmers in this region will (to a degree) trade-off profits for the ability to fulfill stewardship interests (Chouinard et al. 2008). In the context of specific targeted management for MFA, analysis of data on regional farmers’ attitudes toward targeted conservation indicates that most farmers support targeted and coordinated

approaches to improving environmental quality and maintaining farm viability (Arbuckle 2013). Thus regional farmers appear to be generally receptive to the idea of MFA and ecosystem service management. As a mechanism for making this outcome more broadly experienced, ecosystem service management can increase through the efficient translation of research findings concerning multifunctional farming to farmers (Kilpatrick 2000, Vanclay 2004, Ingram 2008, Daily et al. 2009). Based on the findings of Knowler and Bradshaw (2007), extension services can be effective in promoting novel conservation technologies, but only when the technologies are properly promoted and relevant to farmers. Therefore, identifying resistance to ideas, or gaps in knowledge will provide extension agents and crop professionals with knowledge concerning how they can approach MFA topics with farmers.

In order to detect challenges and opportunities for the communication of MFA strategies to farmers, we evaluated the perspectives of public (university) extension agents, private (agribusiness) extension agents, and farmers with respect to MFA management. We hypothesized that the three groups would all have positive attitudes toward some aspects of MFA. We also hypothesized that we would find topics for which there are no significant differences among groups, indicating homophilies in this area. We also hypothesized that we would be able to detect barriers to communication of MFA strategies that need to be addressed for future communication efforts to be successful. These barriers would be indicated by significant differences in perspectives between two groups (heterophilies).

6.2 Methods

We used a combination of targeted and convenience sampling to collect extension agent and farmer perspectives on ecosystem services in multifunctional agriculture.

Participants in our survey were participating in educational experiences that pertained to MFA. Extension agents surveyed were Iowa State University (ISU) extension specialists, with responsibilities to disseminate findings by ISU researchers to farmers and crop professionals (n=19, mean age=55.7). Crop professionals were industry representatives with credentials as certified crop advisors (n=96, mean age=42.1). We surveyed the crop professionals at the 2011 Integrated Crop Management Conference (ICM) in Ames, Iowa. Farmers were individuals who reported farm acreage (mean acres=906 \pm 94, \pm 1 SE), or self-identified as farmers (n=106, mean age=54.1), but did not report employment in agribusiness (seed or chemical sales). Farmers in our study were similar in age to the average age of a farmer in 2007 (57.1), but farmed larger parcels of land than the average corn-soybean farmer in the United States (~500 acres, combined average harvested acres of corn and soybean farms), and much larger than the United States Average harvested acres per farm (140) (2007 Census of Agriculture). Farmers were surveyed at the ICM as well as soil and water management workshops in Iowa. We surveyed all participants prior to any presentation of materials.

The survey consisted of 9 Likert scale questions with options to respond 0-4 from strongly disagreeing to strongly agreeing, or in terms of percent lost revenue that could be recovered by government support (Table 1) and a series of demographic questions. The Likert scale questions were determined to be reliable, with a Chronbach's alpha of 0.72 (Chronbach 1951). Participants in each of the educational activities were informed of the intent of the study, asked to participate, and informed that participation would in no way affect their participation credit. We collected 198 surveys from all groups. All respondents

were residents of Iowa or Iowa border states. Response rate varied by group, but was always greater than 75%.

All analyses were done in JMP Pro 10.0 (SAS). We analyzed all Likert scale questions as continuous numerical responses by three groups (extension agents, crop professionals, and farmers). We used analysis of variance to detect significant differences among groups, and a multiple comparisons corrected pairwise comparison to detect which groups were significantly different (Tukey-Kramer). We coded responses of “I don’t know” or unanswered questions as missing.

6.3 Results

Extension agents and farmers were moderately to highly supportive of federal funds to support agriculture. When asked about federal funds to support productivity, there were no significant differences among groups. Extension agents were the most supportive of federal funds to support productivity (2.94 ± 0.27 , mean \pm SE), and farmers and crop professional were the least supportive (2.56 ± 0.11 and 2.55 ± 0.12 , respectively) ($P = 0.40$). On the question of support for federal funds to support conservation, extension agents were significantly more supportive than crop professionals (3.61 ± 0.23 and 2.90 ± 0.10 , $P = 0.03$), but extension agents were nearly significantly more supportive than farmers (farmers: 3.08 ± 0.09 , $P = 0.09$).

When asked about their ability to manage for an environmental benefit, there were no significant differences among groups ($p = 0.45$). All groups were relatively positive that they could manage a parcel of land in a way that would provide an environmental benefit (2.97 ± 0.07). Farmers were the most confident (3.00 ± 0.09), followed by extension agents and crop

professionals (2.89 ± 0.21 and 2.81 ± 0.10). We found similar, but lower, results when we asked about managing a parcel of land that would provide an environmental benefit and maintain high productivity (2.65 ± 0.07). The pattern of responses among groups remained the same, but group confidences declined, with farmers the most confident, and crop professionals the least (2.73 ± 0.09 and 2.48 ± 0.11).

Next, we asked participants about their perspectives of ecosystem service application. When asked about their comfort with the term “ecosystem service”, respondents were less positive than in previous questions (1.95 ± 0.08). There were no significant differences among groups ($p = 0.17$), but farmers were the most comfortable with the term (2.13 ± 0.12), with crop professionals and extension agents less sure (1.75 ± 0.12 and 1.77 ± 0.29 , respectively). When asked whether they felt confident that they could get funding to support enhancement of services and productivity, respondents were neutral (2.02 ± 0.08). Farmers were the most confident (2.20 ± 0.12), followed by crop professionals (1.92 ± 0.13), and extension agents (1.47 ± 0.27). Farmers were significantly more confident than extension agents ($P = 0.04$). When asked what percent of lost revenues for planting crop land to prairie would be recovered by government funds, respondents felt they could get about 50% of their money back (46.3 ± 2.11). There were no significant differences among the groups ($P = 0.91$).

Finally, we evaluated perspectives on the effect of one acre of prairie adjacent to cropland. All groups on average perceived a positive effect (2.56 ± 0.07). Extension agents perceived the highest positive effect (2.94 ± 0.19), and crop professionals and farmers each perceived a slightly less positive effect (2.49 ± 0.10 and 2.48 ± 0.10 , respectively). None of the pairwise comparisons were statistically significantly different ($P = 0.34$).

Attempts to cluster individual response sets by job category (farmer, crop professional, extension agent) were unsuccessful. However, when we evaluate the totality of the response distributions by question, there is a clear set of homophilic topics (Figure 1). When we summed responses to questions directly related to MFA management and on the same scale (Questions 2,3,4,5,6,8), farmers had the highest score (18.28 ± 0.47), extension agents were slightly lower (18.13 ± 1.08), and crop professionals had the lowest score (17.11 ± 0.50), with a neutral response on all questions resulting in a 12.00. There was no significant difference among groups for the sum of the response ($P = 0.22$). Thus, all respondents on average reported positive attitudes toward concepts of MFA.

Demographics were important determinants for some perspectives (Figure 2). Age had a positive effect on perspectives on supporting government funds for conservation (farmers: $R^2 = 0.06$, $P < 0.001$; crop professionals: $R^2 = 0.09$, $P = 0.003$) as well as terminology (farmers: $R^2 = 0.03$, $P = 0.07$; crop professionals: $R^2 = 0.04$, $P = 0.06$). The acres farmed had a significantly negative effect on the perceived effect of one acre of prairie on adjacent row crop agriculture ($\log(\text{acres})$: $R^2 = 0.04$, $P = 0.03$). However, the average response at the largest farm scales was still a neutral effect. None of the other questions revealed strong trends associated with demographics.

6.4 Conclusion

Rogers (1995) describes the importance of similar perspectives (homophilies) in effective communication. It is clear from our study that the three groups typically involved in the communication of novel agricultural conservation strategies have multiple topics with high levels of homophily. In fact, we could only find two questions, federal conservation

funds and ability to get funding for a habitat that provides an environmental benefit, where the three groups had any significant differences (heterophilies). In these two cases, extension agents were more supportive of conservation funds, and farmers were more confident they could receive funding for conservation efforts. What is unclear in our study is the effect extremely similar perspectives among groups have on the tendency of adoption of novel strategies that support MFA. For example, can extension agents and crop professionals increase the adoption of MFA, when all three groups have similar perspectives about its effectiveness? In contrast, could more positive perspectives promote increased adoption, as long as there were sufficient homophilies? Future research should evaluate the perspectives of new, potential change agents.

Farmers in our study are generally aware that farms provide ecosystem services and that multiple services can be managed within a single agricultural landscape, or that MFA is a feasible concept. The implication is that extension agents and crop professionals can communicate with farmers from a point of common understanding. This knowledge will contribute to understanding how Iowa farmer perspectives may influence the acceptance of new conservation practices that support multifunctional agriculture. Additionally, this study responds to a call by researchers for science and technology communication to operate in a way that promotes “salience, credibility, and legitimacy of the information they produce” (Cash et al. 2003). Our findings suggest that there are many opportunities to communicate research findings that farmers would consider salient, credible, and legitimate. Since farmers are somewhat accepting of the idea that multiple services can be provided by agriculture, and that non-crop habitat may contribute to services, conservation practices that capitalize on these beliefs may be successful.

Farmer information and decision networks are large and diverse webs of interaction (Oreszczyn, Lane, and Carr 2010). While this study only looked at two potential sources of information, they represent common linkages in the dissemination of innovation (Leeuwis, Leeuwis, & Ban 2004, Aflakpui 2007). In addition, while this study does not directly test for adoption of conservation farming techniques, it provides a baseline understanding of how extension agents and farmers in one of the most intensively farmed systems in the world think about conservation strategies and the management of multiple ecosystem services. The adoption of novel technologies and approaches has been extensively studied, and the factors that influence adoption are variable (Rodriguez et al 2009, Garbach et al 2012). Minimally, novel approaches and technology developed at the university require diffusion to and among practitioners. Large gaps in perspectives and missing knowledge elements impede this form of communication, though. Our study suggests that such gaps do not exist, supporting the communication of novel and complex conservation techniques related to MFA.

Demographic results of this study suggest a targeted approach should be taken to find early adopters of MFA strategies. Since older farmers are more likely to find value in government conservation funds and are more comfortable with the term ecosystem service, they may be an important group for early collaboration. Other work has also found that older individuals perceive value in human intervention and problems (Sharp et al. 2011, Gould et al. 1989). However, Gould et al. (1989) found that younger operators were more likely to adopt practices that targeted the problem. More research is necessary to determine whether perspectives lead to implementation in this case. Small-scale farmers are also more likely to perceive a benefit of planting land to diverse vegetation, and thus may be a key collaborator in designing multifunctional landscapes. These results are in contrast to work that has found

farmers with larger holdings more willing to conserve species (Winter et al. 2007). The difference between these findings and our findings however, is that we asked farmers about a perceived impact, rather than a willingness to participate. When possible, extension agents may wish to target their efforts according to these demographic results if they wish to affect farmer management practices.

In conclusion, the perspectives detailed in our study are an important baseline for promoting sustainable agricultural practices to farmers. The results suggest that farmers are most optimistic about management, and that optimism of academic and private extension services could promote more MFA strategies. We also found that on average, conversations between farmers and crop professionals or extension agents will consist of people with similar opinions of conservation and ecosystem service management. While this promotes communication, it fails to provide farmers with an opportunity to hear more positive perspectives. Preliminary research at Iowa State University (unpublished data) shows that students preparing for extension and agricultural education careers are more positive about MFA than any of the groups in this study, but these students still have several areas of homophily with farmers. The inclusion of such positive individuals in extension activities may enhance the acceptance and implementation of MFA strategies.

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Table 2. Summary of Survey Responses. Values are mean (\pm SE) for responses from Midwestern US farmers (predominantly row crop), crop professionals (predominantly seed and chemical sales) and Iowa State University extension professionals. Bolded values are significantly different within question.

Question	Farmers (N = 106)	Crop Professionals (N = 96)	Extension Agents (N = 19)
1. Do you support federal funds to support agricultural productivity?	2.56 (0.11)	2.49 (0.10)	3.00 (0.27)
2. Do you support federal funds to support conservation?	3.08 (0.09)	2.90 (0.10)	3.61 (0.14)
3. How confident are you that you could manage habitat to provide an environmental benefit?	3.00 (0.09)	2.81 (0.10)	2.89 (0.21)
4. How confident are you that you could manage habitat to provide an environmental benefit AND increase agricultural productivity?	2.73 (0.09)	2.48 (0.11)	2.57 (0.21)
5. How comfortable with the term “ecosystem service” are you?	2.13 (0.12)	1.75 (0.12)	1.77 (0.29)
6. How confident are you that you could obtain funding [for] habitat that provides an environmental benefit?	2.20 (0.12)	1.92 (0.13)	1.47 (0.27)
7. Imagine you’ve chosen to convert one acre of cropland to diverse prairie. What percent of lost productivity do you believe you could recoup with government funds?	46.56 (2.97)	42.98 (3.26)	47.50 (7.86)
8. What do you expect the effect of one acre of prairie on the productivity of the adjacent cropland to be?	2.48 (0.10)	2.49 (0.10)	2.94 (0.19)

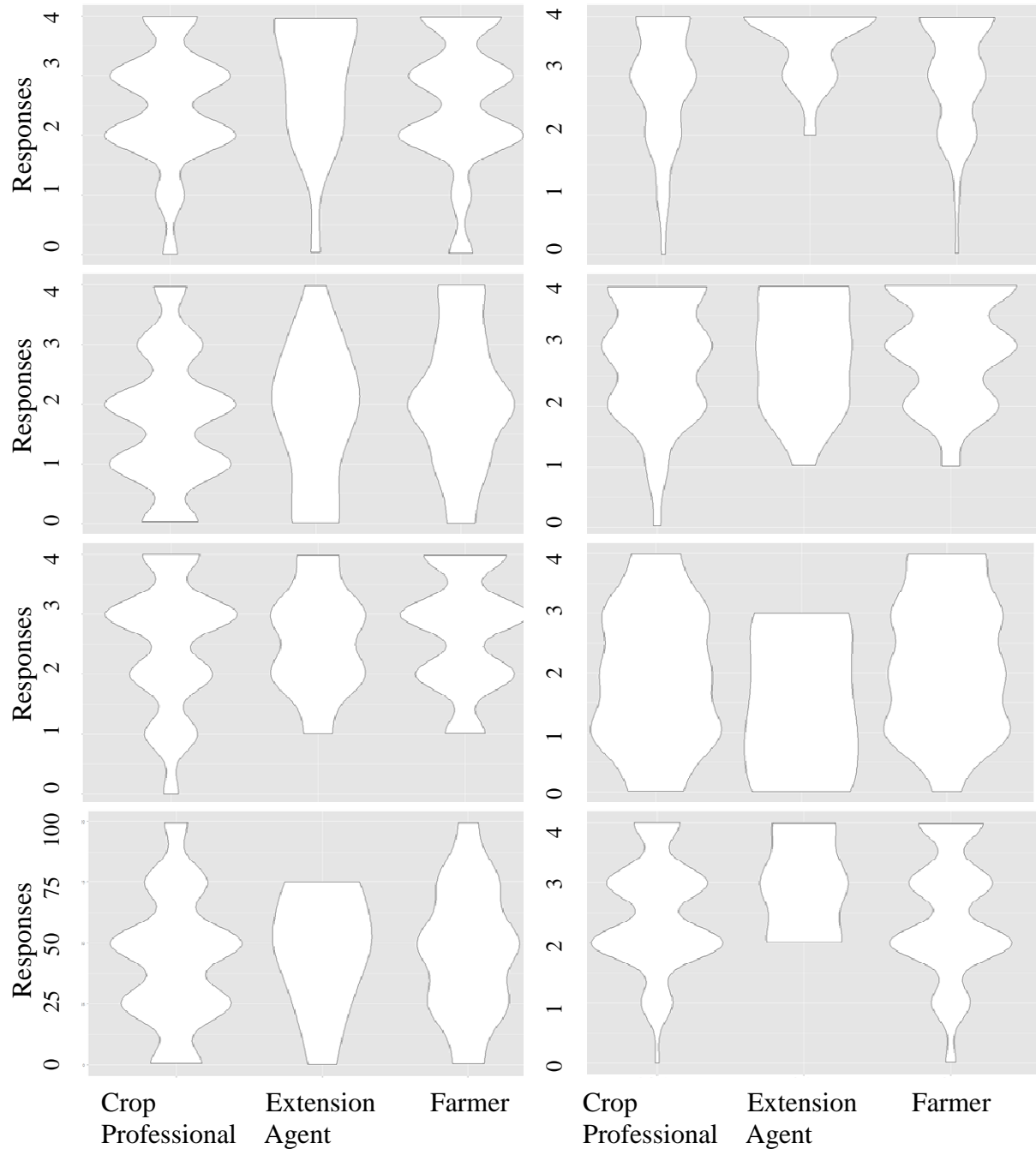


Figure 1. Distributions of responses. The violin plots represent a smoothed representation of response proportions within each group. Midwestern US farmers (predominantly corn-soybean, N=106), crop professionals (predominantly seed and chemical sales representatives, N=96), and extension agents (predominantly production agriculture specialists, N=19) were asked the following questions: A. Do you support federal funds to support agricultural productivity? B. Do you support federal funds to support conservation? C. How comfortable with the term “ecosystem service” are you? D. How confident are you that you could manage habitat to provide an environmental benefit? E. How confident are you that you could manage habitat to provide an environmental benefit AND increase agricultural productivity? F. How confident are you that you could obtain funding [for] habitat that provides an

Figure 1 (continued). environmental benefit? G. Imagine you've chosen to convert one acre of cropland to diverse prairie. What percent of lost productivity do you believe you could recoup with government funds? H. What do you expect the effect of one acre of prairie on the productivity of the adjacent cropland to be?

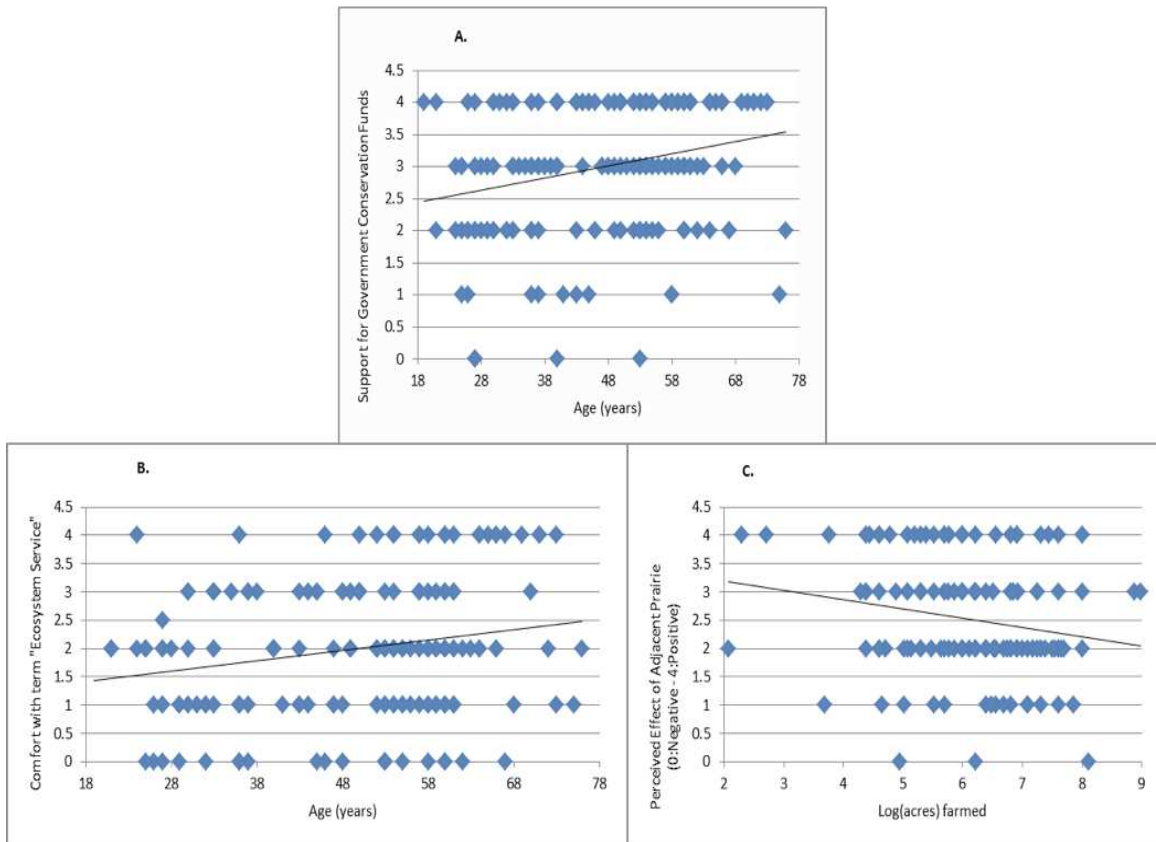


Figure 2. Demographic predictors of A) support for conservation funds based on age, B) comfort with the term ecosystem service based on age, and C) the perceived effect of one acre of adjacent prairie on crop productivity based on farm size (Log(acres)). Data are based on surveys from farmers, crop professionals, and extension agents, as described in Table 1.

CHAPTER 7. CONCLUSIONS

Peromyscus maniculatus bairdii (the prairie deer mouse) is an important organism to corn-soybean agriculture in the Midwestern United States, especially as a buffer against the development of herbicide tolerant weeds. It is also an example of a species that has thrived under novel conditions and high levels of disturbance. Thus, this dissertation provides insight to aspects of commodity agriculture that have promoted its long-term success. It also promotes understanding of how species successfully utilize novel and disturbed environments.

In chapter 2, we found that while prairie deer mice are generalists, the novelty of large, hard waste grain has been a significant natural selection force on jaw morphology, and produced microevolutionary changes in jaw structure in 100 years. This study reveals the rate at which adaptation can occur in this species. This study also indicates that generalization may be sufficient for survival in disturbed systems, but these disturbed systems are also typically simplified and thus are likely to promote evolution to utilize the fewer resources available.

In chapter 3, we found that while in cafeteria trials (where mice have easy access to the totality of seeds) mice show no preferences among seeds, when the rate of return declines over time, mimicking natural conditions, mice show a strong preference for eating and caching complementary seeds when they are part of the same patch. This finding improves our understanding of the role of deer mice in weed control and provides new avenues of research in the effects of caching tendencies. It may also be necessary to revisit models of weed control efficacy on these two weed species.

In chapter 4, we found that fecal isotopes are useful in studying trophic ecology of seed predators in agriculture. Therefore, future research can minimize invasive and costly techniques when studying foraging ecology in simplified systems. We also found patterns linked to nursing that should be considered when studying small mammal stable isotopes.

In chapter 5, we found that erosion of topsoil is not only a threat to continued yield of crop plants, but may also promote weed population growth by deterring the efficient foraging of deer mice. Deer mice recognize the risk associated with foraging on light patches of soil, and given the opportunity, will preferentially forage on darker soils. Given this finding, farmers may need to pay more attention to managing weeds on eroded areas. In addition to the applied consequences of our findings, this species again show rapid adaptation to novel conditions. As it is unlikely that prairie deer mice have experienced this particular risk historically, it is either an evolved behavior, an evolved epigenetic change that allows for ancestral behavioral traits to be turned on, or is a coincidental response to lighting, similar to responses to high moon light.

In chapter 6, we found that farmers and extension agents are amenable to the principles of multifunctional agriculture. This finding supports the value of ecologically based pest management research. Since farmers see potential in managing multiple services on a single parcel of land, and extension agents seem willing to promote such ideas, researchers should feel confident that their findings will enhance the sustainability of farm landscapes and help ameliorate negative outcomes of intensive agriculture.

In sum, it seems that native animals, such as the prairie deer mouse, will have an even bigger role to play in the future of agriculture. Thus, extensive research that integrates these findings will be critical for understanding how management affects the role of deer mice in

agriculture. The prairie deer mouse may be a model organism for studying these roles, as its behavior is consistent and predictable. It is also relatively easy to manipulate both field and enclosure conditions to test behaviors related to potential management changes and degradation of farmland.