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# Genetic diversity and connectivity of white-tailed jackrabbit populations in Iowa with notes on seasonal home ranges

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# Genetic diversity and connectivity of white-tailed jackrabbit populations in Iowa with notes on seasonal home ranges

by

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# TABLE OF CONTENTS

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# ABSTRACT

The loss and fragmentation of Iowa's native prairies has had varied effects on different species as some move more easily through unsuitable habitat than others. Small mammals may be highly affected by isolation as they may not move easily among habitat patches. I studied white-tailed jackrabbits (*Lepus townsendii*) as a representative of Iowa's grassland-adapted species to determine effects of habitat fragmentation on movement patterns, space use, and genetic diversity. I tracked radio-collared jackrabbits from September 2008-September 2009 to determine habitat use in an intensively agricultural landscape. I collected tissue from live-captured animals and road-killed samples across Iowa and South Dakota. Home ranges expanded and shifted following corn harvest (October 2008) and prior to the breeding season (February-May 2009). Home ranges contracted from the end of the breeding season until right before harvest (September 2009) as crop height increased. The population genetic structure analyses suggested there were two populations in Iowa, a central and northwestern population. The northwest Iowa population is not distinct from South Dakota individuals and is moderately differentiated from the less genetically diverse central Iowa population. White-tailed jackrabbit movement patterns are affected by agricultural practices and agricultural fields are a potential barrier to gene flow. These anthropogenic alterations of the landscape in Iowa may also alter other aspects of jackrabbit ecology and other grassland-adapted species.

# CHAPTER 1: GENERAL INTRODUCTION

Declines and extinction of numerous wild species, across taxonomic groups, have been attributed to loss and fragmentation of habitat associated with anthropogenic landscape changes (Fischer and Lindenmayer 2007). For example, North American prairie grasslands have declined greatly in both availability and wildlife diversity. With approximately 99% of all historic prairies eliminated, 55 grassland species in the U.S. are listed as threatened or endangered and 728 species are candidates for listing (Samson and Knopf 1994). This decline in North American prairie is largely attributed to agricultural intensification, the increase in cultivated land and field size for the maximum output of crops, including monoculture plantings, and has become a global issue over the past several decades. The decline of tall-grass prairies is prominent in the midwestern U.S. Prairie once covered 125,000 $km^2$ in the state of Iowa but less than 0.1% of that historic prairie remains. About 60% of Iowa's land is now planted to annual row crops, especially corn (Tucker et al. 2008).

This loss and fragmentation of Iowa's native landscape has had a dramatic effect on its native fauna (Dinsmore 1994). Numerous species adapted to native prairies, particularly those with large space requirements, have long been extirpated from Iowa. However, many have persisted, perhaps due to their ability to maintain viable populations in small habitat patches or their ability to persist in a metapopulation structure. Persistence of remnant populations may be threatened as the national impetus to move toward a bioeconomy forecasts major changes in Iowa's landscape once more (Heisey 2009). In order to predict how changes in the landscape and land use will affect Iowa's wildlife diversity, it is

necessary to understand current relationships between wildlife species and their fragmented environment.

One grassland species still found in Iowa is the white-tailed jackrabbit (*Lepus townsendii*), also referred to as the prairie hare. Suitable jackrabbit habitat is likely scarce and isolated across the state. White-tailed jackrabbits prefer open habitats (Kline 1963; Lim 1987) and rely on vision to detect, and speed to avoid, predators rather than making use of burrows (Rogowitz and Gessaman 1990) as do cottontail rabbits (*Sylvilagus floridanus*). The extent to which jackrabbits may be able to move across the expansive corn fields that now dominate Iowa's landscape is unknown. Small mammals may, in many cases, be more restricted than birds with respect to movement across unsuitable habitat. Among small mammals, jackrabbits are likely to be more sensitive to fragmentation due to larger space needs, with annual home ranges of $\geq 2$ km in grasslands (Donoho 1972; Schaible 2007). Furthermore, jackrabbits may have more restrictive habitat requirements than many smaller mammal species in altered habitats. Jackrabbits in Iowa occur at the eastern boundary of their historic range (Lim 1987), they may be more susceptible to fragmentation and isolation due to the decline in suitable habitat that typically defines species' range boundaries (Swihart et al. 2003). The Iowa Department of Natural Resources (IDNR) listed the white-tailed jackrabbit as a species of greatest conservation need in the Iowa Wildlife Action Plan (Zorher 2006).

White-tailed jackrabbits may also be vulnerable to detrimental genetic effects associated with fragmentation. As suitable habitat decreases, a population's size may decrease. With the loss of individuals comes a loss of some rare alleles, making the population susceptible to reduced gene flow and loss of genetic diversity (Garner et al. 2005;

Lacy 1997). Furthermore, small isolated populations are at risk of local extinction due to increased susceptibility to stochastic demographic or environmental variability alone or combined with loss of genetic variation (Frankham 2005; Laikre et al. 2009; Templeton et al. 1990). The extent to which populations of white-tailed jackrabbits in Iowa are genetically isolated due to land use changes is unknown. These characteristics make the white-tailed jackrabbit a particularly suitable species in which to investigate the effects of fragmentation, changing land use, and agricultural practices on small mammal populations.

The distribution of the white-tailed jackrabbit ranges from the Great Plains in central Saskatchewan south to the Rocky Mountains at the northern border of New Mexico and inland from the west coast to Lake Michigan in Wisconsin (Lim 1987). While the historic range of white-tailed jackrabbits is thought to have included only northwest Iowa, breaking up the tall-grass prairie for cultivation of diverse crops in the late 19$^{th}$ and early 20$^{th}$ century increased the range of jackrabbits into eastern and southern portions of Iowa (Lim 1987). However, jackrabbit populations appear to have declined dramatically across Iowa, as the proportion of acres in small grains and hay, which is more suitable white-tailed jackrabbit habitat, has declined relative to the proportion of acres planted to corn and soybeans (Bogenschutz et al. 2007). White-tailed jackrabbit densities have been estimated to average 7/ km² in parts of Wyoming, 0.4-2.3/km² in Colorado (Flinders and Hansen 1973, 1975), 4-8/km² in Minnesota (Mohr and Mohr, 1936), and 27.12/km² in northwest South Dakota where habitat is most ideal for jackrabbits (Schaible 2007). Kline (1963) estimated population densities in Iowa at 2-6 hares per square km to be common, with occasional highs of 12 per square km, based on records of circle hunts and personal observations. Records of jackrabbit densities, obtained from the IDNR roadside surveys conducted from 1962 to 2007

(Bogenshutz et al. 2007), show a significant decline in jackrabbits across the state, with < 1 observed per 78-km stretch of road by 2007. The jackrabbit estimates from the roadside surveys can be considered an underestimate, however, as they only count jackrabbits observed by the road during the day and *L. townsendii* tends to be crepuscular (Rogowitz 1997). Estimates of jackrabbit numbers in the state of Iowa do not exist but populations of jackrabbits still persist in northwest and central Iowa with occasional sightings in northeast and southern Iowa (Fairbanks, unpublished data; E. Colboth, USDA-APHIS Wildlife Services, personal comm.).

White-tailed jackrabbit biology has not been studied in-depth, but some basic information is known. White-tailed jackrabbits have a brownish-gray summer coat with white underparts. They undergo two molts a year. The winter molt occurs in November to early December, in Iowa (Kline 1963), when the summer coat is replaced with a white winter coat, although the tips of the ears remain black. This winter coat is thicker and reduces heat loss (Rogowitz and Gessaman 1990). The winter coat is replaced by the summer coat in March to early April (Kline 1963). White-tailed jackrabbits acquired their name from their distinctive white tails that do not change color with molting. The sexes are not distinguishable without inspection of their reproductive anatomy.

White-tailed jackrabbit diets consist of forbs, shrubs and grasses. In natural habitats, forbs account for the majority (70%) of their summer diet with a shift to shrubs (76%) in winter months (Bear and Hansen 1967), although foraging habits in highly agricultural settings remain unknown. Duration of foraging increases in winter months with persistent snow cover, but is not affected by precipitation (Rogowitz 1997). Predators include red foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), grey wolves (*Canis lupus*), weasels *(Mustela*

*sp*.), martens (*Martes americana*), bobcats (*Lynx rufus*), lynx (*Lynx canadensis*), golden eagles (*Aquila chrysaetos*), and hawks (*Buleo sp.;* Lim 1987). Red foxes, coyotes, weasels, bobcats, and hawks are all found in Iowa, although coyotes are most likely the main predators in the state due to their size, abundance and the size of adult jackrabbits. Adult white-tailed jackrabbits in Iowa weigh 3.10 to 3.80 kg, on average, with females being the larger sex (Kline 1963). The range of bobcats in Iowa is generally restricted to the southern one-third of the state (Tucker et al. 2008), limiting their potential as jackrabbit predators.

White-tailed jackrabbits are promiscuous (Chapman et al. 1982). Breeding behavior is characterized by chasing, circling and jumping exhibited by two or more jackrabbits (Blackburn 1968); personal observation) between February and July (James and Seabloom 1969; Rogowitz 1992). The specific length and timing of the breeding season in Iowa is not known, but is likely dependent on environmental factors such as snow melt (Rogowitz 1992). White-tailed jackrabbits can produce up to four litters a year (James and Seabloom 1969) and gestation lasts 42 days in Iowa, according to Kline (1963). This species displays synchronous breeding and postpartum estrous (James and Seabloom 1969; Kline 1963; Rogowitz 1992). Litter size ranges from 1 to 11 leverets, averaging 4 to 5 (Kline 1963), although lower fertility may be exhibited in the first and last litters of the season (Rogowitz 1992). Juveniles are not known to reproduce in their first year (James and Seabloom 1969, Rogowitz 1992) and the typical lifespan of a white-tailed jackrabbit is < 1 year in Wyoming (Rogowitz and Wolfe 1991). These demographic parameters will be important to verify for any given population, as the published values suggest that only a small percentage of juveniles born in a particular year will survive long enough to contribute offspring the following year.

Genetic studies of white-tailed jackrabbits have been limited to a handful of descriptive karyotyping analyses (Lim 1987). This species is diploid and has a total of 48 chromosomes with lengths ranging from 1.5-6.5 microns (Jalal et al. 1967). A single genetic sample from a white-tailed jackrabbit was also used to assess cross-species transferability of microsatellites developed for the European rabbit (*Oryctolagus cuniculus*; (Surridge et al. 1997). Microsatellites are genetic markers that consist of nucleotide repeats that are generally characterized by high levels of polymorphism. A study of population genetic structure using microsatellites could yield valuable information about genetic diversity, levels of inbreeding, and population differentiation among populations of white-tailed jackrabbits.

As an initial step toward understanding the dynamics of grassland-adapted small mammal populations in a changing, human-dominated landscape, we undertook a study of movement patterns of white-tailed jackrabbits in Iowa. Our study integrated population genetic and radio-transmitter tracking methods to assess connectivity among populations as well as potential effects of seasonal land cover changes on movements of animals. Genetic samples collected opportunistically from across Iowa and South Dakota, and from jackrabbits captured live on the Iowa State University Agronomy and Agricultural Engineering Research Farm (hereafter, Research Farm, Fig. 1) in Boone County, IA, were used to investigate regional genetic differences. The objectives of this study were to 1) determine the movement and seasonal land use patterns of white-tailed jackrabbits in Iowa's intensively agricultural landscape by means of radio-telemetry field studies and 2) quantify genetic diversity and connectivity of white-tailed jackrabbit populations in Iowa by assessing the number of populations in Iowa, their genetic diversity, and the degree of genetic connectivity between

these populations. Collectively, the results of this study are expected to be useful in the development of conservation management strategies for the white-tailed jackrabbit in Iowa, serve as an indicator of the potential effects of fragmentation on small mammals restricted to grassland habitats, and provide baseline data to assess impacts of impending land use change in Iowa.

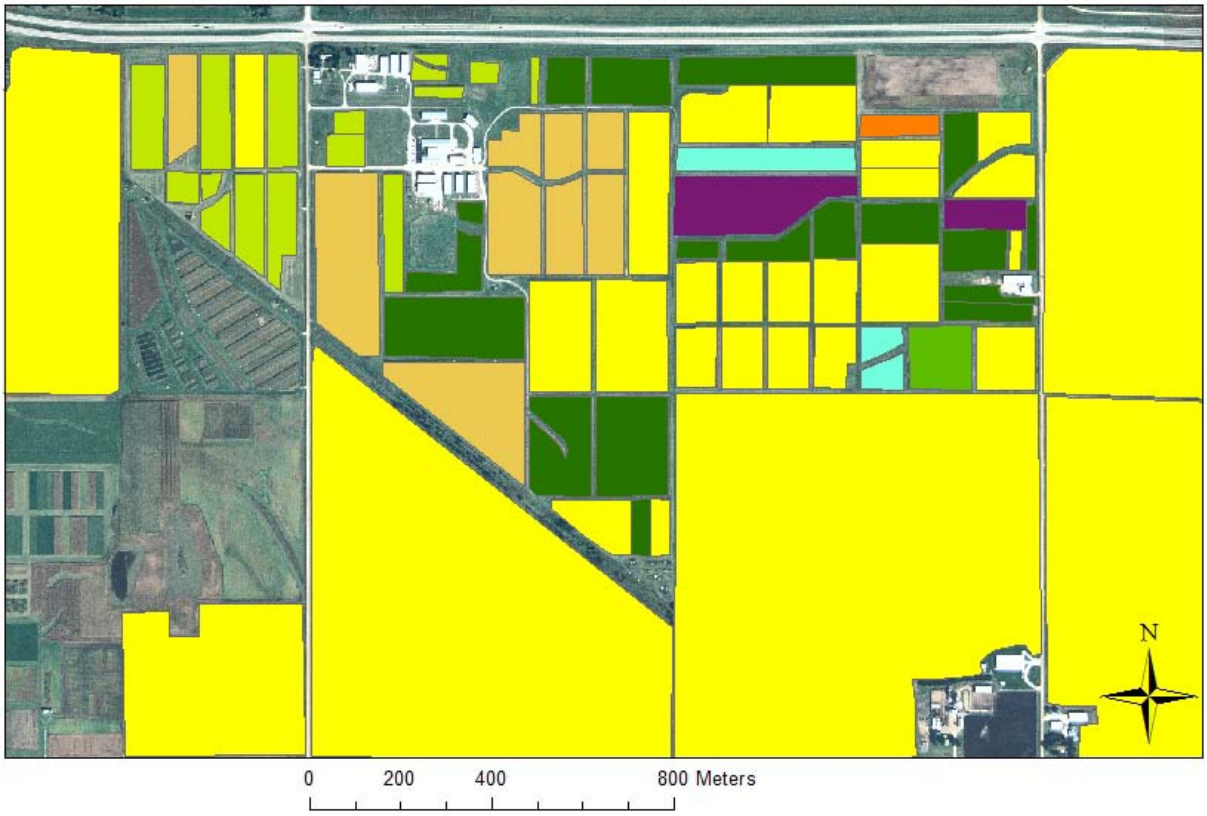


Figure 1. Map of the Iowa State University Agronomy and Agricultural Engineering Research Farm and surrounding private lands in Boone, County, Iowa. Fields known to be planted to corn in 2008 are represented in yellow. Oat fields are shown in tan, soybean fields in dark green, and alfalfa fields in purple. All remaining fields were planted to some combination of these crops or were unknown for 2008.

## THESIS ORGANIZATION

This thesis consists of four chapters. Chapter 1 is an introduction to my study. Chapter 2 reports results of a telemetry study documenting seasonal shifts in home range size, degree of overlap between individuals, and seasonal shifts in land use exhibited by white-tailed jackrabbits in central Iowa. Chapter 3 investigates genetic diversity and genetic differentiation of white-tailed jackrabbit populations across Iowa and South Dakota. Chapters 2 and 3 are intended for publication in peer-reviewed journals with co-authors listed at the beginning of each chapter. W. Sue Fairbanks and Julie Blanchong contributed to the development of this study, field and lab assistance, and were editors on chapters. Todd Bogenshutz and Mark McInroy (IDNR) assisted with field research and are contributing authors for chapter 2. Chapter 4 is an overall discussion of the results and their implications for the persistence of white-tailed jackrabbits in Iowa, with suggestions for future research.

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# CHAPTER 2: SEASONAL HOME RANGES AND MOVEMENT PATTERNS OF AN IOWA SPECIES OF GREATEST CONSERVATION NEED, THE WHITE-TAILED JACKRABBIT

A paper to be submitted to *The Journal of Mammalogy*

Irma Tapia, W. Sue Fairbanks, Julie Blanchong, Mark McInroy and Todd Bogenschutz

## ABSTRACT

Habitat fragmentation can have varied effects on different species as some move more easily through unsuitable habitat to reach fitting environments. Small mammals may be highly affected by isolation as they may not easily move among habitat patches. We investigated the white-tailed jackrabbit (*Lepus townsendii*) as a representative grassland-adapted species to determine effects of habitat fragmentation on movement patterns and space use in an intensively agricultural landscape. Home range sizes increased following corn harvest when jackrabbits also increased their use of those harvested fields. However, jackrabbits selected against corn fields, compared to availability in the pre-harvest (September 2008 - October 2008), post-harvest (November 2008 - January 2009), and growth seasons (June 2009 – September 2009). Agricultural practices altered movement patterns and space use of white-tailed jackrabbits and may affect timing of dispersal.

## INTRODUCTION

Shifts in land cover from natural to agricultural habitats have had diverse effects on wildlife. Medium-sized generalist predators tend to thrive in farmlands rich in accessible forage (Heske et al. 1999; Litvaitis and Villafuerte 1996; Oehler and Litvaitis 1996). On the other hand, large proportions of agricultural land have been implicated in high losses of

imperiled species (Gibbs et al. 2009). Agriculture has also been listed as one of the most frequent contributors to species declines in the U.S.A. (Czech et al. 2000). The conversion of natural habitats to agriculture may reduce suitability of the land to various species and limit movement of wildlife between remnant habitat patches. Agricultural intensification, the increase in cultivated land and field size for the maximum output of crops, including monoculture plantings, has become a global issue over the past several decades. This intensification has decreased habitat availability and increased fragmentation for many species around the world, particularly in the Midwestern U.S. (Basore et al. 1986; Fahrig and Merriam 1985; Grixti et al. 2009; Woolf and Hubert 1998).

Iowa's landscape has been changing dramatically for over 150 years, first with European settlers breaking up the tall grass prairie to plant crops for their livelihood and more recently with the intensification of agriculture. In the 1850's, prairie made up over 60% of Iowa's landscape. By the turn of the 21$^{st}$ century, a mere 0.1% of historic prairie remained (Zorher 2006), with the small remnant patches of natural prairie typically isolated from one another by large agricultural fields. This loss and fragmentation of Iowa's native landscape has had a dramatic effect on its native fauna. Numerous species adapted to native prairies, particularly those with large space requirements like bison (*Bos bison*) and elk (*Cervus elaphus*), have long been extirpated from Iowa (Dinsmore 1994) and many that remain are also declining, such as prairie chickens (*Tympanuchus cupido pinnatus*), least shrews (*Cryptotis parva*), and white-tailed jackrabbits (*Lepus townsendii*) .

The white-tailed jackrabbit is considered an Iowa Species of Greatest Conservation Need (Zorher 2006). This species has declined dramatically in the state, according to August roadside surveys conducted by the Iowa Department of Natural Resources (IDNR),

for over half a century (Bogenschutz et al. 2007). Habitat changes associated with agricultural intensification have been named as the ultimate cause of declines in European hare (*Lepus europaeus*) populations (Smith et al. 2005). Iowa's landscape has been stripped of its natural diversity and does not maintain much crop diversity, as it is now dominated by monocultures of soybeans and tall corn, likely reducing habitat suitability for jackrabbits. White-tailed jackrabbits prefer open habitats (Kline 1963; Lim 1987) and rely on vision to detect, and speed to avoid, predators rather than making use of burrows (Rogowitz and Gessaman 1990) as do cottontail rabbits (*Sylvilagus floridanus*). Small mammals may, in many cases, be more restricted than birds with respect to movement across unsuitable habitat. Among small mammals, jackrabbits are likely to be more sensitive to fragmentation due to larger space needs and more restrictive habitat requirements than many smaller mammal species. White-tailed jackrabbits in open, grassland habitats have annual home ranges $\geq 2.00$ km² (Donoho 1972; Schaible 2007). Many species of grassland rodents have been shown to have much smaller home ranges, including the hairy-tailed bolo mouse (*Necromys lasiurus*), the grassland mouse (*Mus spretus Lataste*), and the meadow vole (*Microtus pennsylvanicus*) with respective home ranges of $\leq 0.005$ km², $\leq 438$ m², and $\leq 0.002$ km² (Blair 1940; Gray et al. 1998; Pires et al. 2010). Further, as jackrabbits in Iowa occur at the eastern boundary of their historic range (Lim 1987), they may be more susceptible to fragmentation due to the decline in suitable habitat that typically defines species' range boundaries (Swihart et al. 2003).

Suitability of habitat may vary temporally, especially in agricultural settings where farming practices dramatically change the landscape on a seasonal basis. Hairy-footed gerbils (*Gerbillurus paeba*) responded to annual changes in matrix habitat structure, brought on by

rainfall, colonizing once unsuitable habitat between otherwise fragmented populations (Blaum and Wichmann 2007). The authors suggested that temporal heterogeneity of habitat should be considered in other studies of fragmentation. Harvest is a major contributor to the change in Iowa's landscape as tall corn fields, that may represent unsuitable jackrabbit habitat, may be converted to more fitting habitat in the form of short stubble fields following harvest. To predict how changes in the landscape and land use have, and will continue to, affect Iowa's wildlife diversity, it is necessary to understand current relationships between wildlife species and their fragmented environment. In addition, understanding the conditions under which remnant species are able to co-exist with intensive agriculture on the landscape will assist with management and recovery plans for species of conservation need.

In this study, we investigated how a grassland-adapted species, the white-tailed jackrabbit, uses Iowa's intensively agricultural landscape. We chose to study this species as it has large space needs in non-agricultural habitats, relative to other small mammals (Donoho 1972). Jackrabbits may also alter their space use in response to agricultural practices as has been seen in European hares (Chapuis 1990; Marboutin and Aebischer 1996; Reitz and Leoanrd 1994; Tapper and Barnes 1986). The objectives of this study were to 1) estimate home range size on a seasonal basis, 2) investigate field type use on a seasonal basis, 3) estimate survival rates and investigate temporal patterns in survival, and 4) estimate population size in recent years.

## MATERIALS AND METHODS

*Study Area*- Our study site was the Iowa State University Agronomy and Agricultural Engineering Research Farm (Research Farm, 42°3′40″N 93°53′10″W, Fig. 1) and adjacent farm land in Boone County, Iowa. The Research Farm lands encompass 2.75 km² and are planted to variable-sized,

but relatively small plots (0.13 $m^2$ - 0.098 $km^2$) of corn, soybeans, oats, alfalfa and wheat. The fields are divided by large, mowed grassways (tall fescue, *Schedonorus phoenix* (Scop.) Holub) about 10-15 m wide. The Research Farm is surrounded by private farmlands that grow primarily corn in 0.65 $km^2$ fields.

*Data Collection*- Jackrabbits were live-captured on the Research Farm by drive-netting, beginning in September 2008. One-meter-high wing fences were set extending from the corner of a harvested oat or alfalfa field, forming a large V. Wing fences were approximately 100 m long on each side, composed of nylon netting, with a stake about every 2 m. The stakes were set and the nets were laid out, rolled up on the ground to permit entrance to the field, a day or two before the drive. The day of the drive, 2-4 people set the net on wire barbs attached to the tops of the stakes so that, when a jackrabbit ran into the net, the top of the net fell on the animal. The same people positioned themselves behind the net to quickly reach a jackrabbit captured in the net before it could escape. A crew of 10-15 people moved toward the net from the opposite edge of the field, making noise to flush jackrabbits from their resting sites. When a jackrabbit ran into the net, one of the people positioned behind the net held it down by placing one hand anterior to the hips with fingers facing posteriorly and another hand extending the hind legs with two digits between the legs for a firm grip.

Once the animals were extracted from the net they were placed in a restraint cone, scaled up from guidelines in Koprowski (2002), to facilitate handling of the animals. A livestock ear-notch tool was used to remove a piece of ear tissue for genetic studies (see Ch.3), and a radio-collar (Advanced Telemetry Systems, Isanti, MN) was attached. We collected basic information from live-captured animals including: sex, standard size measurements (body length, hind-foot length, ear and tail length), and weight (measured with a hand-held spring scale). A protocol

specific to this project outlining all procedures performed on animals was approved by Iowa State University's Institutional Animal Care and Use Committee (# 2-08-6502-W) and met guidelines approved by the American Society of Mammalogists (Gannon and Sikes 2007).

We tracked radio-collared animals for a period of one year, September 2008-September 2009. Collars had a battery life of 894 days and weighed about 40g (0.08-0.10% of the weight of an adult jackrabbit). Radio-collared animals were located by sight or triangulation 3 times a week on random days and at random times throughout the day and night. Radio-tracking occasions were separated by a minimum of 24 hrs to maintain independence. Field types in which jackrabbits were located were also recorded.

The tracking year was divided into 4 seasons likely to result in changes in food availability and cover for jackrabbits. The pre-harvest season, September-October 2008, was prior to the corn harvest. In the post-harvest season, November 2008-January 2009, the corn had been harvested, reducing those fields to stubble. In the breeding season, February-May 2009, the jackrabbits were breeding and the crops were just being planted and beginning to grow. By the growth season, June-September 2009, the breeding season was ending and the crops, especially the corn, were gaining height, providing more cover and reducing visibility.

We estimated population densities on the Research Farm using a line-transect spotlighting method. Spotlighting was performed on the Research Farm, after the breeding season and before corn harvest, for 4-6 days per month: July-October 2008 and July-October 2009. A straight-line transect was not possible on the Research Farm, so a survey route through the farm (Fig. 1) was used. We established the route to minimize recounting the same individuals by avoiding tight turns and bends as much as possible (Smith and Nydegger 1985). The same survey route had been used for 2 years before this study began (Fairbanks,

unpublished data). We drove the survey route at speeds of <15 kph between 1900 and 2300 h. A 1,000,000- watt spotlight was used to sight the jackrabbits along the transect. Leupold laser rangefinders were used to determine the bearing and distance from the observer and the offset function of a Trimble Geo Explorer 3 GPS unit was used to record the location of jackrabbits spotted. The number of jackrabbits in a group and field types being used were also recorded. Likelihood of recounting was minimized by observing directional movement of jackrabbits after spotting.

*Analyses*- A maximum likelihood estimator in LOAS 4.0 (LOAS 2007) was used to estimate triangulated locations . Home ranges were delineated using the Fixed Kernel Density Estimator in the Hawth's Analysis Tools Extension for ArcGIS® 9.2. The fixed kernel density estimator calculates an individual's probability distribution of use given the locations in which it was observed. Least squares cross validation was used to determine the appropriate smoothing parameter (White and Garrott 1990) and 50% and 95% kernel isopleths, areas encompassing 50% and 95% of the distribution of use, were created for individuals in all seasons. We used 95% kernels to test for differences in home range size among seasons and differences in home range size between the sexes with Mann-Whitney U tests.

We used a chi square contingency table to test for differences in field type use in day versus night and in males versus females. Habitat selection was tested by comparing habitat use and habitat availability within each season. Habitat use was established with the records of field types used by all jackrabbits in each season. A minimum convex polygon of all jackrabbit locations throughout the year of tracking was created and the percent cover of each field type within that polygon was used as an index of habitat availability. Habitat

availability was consistent for the pre-harvest, post-harvest, and breeding seasons. However, crop planting took place in the spring when some fields were planted to different crops. Therefore the habitat availability for the growth season was based on the same area but has a different field type composition. A chi-square contingency table was used to test for differences between the observed habitat use and the expected habitat use (based on availability) in each season.

Seasonal shifts in home ranges were investigated by calculating volume of intersection (VOI), defined as the area of home range overlapping with another home range, for the same individual from season to season. We also compared VOI between individuals by season for the males overlapping with males, females overlapping with females, and males overlapping with females and VOI between the groups of males overlapping with males, females overlapping with females, and males overlapping with females within seasons. All VOI calculations were expressed as the percent of an individual's home range overlapped by the same individual in a different season, or by another individual in the same season. Differences in median percent VOI were tested with a Kruskal-Wallis test.

We used radio-tracking data from individuals to estimate daily survival rates (DSR) using nest survival models in program MARK (White 2010). MARK uses maximum likelihood methods to estimate DSR. The nest survival model is often used in birds to estimate survival to fledging, but is not limited to such studies. This model was used as it does not require the exact dates of mortality, which were unknown for our study. Survival for individual jackrabbits was assumed to be independent. We created 7 models using the factors season, daily minimum temperature, and sex as they were expected to have significant effects on survival (Table 1). Season was expected to have a significant effect

on survival because agricultural practices are associated with specific times of year in which availability of forage and cover shift, potentially changing a jackrabbit's susceptibility to predation. Daily minimum temperature was included as a factor to account for shifts in energy expenditure related to thermoregulation and forage availability. Sex was included to account for differences in survival associated with differences in energy allocated to reproduction. Models were compared using Akaike's information criterion adjusted for small sample size ($AIC_C$), $\Delta AIC_C$, and the Akaike weight (Burnham and Anderson 2002) to determine variation in survival. AIC measures the fit of the model to the data but penalizes for complexity. If competing models are similar in accuracy but vary in complexity (number of parameters), the model with the fewest parameters will have the higher AIC score and be termed the "best" model. "Best" here implies that, of the models being tested, it represents the data the most accurately. However, models with an $AIC_C$ score within 2 points are arbitrarily considered indistinguishable.

Perpendicular distances from the jackrabbit locations to the spotlight survey route (calculated in ArcGIS), for all nights in a given month and year were used to calculate a detection function in program DISTANCE (Thomas et al. 2009). From this detection function, DISTANCE calculates the effective strip width, $\mu$, the distance from the transect at which the number of animals observed beyond that distance is equal to the number that go undetected within that distance. Therefore, the total number of animals observed, n, is equal to the total number of animals, both seen and unseen, within the effective strip width. These parameters were used to calculate the population density, D, for each night separately.

$$D = n/2\mu L$$

L is the distance of the transect route. Density estimates were used to create population estimates for each night and the estimates were then averaged across all survey nights within a month. We compared the results of our surveys and previous surveys conducted using the same equipment and methods (Fairbanks, unpublished data) in 2006 and 2007 using a Wilcoxon Rank Sum test in program R (Team 2009). Only fall estimates (September and October) were compared across the 4 years to maintain consistency, as all young of the year were easily observable by the fall.

## RESULTS

Between September 2008 and September 2009, we captured and collared 13 jackrabbits (9M, 4F). A total of 450 locations were recorded and the average number of points used to create seasonal home ranges was 23.68 ± 10.42 (range: 6-39). We estimated seasonal home ranges for 8 jackrabbits (4M, 4F). The remaining 5 jackrabbits were captured in late summer 2009 as the growth season was ending so adequate information for home range delineation could not be collected. We used only 6 points to create a home range for a diseased jackrabbit that did not move far from a particular corn field. The exact location of this jackrabbit could not always be acquired due to signal bounce-back from the corn, but it was evident that the same corn field was being used. In the pre-harvest season, home ranges were calculated using a low number of locations (<20). The pre-harvest season was the shortest season in the study and jackrabbits were captured beginning 10 September 2008 through 30 October 2008, reducing the possible number of locations that could be acquired in that season.

The median size of jackrabbit home ranges expanded following the corn harvest (Fig. 2). The median home range size increased 287% from the pre-harvest to post-harvest season.

However, the difference between the pre-harvest and post-harvest home ranges was not statistically significant (Mann Whitney W=30, p = 0.17, n = 12). In the breeding season, the median home range size increased 27% from the post-harvest size and finally decreased 46% from the breeding to growth seasons. Median home range size in the breeding season was significantly greater than that in the pre-harvest season (W=23, p = 0.04, n = 10).

At the beginning of the post-harvest season, males moved off the Research Farm, incorporating large portions of harvested corn fields on private lands into their home ranges. The females expanded their home ranges to include adjacent harvested corn fields but remained primarily on the Research Farm. Male home ranges were not significantly different in size from female home ranges in the pre-harvest (W = 3, p = 0.80, n = 2F, 4M), post-harvest (W = 2, p = 0.53, n = 4F, 2M), breeding (W = 1, p = 1, n = 3F, 1M), or growth season (W = 0, p = 0.67, n = 2F, 1 M), however, sample sizes were small for these comparisons.

The average percent VOI between an individual's pre-harvest and post-harvest home ranges was 71%. This decreased to 39% and 31% in the post-harvest-breeding and breeding-growth season transitions, respectively, suggesting shifts in individual home ranges between seasons. VOI varied between individuals across the seasons, as well. There was a large decrease in VOI between males from the pre-harvest to post-harvest season, when the males moved off the main Research Farm (Fig 3). There was also a large increase in VOI between males and females from the post-harvest to breeding season. However, we did not detect any statistically significant differences in VOI across the seasons (Kruskal-Wallis H = 3.13, df = 3, p = 0.37). VOI across the groups of males overlapping with males, females overlapping with females, and males overlapping females were significantly different in the pre-harvest

((Kruskal-Wallis H = 11.36 df = 2, $p < 0.001$), post-harvest (Kruskal-Wallis H=16.07, df = 2, $p < 0.001$), and the breeding seasons (Kruskal-Wallis H = 10.11, df = 1, p = 0.001).

Male and female jackrabbits increased their use of corn fields in the post-harvest and breeding seasons (Fig 4). Field type use was not significantly different between males and females in the pre-harvest ($X^2 = 5.23$, df = 4, p = 0.26), post-harvest ($X^2 = 2.13$, df = 4, p = 0.71), breeding ($X^2 = 0.92$, df = 3, p = 0.82), or growth season ($X^2 = 1.18$, df = 4, p = 0.88). Field type use did not differ significantly between day and night in the pre-harvest ($X^2 = 6.98$, df = 3, p = 0.07), post-harvest ($X^2 = 3.51$, df = 4, p = 0.48), or breeding season ($X^2=3.14$, df = 3, p = 0.37). However, the jackrabbits used field types significantly differently in day versus night during the growth season ($X^2 = 20.30$, df = 4, $p < 0.001$). Soybean fields were used significantly more in the day versus night, during the growth season (Bonferroni Z = 2.83, p = 0.002).

Jackrabbits displayed habitat selection in the pre-harvest ($X^2 = 63.70$, df = 3, $p < 0.0001$), post-harvest ($X^2 = 22.89$, df = 4, $p < 0.0001$), and growth seasons ($X^2 = 15.27$, df = 4, $p < 0.01$). However, jackrabbits did not appear to exhibit habitat selection during the breeding season ($X^2 = 1.54$, df = 3, p = 0.67). Jackrabbits selected against corn fields (Bonferroni Z = 7.32, $p < 0.001$) and selected for soy bean fields (Bonferroni Z = 2.29, p = 0.01) and oat fields (Bonferroni Z = 5.32, $p < 0.001$) in the pre-harvest season. In the post-harvest season, jackrabbits selected against corn fields (Bonferroni Z = 3.72, $p < 0.001$) and selected for oat fields (Bonferroni Z = 1.23, p = 0.01) and alfalfa fields (z = 2.98, p = 0.001). In the growth season, jackrabbits selected against corn fields (Bonferroni Z = 3.00, p = 0.001) and selected for alfalfa fields (Bonferroni Z = 2.4, $p < 0.01$).

Radio-tracking data from all 13 individuals captured during the study period were used to estimate DSR. A model representing constant mortality was the most supported model (Table 1) of jackrabbit DSR on the Research Farm. All other models, except the quadratic model, were comparable (within 2 ΔAIC values), however, the constant model contained the only statistically significant parameter coefficient (β). Though other models were comparable in ΔAIC values, the factor they were modeling did not significantly represent jackrabbit survival rates. This lack of significance may be due to small sample size. DSR in the constant survival model was 0.997 (95% CI 0.993- 0.999) with a 0.330 (95% CI 0.103-0.677) probability of surviving the 385-day study period.

The number of jackrabbits spotted per survey night in 2008 and 2009 varied from 5-33 individuals. The largest group of jackrabbits observed consisted of 8 individuals (average 1.36 ± 0.87 jackrabbits, with groups greater than 3 being very rare). We detected an overall declining trend across years in the population size on the Research Farm (Fig. 5).

## DISCUSSION

Due to the dramatic conversion of historic prairie to row crops, white-tailed jackrabbits can be found living in corn dominated landscapes in Iowa. Previous studies have shown that jackrabbits maintain smaller home ranges in agricultural landscapes than in grasslands (Donoho 1972, Schaible 2007). Annual home ranges for white-tailed jackrabbits in South Dakota's agricultural fields were estimated to be less than half the size of home ranges in grasslands (0.61 km² and 0.88 km² versus 2.00 km², Schaible 2007). The potential for high quality food and shelter in agricultural land was suggested as a cause for smaller home ranges (Schaible 2007). Similar-sized grassland home ranges (2.59 km²) were seen in

Colorado grasslands during the breeding season, although these estimates did not distinguish black-tailed jackrabbit (*Lepus californicus*) from white-tailed jackrabbit home ranges (Donoho 1972). Small seasonal home ranges (0.21 km²) have also been reported in an agricultural landscape for the European hare (Ruhe and Hohmann 2004). Harvest of crops did not change home range size of these hares, however, the crops being cultivated in these fields were predominately cereals and sugar beets, which differ structurally from corn fields, and so may affect hare behavior differently. During the pre-harvest and growth seasons, the jackrabbits in our study had a median of 0.32 km² and 0.73 km² home ranges, respectively, which are comparable in size to those of South Dakota jackrabbits in agricultural land. However, median post-harvest and breeding home ranges were 1.24 km² and 1.58 km², slightly smaller than those observed in grassland habitats in South Dakota and Colorado. Hares typically respond to seasonal changes in their habitat by changing their patterns of space use, with shorter field types being used more in winter months or cores of home ranges shifting following harvest (Chapuis 1990; Marboutin and Aebischer 1996; Reitz and Leoanrd 1994; Tapper and Barnes 1986). The seasonal changes in home range size in our study may be related to either the striking changes in structure on the landscape following harvest when tall corn is reduced to stubble, the seasonal differences in food availability, or the reproductive strategies of the sexes.

Iowa winters are harsh, with mean daily temperatures ranging from approximately -3˚ C to -11˚C between December and February (National Climatic Data Center). In these conditions, it is expected that resources would be limited and cause jackrabbits to expand their home ranges to find forage. This is unlikely on the Research Farm, however, as waste corn and hay are found all winter in great supply. Waste corn is collected in a central

location on the Research Farm where aggregations of jackrabbits can be observed feeding on winter nights. Shifts in core areas of home ranges and increased size of home ranges among other small mammal species have been attributed to greater refuge availability (Lombardi et al. 2007) and older age (Hoset et al. 2008) rather than food availability and seasonal changes.

Age is generally a factor in reproduction. Jackrabbits on the Research Farm may have expanded their home ranges in search of potential mates for the breeding season (February-May). Home ranges did increase in the breeding season but not nearly to the same degree as in the post-harvest season. Post-harvest home range expansion occurred well before the breeding season and males' testicles do not begin to descend until just before females become receptive in February to mid-March (Lim 1987, Rogowitz 1992). Timing of home range expansion, therefore, does not appear to be consistent with breeding behavior.

The most likely explanation for the observed increase in home range size in the post-harvest season is that corn fields were converted into suitable jackrabbit habitat by the corn harvest. The corn fields were used more by both sexes after the harvest than before the harvest. Immediately following the conversion of these fields into suitable open habitat, we observed the expansion of home ranges and increased proportion of corn fields in home ranges. This trend of increasing home range size following crop harvest has been seen in other species as well: European rabbits (Smith et al. 2004), white-tailed deer, *Odocoileus virginianus* (Vercauteren and Hygnstrom 1998). European rabbits also decrease home range size with increasing population density (Ruhe and Hohmann 2004). If the unharvested corn fields are indeed unsuitable habitat, then the home ranges observed during the pre-harvest season may have been artificially small due to high densities imposed by corn height. Home range studies of white-tailed jackrabbits have previously only calculated annual home ranges.

Data on seasonal home ranges for white-tailed jackrabbits in more natural, grassland habitats are needed for comparison to determine whether seasonal home range expansions occur in the absence of profound changes in vegetation structure resulting from crop harvest.

In addition to increasing the size of their home ranges, male jackrabbits also decreased home range overlap with other males in the post-harvest season. This decrease in VOI after corn harvest may have been due to natal dispersal by males. However, the age of animals at capture was unknown and most males did not survive long enough to determine if their post-harvest home ranges represented permanent shifts away from pre-harvest home ranges. Alternatively, the decrease in VOI might have been a result of males establishing territories, as in some other lagomorph species (Holley 1986; Monaghan and Metcalfe 1985). Voles also exhibit stronger territoriality in the breeding season, when home range overlap between males declines (Salvioni and Lidicker 1995). Female jackrabbit home ranges also increased after corn harvest but home range overlap between females did not decrease to the same extent as between males, suggesting that if territoriality plays a factor in home range overlap it may be more prominent in males. There were significant differences in median VOI among the males overlapping with males, females overlapping with females, and males overlapping with females within seasons. However, we expect the observed decrease in VOI among males may have been due to the lack of suitable habitat when the corn was tall, forcing jackrabbits to exist at artificially high densities in small/highly overlapping home ranges until corn harvest. Male home ranges increased in size and home range overlap between males decreased immediately following harvest, suggesting that crop height limited field use. Only one radio-collared male survived from the beginning of the study to its completion, but this animal's growth season home range was a highly condensed portion of

its breeding home range, and was surrounded by tall corn fields. Although VOI among females did not decrease to a great extent following corn harvest, both sexes increased use of corn fields at that time, supporting a temporal aspect to suitability of corn fields as habitat for jackrabbits.

The increase in VOI between the sexes in the breeding season might be attributed to jackrabbits searching for potential mates. Mt. Graham red squirrels, *Tamiasciurus hudsonicus grahamensis,* increase home range overlap between the sexes during the breeding season (Koprowski et al. 2008), while other species maintain proportionally large home range overlaps between the sexes year-round : Alpine hare, *Lepus timidus* (Gamboni et al. 2008); raccoon, *Procyon lotor* (Chamberlain and Leopold 2002). These long-term home range overlaps have been potentially influenced by extended breeding behavior in summer. Environmental factors, such as timing of snow melt, available cover, and forage quality, likely limit the timing and duration of the breeding season in white-tailed jackrabbits (Rogowitz 1992). In our study, both male and female jackrabbits expanded their home ranges and increased inter-sex home range overlap in conjunction with the breeding season. Male home ranges were not significantly larger than female home ranges. Although lack of significance may be due to small sample sizes, we would not expect a significant difference between male and female home ranges of a promiscuous species, as both sexes have equal opportunity for mating events (Chapman et al. 1982). Unfortunately, there are no data on white-tailed jackrabbit breeding behavior in natural habitats in this region with which to compare our data. Breeding behavior in the white-tailed jackrabbit has not been well studied, in general (James and Seabloom 1969; Rogowitz 1992), and more research is needed

to understand how reproduction may be shaped by dynamic habitat structure as occurs in agricultural landscapes.

Populations of several hare species have been declining across the world (Dingerkus and Montgomery 2002); alpine hare, (Newey et al. 2007); European hare, (Smith et al. 2005); snowshoe hare *(Lepus americanus),* (Keith et al. 1993; Keith et al. 1984)*;* white-tailed jackrabbit *(*Kline 1963*;* this study). These declines have largely been attributed to three main causes: habitat degradation, disease, and predation. All but one mortality of radio-collared jackrabbits, in this study, were attributed to coyote predation. In small, isolated jackrabbit populations, predation may be a significant contributor to further decline of jackrabbits in Iowa. Different forms of mortality may be additive in smaller populations (Smart et al. 2010). While there is currently still a hunting season on white-tailed jackrabbits in Iowa, hunting is not permitted on the Research Farm. Hunting may not account for a large proportion of jackrabbit mortality in the rest of the state either as the bag limits have been lowered in recent years to 1 daily and 2 in possession. Although reporting jackrabbit harvesting is not mandatory, no reports of harvested jackrabbits were recorded in 2009 when a request was submitted to licensed small game hunters. Disease was observed in two radio-collared individuals during the study period, one with a postmortem diagnosis of kidney disease and bacterial infection, and one that showed symptoms of illness, but was consumed by a coyote, preventing diagnosis. Whether these occurrences of disease are rare is not known and further investigation into causes of mortality is needed to determine disease effects on this population. A third, more likely, cause of decline is loss of suitable habitat due to agricultural intensification and increased corn planting. Jackrabbits selected for shorter crop fields in the pre-harvest, post-harvest, and growth seasons and selected against

corn fields in the pre-harvest, post-harvest and growth seasons. No habitat selection was detected during the breeding season when the landscape becomes more homogenous with all fields being barren or reduced to stubble early in the season and all fields having only short crop shoots later in the season. The Research Farm has had relatively stable corn plantings in the past 10 years (Mike Fiscus, Research Farm Manager, personal comm.), although there is an overall trend of increased acres planted to corn in Iowa. It has been argued that similar changes in agricultural habitats have contributed to the population declines of European hares in the past decades (Boag and Tapper 1992; Hansen 1992; Ruhe 1999). These declines can further be exacerbated by susceptibility to genetic drift and inbreeding in small populations (Lacy 1997).

With the small number of radio-collared jackrabbits in this study, we were unable to detect an impact of daily minimum temperature, season, or sex on survival rates. These factors may prove to be statistically significant with larger sample sizes. The annual survival for our population was greater than estimates from the only other published study of white-tailed jackrabbit survival. Rogowitz (1991) estimated annual survival to be 0.093 in 1985 and 0.20 in 1986 (compared to 0.33 in our study) in southwestern Wyoming, where mountains, sagebrush steppe and high desert terrain cover the land. The vast differences in habitat between our study and the Rogowitz study may affect survival via factors such as forage availability, predator density, and susceptibility to predation. Our annual survival estimates lie between rates for other species of hares in natural (0.22) and agricultural (0.51) habitats (Marboutin and Peroux 1995), respectively. Estimates of hare survival appear to be higher in agricultural landscapes than in more natural habitats, potentially due to high quality forage and shelter in agricultural lands as suggested by Schaible (2007). Yet the population

on the Research Farm is declining. The cause of this decline may not be directly linked to adult survival but may be affected by fecundity or juvenile survival, which can be affected by aspects of agricultural practices such as field size or types of crops being planted. In addition to survival estimates, a better understanding of this species' reproductive ecology in different habitats is needed for accurate population modeling.

Our study identified potential seasonal barriers to jackrabbit movement in Iowa's intensively row-crop dominated landscape. An important question to the conservation of this species is: Are jackrabbits able to expand their home ranges or disperse after corn harvest, to the extent that population connectivity is maintained, or are these populations isolated by expansive corn fields? As jackrabbits increased their use of corn fields following harvest, the degree of connectivity between white-tailed jackrabbit populations may vary temporally, thus permeability of corn fields and the degree of isolation may vary with agricultural seasons. Continued research should focus on timing of white-tailed jackrabbit dispersal relative to corn height.

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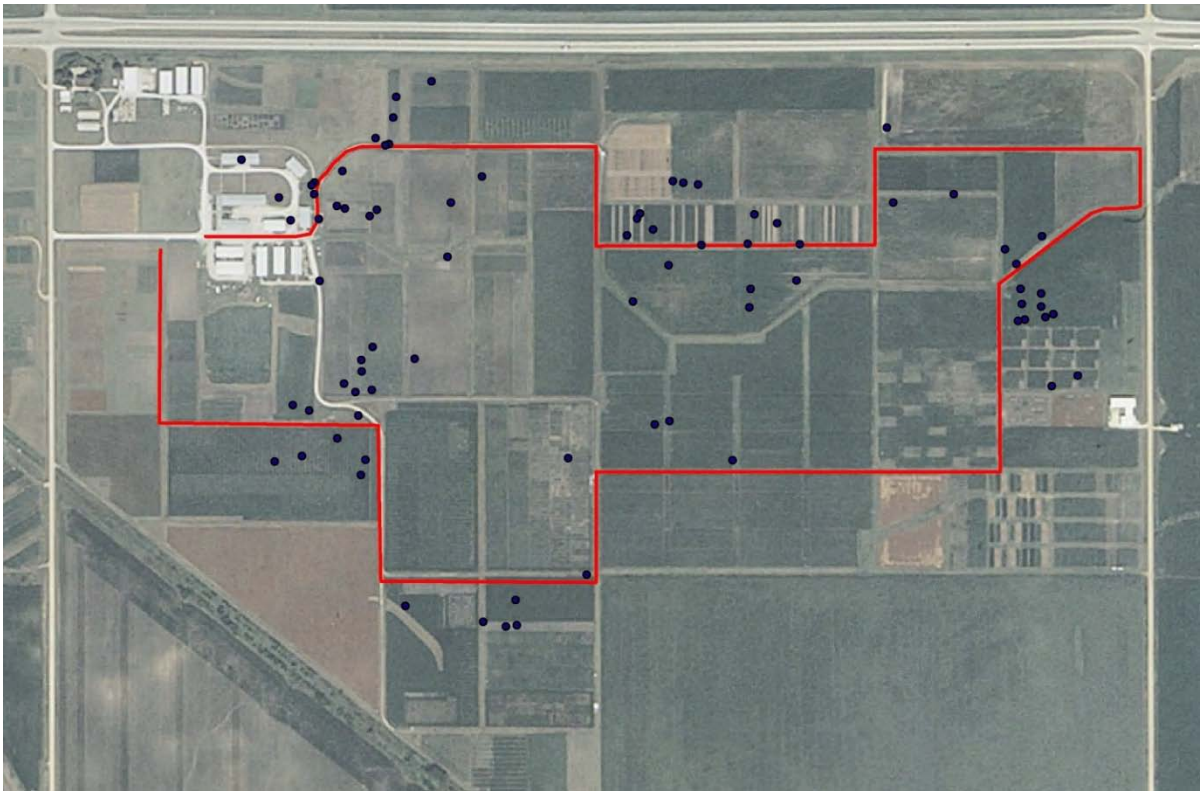


Figure 1. The spotlighting transect route (red line) used to estimate population size on the Iowa State University Agronomy and Agricultural Engineering Research Farm in Boone County, Iowa, in late summer-early fall, 2006 to 2009. The dots represent individuals or groups of jackrabbits sighted in September 2006.

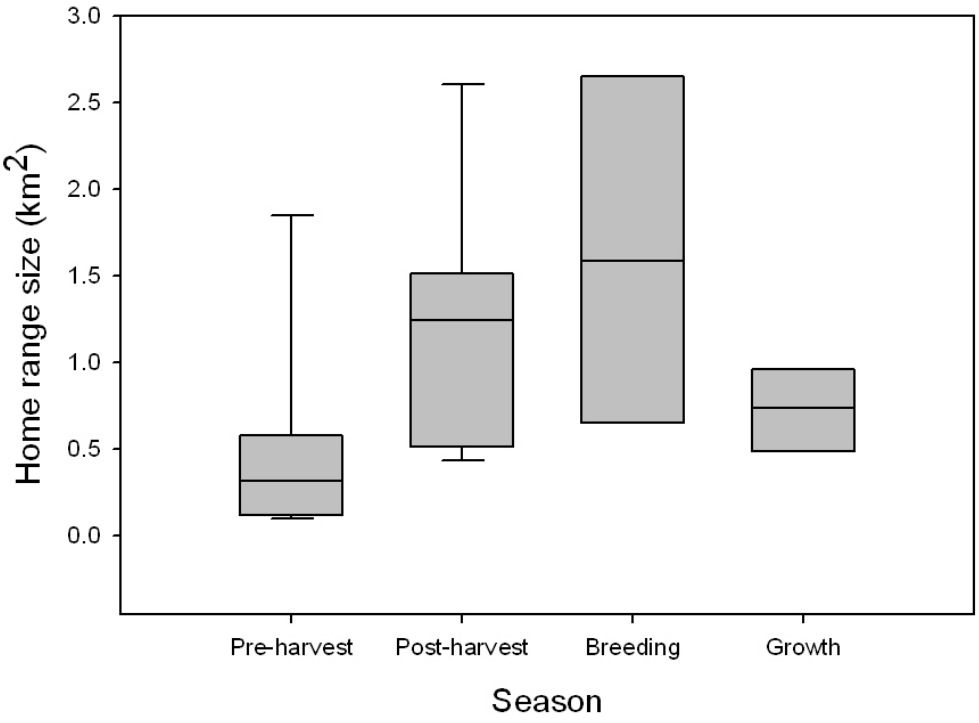



Figure 2. White-tailed jackrabbit home range sizes, based on 95% kernel isopleths, across 4 seasons (male and female home ranges combined). Boxes represent 50% of the data and are intercepted by median values. Whiskers indicate 80% of the data. The pre-harvest season spanned from September-October 2008, followed by the post-harvest season which covered November 2008-January 2009. The breeding season began in February 2009 and ended in May 2009 and was followed by the growth season which spanned from June-September 2009. Harvest is used in reference to crops, particularly corn.

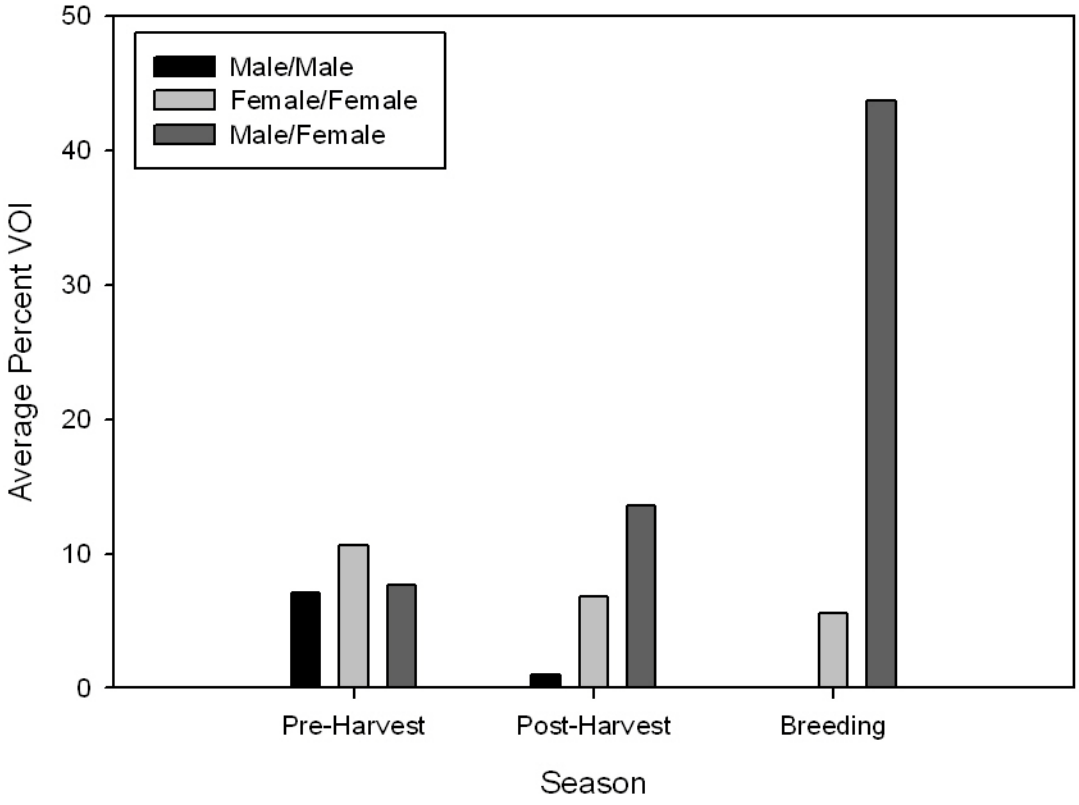
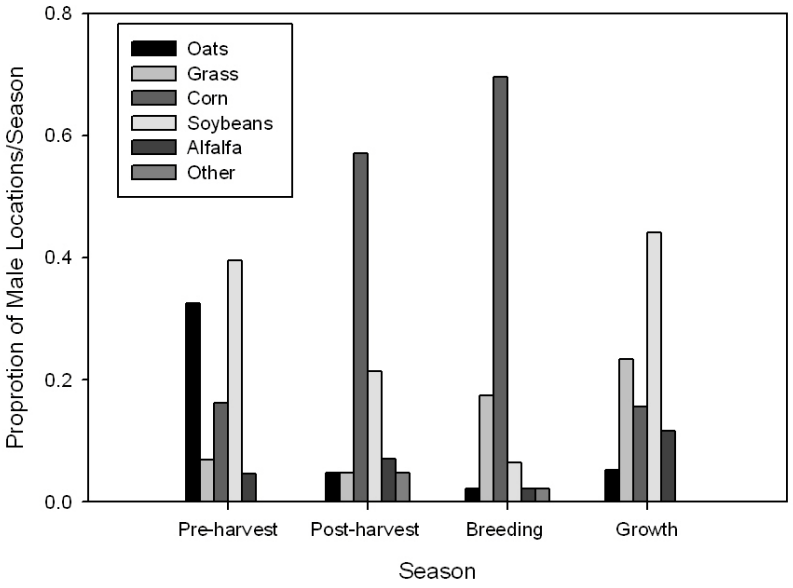



Figure 3. Volume of intersection (VOI) of white-tailed jackrabbit home ranges within and between the sexes. Seasons as in Figure 2.

A

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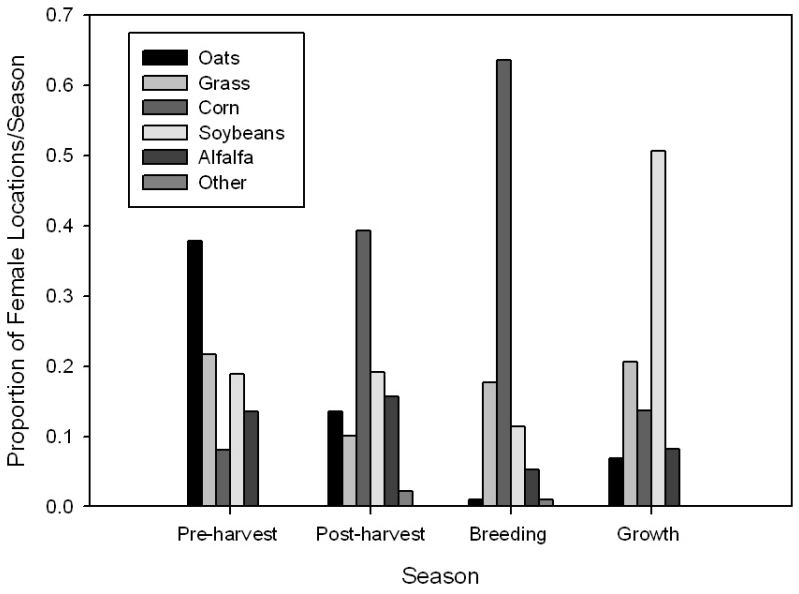


Figure 4. Proportions of locations of A) males and B) females in the available field types across the seasons. See Figure 2 for season dates. The number of locations used for comparisons varied across the seasons: pre-harvest, n = 43M, 37 F locations; post-harvest, n = 42M, 89F; breeding, n = 46M, 96F; growth, n = 77M, 73F.

Table 1. Models of daily survival rate tested for jackrabbits on the ISU Research Farm September 2008-September 2009. Constant model: constant survival over time; Min Temp: survival is related to daily minimum temperature as energetic constraints may vary with temperature; Linear model: survival decreases in a linear fashion; Breeding model: survival differs in the breeding season from the rest of the year; Sex model: difference in survival of the sexes; Harvest model: survival after corn harvest, the post-harvest and breeding seasons combined, differs from the rest of the year; Quadratic model: survival increases with time until a certain point at which it drops off. A seasonal model including all four seasons was not tested due to lack of data.

| Model | AICc | Delta AICc | AICc Weights | Model Likelihood | K | Deviance |
|---|---|---|---|---|---|---|
| Constant | 60.53 | 0.00 | 0.31 | 1.00 | 1 | 58.52 |
| Min. Temp | 62.08 | 1.55 | 0.14 | 0.46 | 2 | 58.07 |
| Linear | 62.28 | 1.75 | 0.13 | 0.42 | 2 | 58.27 |
| Breeding | 62.31 | 1.78 | 0.13 | 0.41 | 2 | 58.31 |
| Sex | 62.50 | 1.98 | 0.15 | 0.37 | 2 | 58.50 |
| Harvest | 62.52 | 1.99 | 0.11 | 0.37 | 2 | 58.52 |
| Quadratic | 63.62 | 3.10 | 0.07 | 0.21 | 3 | 57.61 |

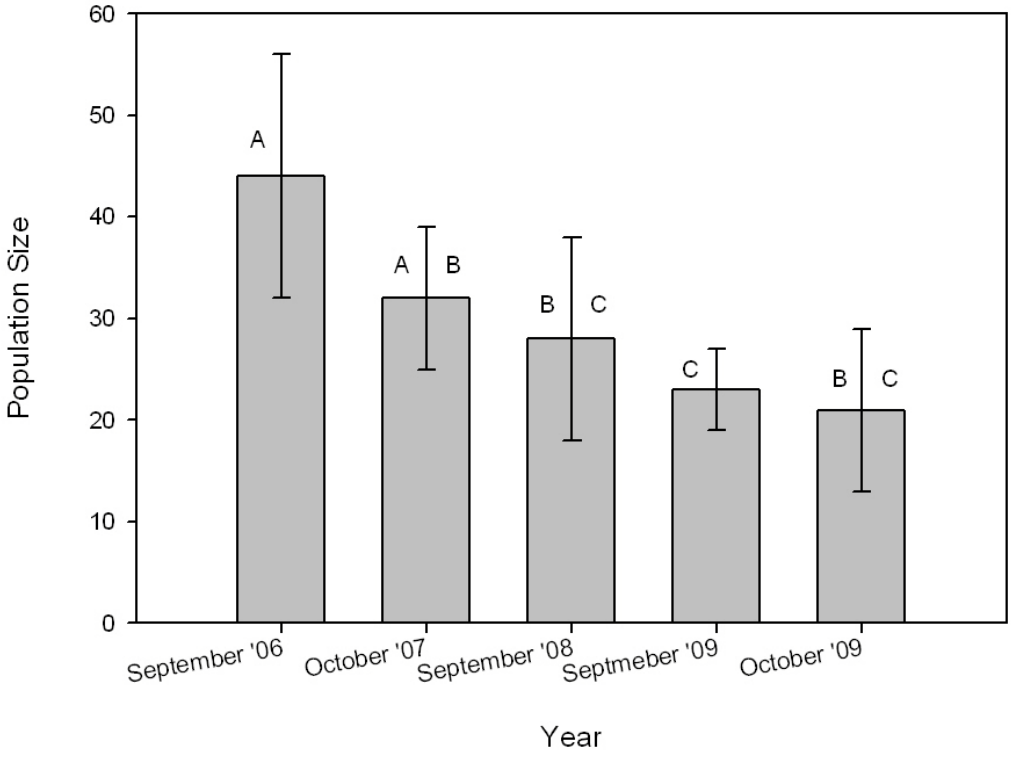


Figure 5. Fall population estimates on the ISU Research Farm between 2006 and 2009 with standard deviations. Bars with different letters are significantly different (Mann-Whitney U, $p < 0.05$).

# CHAPTER 3: GENETIC STRUCTURE OF WHITE-TAILED JACKRABBITS IN AN AGRICULTURALLY DOMINATED LANDSCAPE

A paper to be submitted to *Conservation Genetics*

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## ABSTRACT

Agricultural intensification has reduced availability of natural grassland habitats in the midwestern U.S. and fragmented remnant patches. As grassland habitats become less available and more discontinuous, remnant wildlife populations may exhibit a loss of genetic diversity and connectivity. We investigated the effects of fragmentation on the genetic structure and diversity of a grassland adapted species, the white-tailed jackrabbit (*Lepus townsendii*), in the midwestern states of Iowa and South Dakota. Our population genetic structure analyses suggested there were two jackrabbit populations; one central Iowa population and one population consisting of northwestern Iowa and South Dakota jackrabbits. These populations were moderately genetically differentiated ($F_{ST}$ = 0.06) and exhibited low recent migration rates (0.01-0.04). Potential barriers to gene flow among these jackrabbit populations include highways, agricultural fields, and physical distance.

## INTRODUCTION

Fragmentation of natural habitats has become a vital issue in wildlife conservation, having serious negative impacts on native wildlife, including genetic isolation. As suitable habitat decreases, a population's size may decrease and its spatial seperation to other populations may increase. Small fragmented populations are more difficult to find by dispersers reducing gene flow and leading to a loss of genetic diversity (Garner et al. 2005;

Lacy 1997). Fragmentation can isolate small remnant populations, increasing their risk of local extinction due to loss of genetic variation, to increased susceptibility to stochastic demographic or environmental variability, or a combination of these factors (Frankham 2005; Laikre et al. 2009; Templeton et al. 1990). Metapopulations, or subpopulations existing in discrete habitat patches that interact by means of immigration and emigration, can alleviate some of these effects. Occasional exchange of individuals among remnant populations can mitigate the negative effects of fragmentation by "rescuing" declining populations or recolonizing vacant patches via immigration of animals that serve to increase a remnant's genetic diversity (Hanski and Gaggiotti 2004). However, dispersal among habitat patches depends on the matrix habitat (intervening unsuitable landcover) and a species' willingness/ability to cross it (Haddad et al. 2003).

The issues surrounding population fragmentation may be especially important in the midwestern U.S. where agricultural intensification, the increase in cultivated land and field size for the maximum output of crops, including monoculture plantings (primarily corn and soybeans), has reduced the availability of natural habitats and increased fragmentation (Zorher 2006). Agricultural fields may increase isolation of remnant habitat patches as they may be perceived as matrix habitat with limited permeability by some wildlife. For example, bobcats (*Lynx rufus*) in Iowa incorporate < 3% of agricultural land in their home ranges and generally avoid areas with large proportions of row crop agriculture (Tucker et al. 2008). Further, bobolinks (*Dolichonyx oryzivorus*) using grassland edge habitat adjacent to agricultural fields, in Iowa, did not include the fields in their territories but used them as boundaries to territories (Fletcher and Koford 2003).

The landscape of the midwestern U.S. has changed drastically with agricultural intensification. This change is more prominent in some states than in others. Tall-grass prairies once dominated over 60% of Iowa's land. By the turn of the 21st century, historic prairie had been diminished to a mere 0.1% of its historic range (Zorher 2006) being replaced by expansive corn fields. In the 1950's, approximately 40% of South Dakota's land was considered cropland and that has not markedly changed in over 50 years (USDA), Census of Agriculture). However, as the demand for corn for biofuel increases, acres planted to corn are expected to increase in all regions, especially in the Corn Belt and Northern Plains, by 2016 (Heisey 2009). As portions of remaining natural habitats are converted to agriculture, the persistence of grassland adapted populations will likely be threatened if there is reduced dispersal/gene flow between suitable habitat patches that are separated by increasingly larger fields of potentially unsuitable corn habitat.

The white-tailed jackrabbit, *Lepus townsendii*, is a model species in which to study the impacts of fragmentation, due to agricultural intensification, on genetic diversity and genetic structure. The historic range of white-tailed jackrabbits extended only as far east as northwest Iowa (Lim 1987). When tall-grass prairies began to be replaced with diverse, small fields including short grain crops over 150 years ago, the landscape became more suitable for this short-grassland adapted species, allowing it to expand its range across Iowa, and even into Wisconsin, Illinois, and Missouri (Lim 1987). However, white-tailed jackrabbits have more recently been extirpated from Illinois and Missouri and are considered a Species of Greatest Conservation Need in Wisconsin. Currently, white-tailed jackrabbits are also considered a Species of Greatest Conservation Need in Iowa (Zorher 2006). Agricultural practices, in Iowa, have shifted to planting row crops (e.g. corn and soybeans)

that now cover 60% of the state's land, greatly reducing and fragmenting suitable jackrabbit habitat. The lack of suitable jackrabbit habitat and its fragmentation may be at least partially responsible for apparent reductions in jackrabbit numbers across the state. According to the Iowa Department of Natural Resources (IDNR), data from August roadside surveys conducted from 1962 to 2007 (Bogenshutz et al. 2007), there has been a considerable decline in jackrabbits across Iowa with a high of ~1 jackrabbit observed per 78 km stretch of road in 1964, 0 jackrabbits observed during the surveys in 2008, and 1 jackrabbit observed in the state in 2009 (Todd Bogenshutz, personal comm.). This species is still found in pockets of habitat in central and northwest Iowa, generally on small farms that still plant shorter crops such as alfalfa, oats and wheat and at airports where large areas of grass surrounding landing strips are regularly mowed (Fairbanks, unpublished data; E. Colboth, USDA-APHIS Wildlife Services, pers. commun.).

In general, little is known about the white-tailed jackrabbit across its range, which spans from Iowa west to Washington and southern Canada south to northern New Mexico (Lim 1967). Further, no published data currently exist on the genetic diversity or structure of the species. Dispersal patterns of this species are also unknown. Relative to Iowa, white-tailed jackrabbits are more abundant west of the Missouri River, including South Dakota (Schaible 2007). Genetic diversity and population structure may vary in white-tailed jackrabbit populations that occur at the periphery of their range in Iowa compared to those populations that are more continuously distributed in less agriculturally fragmented areas in the core of their range, such as in South Dakota, due to fluctuating environmental conditions as has been seen in the chukar partridge, *Alectoris chukar* (Kark et al. 2008). Fluctuating environmental conditions can include suitable habitat availability, density of con-specifics,

and successful migration that shift from core to peripheral populations and influence patterns of genetic diversity and connectivity. The authors recommend sampling from peripheral, sub-peripheral, and core populations in order to clearly identify patterns of genetic diversity across a species range. Although there is an ever-growing body of literature on the effects of fragmentation on genetic diversity and connectivity in wildlife, the effects on gene flow of fragmentation due to agricultural intensification have not been well documented in mammals. Fragmentation due to agriculture can vary in its effects as agricultural practices change the permeability of the landscape on a seasonal basis. This seasonal change in permeability can affect timing and success of dispersal, which can shape patterns of genetic diversity and structure among populations.

In this study we investigated population genetic diversity and structure of a grassland-adapted species, the white-tailed jackrabbit, across a portion of its range using bi-parentally inherited microsatellite markers. We sampled individuals from the eastern periphery of the white-tailed jackrabbit's range in central Iowa, from the sub-periphery of its range in northwest Iowa, and core areas of its range in South Dakota and Montana. The objectives of this study were to 1) quantify and compare genetic diversity in Iowa and South Dakota populations of white-tailed jackrabbits, 2) quantify the number of genetically distinct populations in our sample, and 3) characterize spatial genetic structure. We hypothesized that genetic diversity would be lower in white-tailed jackrabbits in Iowa relative to South Dakota and that each regional sampling area (central Iowa, northwest Iowa, South Dakota and Montana) would be a distinct genetic population due to limited movement across matrix habitat imposed by fragmentation, and that spatial genetic structure would follow an isolation-by-distance pattern.

# METHODS

We collected tissue samples in Iowa from live-captured jackrabbits on the Iowa State University Agronomy and Agricultural Engineering Research Farm (Research Farm) in Boone County, Iowa (as described in Ch. 2) and from road-killed samples acquired opportunistically by the Iowa Department of Natural Resources and County Conservation Boards. Iowa samples came from central counties (Boone, Marshall, and Polk) and northwestern counties (Clay, Dickinson, Emmet, Lyon, O'Brien, Osceola, and Pocahontas). Tissue samples from road-killed and harvested white-tailed jackrabbits across South Dakota were obtained with the aid of the South Dakota Game, Fish, and Parks Department and Charles Dieter of South Dakota State University. We also collected 7 samples from harvested jackrabbits in Montana. We obtained a total of 113 samples from the three states (Fig. 1A). UTM coordinates were acquired for point locations of most samples, although a small portion of sample locations could only be identified to the county level. For these samples, UTM coordinates for the central point of their respective counties were assigned. Based on the locations from which our samples were collected, we grouped them into 4 putative white-tailed jackrabbit populations: central Iowa, northwest Iowa, South Dakota and Montana. All samples were collected between September 2008 and January 2010 and stored in a -80ºF freezer until DNA extractions could be performed.

We extracted DNA from ear, tongue or liver tissues using Qiagen DNEasy extraction kits. Eleven genetic markers (microsatellites) developed for the European rabbit, *Oryctolagus cuniculus*, were optimized to amplify *L. townsendii* DNA : sol03, sol08, sol28, sol30 (Rico et al. 1994), sol33, sol44 (Surridge et al. 1997), sat02, sat03, sat12, sat13, sat16 (Mougel et al. 1997). Microsatellites are presumably neutral fragments of bi-parentally

inherited DNA made up of 2-6 nucleotide repeats. Each distinct repeat length is considered a respective allele. Microsatellites tend to be highly polymorphic and have high mutation rates. Due to their high level of allelic diversity, they can be very informative about a population's genetic diversity and structure. Polymerase chain reaction (PCR) products were sent to the Iowa State University DNA Facility where genotypes were visualized on an ABI 3730 Genetic Analyzer. We determined genotypes and allele sizes from peaks using PeakScanner© v.1.0 software.

Quality control measures were put in place to reduce genotype scoring errors. First, all plates of PCR products (96 samples) submitted to the DNA facility for genotype visualization included 4 known samples per locus; the number of known samples was prorated for plates that were not full. Known samples were established as individual samples that were analyzed during optimization more than 5 times and provided consistent genotypes. Genotypes were scored by two people and any samples with inconsistencies in scoring were submitted for genotyping again. We genotyped 10% of homozygous individuals a second time to verify that only one allele amplified.

We screened genotype data for null alleles, allelic drop-out and allelic stuttering using the program MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004). Null alleles are alleles that fail to amplify due to mutations at the primer binding site. Allelic drop-out refers to alleles that fail to amplify due to sampling errors such as low quality or quantity of DNA. Allelic stuttering occurs when minor products are produced that are within a few repeat units of the main allele. The presence of these genotypic inconsistencies causes scoring errors which may lead to deviations from Hardy-Weinberg equilibrium (HWE). Deviations from HWE can also be detected in the case of true homozygosity or heterozygosity excess. A

population is in HWE when allele frequencies and genotype frequencies do not change from generation to generation due to the assumptions that mating is random, natural selection is not acting on the loci being investigated, mutation and migration do not occur, and the population is infinitely large. HWE is often a basic assumption in genetic analyses and was so in our analyses of F-statistics and Bayesian clustering. Violation of the assumption of HWE may reduce reliability of the results of analyses that assume HWE. Null alleles and allelic drop-out will decrease the observed heterozygosity and will lead to an underestimation of genetic diversity, an overestimation of population differentiation, and an overestimation of the inbreeding coefficient. Linkage equilibrium was also an important assumption in our analysis of migration rates. Linkage equilibrium occurs when the genotypes of loci are independent of one another. If two or more loci are linked, their dependency can bias results.

We tested for deviations from HWE and linkage equilibrium in our populations using a Markov chain estimator with 10,000 dememorizations steps, 100 batches and 10,000 iterations per batch in GENEPOP version 4.0.10 (Raymond and Rousset 1995). We tested for these deviations in the global population (i.e., all samples collected), in our 4 putative populations, and in the populations identified as genetically distinct (refer to Bayesian clustering results). Significance levels were adjusted using the sequential Bonferroni correction for all statistical tests involving multiple comparisons (Rice 1989). We used GenAlEx 6.2 (Peakall and Smouse 2006) to calculate the number of distinct alleles ($N_A$), their frequencies, and observed ($H_O$) and expected ($H_E$) heterozygosities for each locus in each sampling region and in the identified genetically distinct populations. Allelic richness ($A_R$) was also calculated in program FSTAT (Goudet 2001). Allelic richness adjusts $N_A$ for sample size, which was necessary to compare allelic diversity across regions in which sample

sizes were uneven. These measures served as descriptive parameters of genetic diversity within the four putative populations.

We quantified the degree of genetic differentiation among the four regions (central Iowa, northwest Iowa, South Dakota, and Montana) using F statistics in program FSTAT. F statistics are measures of variance between different levels of groupings, in this case, individuals, subpopulations and populations. For this study, we quantified $F_{ST}$, the measure of genetic differentiation between subpopulations (i.e., our four regions), in a pair-wise fashion and globally across the four regions. $F_{ST}$ values range from 0 to 1 with larger $F_{ST}$ values signifying greater genetic differentiation. FSTAT tests for significant deviations of the estimated $F_{ST}$ from $F_{ST}$ values obtained by randomizing multi-locus genotypes among populations. We also quantified $F_{IS}$ within each population. $F_{IS}$ is the measure of differentiation between pairs of individuals within a subpopulation, also referred to as the inbreeding coefficient. $F_{IS}$ values can range from -1 to 1 with negative values indicating higher levels of inbreeding than expected and positive values indicating lower levels of inbreeding than expected. FSTAT tests for significant deviations of the estimated $F_{IS}$ from $F_{IS}$ values obtained by randomizing alleles among individuals within designated populations.

In order to identify if our 4 putative populations were genetically distinct populations, we used two methods of clustering algorithms. In both of these methods, distinct populations are identified using the genetic data as opposed to the F-statistics approach to population differentiation where we defined the potential populations and evaluated how different they were. Program STRUCTURE 2.3.2 (Falush et al. 2003; Pritchard et al. 2000) uses a Bayesian clustering method to identify the number of distinct genetic populations (K) while minimizing deviations from HWE and linkage equilibrium in each population. We used a

burn-in of 100,000 steps followed by 1,000,000 Monte Carlo steps. Initially, data were analyzed in structure with K=1-8 to assure that the number of populations was not underestimated. Population assignment tests for higher K values revealed that the majority of individuals were assigning strongly to only a portion of the population clusters, meaning some populations were not being represented by any individuals in the sample, so the number of K was lowered for final analysis. Final analysis included K=1-5, with 5 iterations for every value of K using the admixture and correlated allele models. The admixture model assumes that ancestry for an individual may be spread between various populations and the correlated model assumes allele frequencies are likely to be similar among populations. Our jackrabbit populations should be well represented by these models as they were likely more genetically connected before habitat fragmentation. We identified the most likely K using mean log likelihood (LnP(D)) as described in Pritchard et al. (2000) and $\Delta K$, which is a measure of the rate of change in (LnP(D)) between successive K values (Evanno et al. 2005).

We further assessed the number of genetically distinct populations with an added geographic component in program GENELAND 2.9.2 (Guillot 2008; Guillot et al. 2005a; Guillot et al. 2005b; Guillot et al. 2008). GENELAND uses a similar Bayesian clustering algorithm to STRUCTURE, but it has the added option to incorporate geographic locations of samples in the analysis, so that geographic proximity increases the likelihood that individuals will be assigned to the same population. In addition, GENELAND outputs a map outlining the boundaries of distinct genetic populations, which can be used to infer potential landscape features serving as barriers to gene flow. Initial analyses were undertaken in GENELAND with the correlated model (as defined for the STRUCTURE analysis), however, it overestimated K and created ghost populations to which no individuals were assigned. For

final analyses, we used the uncorrelated model with a burn-in of 10,000 steps followed by 100,000 MCMC iterations (with thinning = 100). We tested for K=1-5 and these runs were repeated 5 times, as in the STRUCTURE analysis. We also calculated F-statistics for the genetically distinct populations identified in these analyses.

We used three methods of population assignment to identify potential migrants in the populations identified by the clustering methods. First, individual posterior probabilities of assignment to each population were averaged across the 5 iterations in STRUCTURE and used to identify potential migrants among these clusters. We arbitrarily defined potential migrants and their offspring as individuals with assignment probabilities of <50% to the population from which they were collected. We used program Geneclass2 (Piry et al. 1999) to identify first generation migrants and compute the individual probabilities of residence to the population from which they were sampled. We used a Monte Carlo simulation described in Rannala and Mountain (1997) to create 10,000 individual genotypes randomized from our data. Posterior probabilities of observed genotypes were then used to assign migrant or resident status. Individuals whose genotypes had a probability $\alpha < 0.01$ of being encountered in the population of origin, based on the simulated data, were defined as migrants. Lastly, we quantified recent rates of migration between genetic populations and identified potential migrants using program BAYESASS 1.3 (Wilson and Rannala 2003). This program uses maximum likelihood theory to estimate migration rates, or the fraction of a population that is first or second generation migrants. We used 3,000,000 MCMC iterations, a burn-in of 1,500,000 steps, and a sampling frequency of 3000. BAYESASS does not assume symmetrical migration among populations or HWE, but does assume linkage equilibrium among the markers. Delta values were adjusted (delta p = 0.1, delta m =

0.05, delta F = 0.1) from default values to maximize Log likelihood values. Delta values represent the largest amount a respective parameter (p: allele frequencies; m: migration rates; F: inbreeding coefficient) can change in each iteration of the chain. Migrants were defined as individuals with assignment probabilities of <50% to the population from which they were collected as in the STRUCTURE method.

To examine finer-scale genetic structure in white-tailed jackrabbit populations, we tested for correlations between genetic and geographic distance in jackrabbits sampled, excluding central Iowa animals. The central Iowa population was excluded from these analyses as >80% of these samples were collected within 2 km from each other and all were at least 109 km from the nearest northwest Iowa jackrabbit. Under isolation by distance (IBD) theory, it is expected that as geographic distance between pairs of individuals increases so should genetic distance due to limited dispersal distances of individuals. We tested for IBD with Mantel tests in GenAlEx. Mantel tests are regressions of matrices, in our case matrices of genetic and geographic distance. The matrices are subjected to random permutations of the columns and rows and their correlation coefficients determined. The significance of the regression is assessed by the proportion of correlation coefficients in the permuted matrices that are higher than that of the observed matrices. If Mantel tests suggest a significantly positive correlation between genetic distance and geographic distance then IBD is supported.

We further assessed spatial autocorrelation, the extent of correlation between genetic and geographic distances across various distance classes, in all but central Iowa jackrabbits, using GenAlEx. We calculated estimates of ‘r’, a measure of genetic similarity among pairs of jackrabbits over distance classes of 10, 40, 70, 100, 150, 200, 300, 500, 700, 1000, and

1450 km. Geographic distance classes in all analyses were established to maximize geographic distances being examined and number of pair-wise comparisons being tested at each interval (Table 1). We tested for significance of observed r values with 1000 bootstraps to create 95% confidence intervals around the mean value of r and 1000 permutations to create 95% confidence intervals around the null hypothesis of r = 0. We also calculated estimates of Moran's I and Rousset's distance 'a' in program SPAGeDi 1.3 (Hardy and Vekemans 2002). Moran's I is a measure of correlation between genetic similarity and distance ranging from -1 to 1. Positive values indicate that genetic similarity between pairs of individuals is higher than expected if no correlation existed and negative values indicate that pairs of individuals are less genetically similar than expected if no correlation existed. Rousset's distance is a measure of genetic differentiation, or dissimilarity, between pairs of individuals. We ran 20,000 permutations over the same geographic distance classes given above. Observed values that fell outside the 95% confidence intervals of the permuted values were significantly correlated with geographic distance.

Jackrabbits were not sampled between central and northwest Iowa. Due to our opportunistic sampling scheme, it is unclear whether or how many jackrabbits may occur between the two regions. Taking into account this unknown and the recent decline in central Iowa jackrabbits (see Ch.2), we used program BOTTLENECK (Cornuet and Luikart 1996) to determine if the central Iowa population displayed a heterozygosity excess consistent with the occurrence of a population bottleneck. Heterozygosity excess is expected after a bottleneck event as the steep drop in numbers of individuals will eliminate some proportion of the rare alleles that had been present in the population (Cornuet and Luikart 1996). While the number of alleles decreases rapidly, the amount of heterozygosity in the population is

expected to decrease at a much slower rate. If a population has recently undergone a bottleneck event, it should exhibit greater heterozygotsity than expected based on the number of alleles present in the population. We used a two-phased model of mutation (TPM) as it has been shown to be the most appropriate model for microsatellite data (Dirienzo et al. 1994). The TPM is a combination of the infinite alleles model and the stepwise mutation model. The infinite alleles model assumes that each mutation produces a novel allele different from all existing alleles and the stepwise mutation model assumes that mutation occurs in one-step processes so that new alleles can only be either one step larger or smaller than an existing allele. We ran 1000 TPM iterations with 95% single-step mutations and a variance between 3-36% in 12 steps as recommended by Piry et al. (1999). Wilcoxon signed-rank tests were used to test for significance of heterozygosity excess at each locus. We also tested for a second indicator of a population bottleneck, a shift from an L-shaped distribution in allele frequencies. In a population at mutation-drift equilibrium, a large proportion of alleles should occur at low frequencies and the number should decrease sharply with increasing frequency providing a graphical L shape.

## RESULTS

We collected 28 white-tailed jackrabbit samples from central Iowa and 17 from northwest Iowa. There was a distinctive spatial gap (> 100 km) between regions sampled in Iowa (Fig.1B) that may be due to the absence of jackrabbits in this area or uneven effort or opportunity to collect road-killed animals in that area. We acquired 60 samples from South Dakota and 7 from Montana. There was also a distinctive spatial gap (434 km) between samples collected in South Dakota and Montana (Fig. 1A ) that is due to lack of sampling

effort in Montana. We eliminated 3 South Dakota samples from the study as 2 were believed to be black-tailed jackrabbits (based on mtDNA data, unpublished) and 1 did not amplify across sufficient loci. Due to the small number of samples obtained from Montana, we conducted all analyses both with and without those individuals. We were able to optimize 8 (Sol08, Sol28, Sol33, Sat2, Sat3, Sat12, Sat13, and Sat16) of the 11 microsatellites for amplification in white-tailed jackrabbits (Table 2). We eliminated the remaining 3 microsatellites (Sol03, Sol 30, and Sol44) from our study as we could not obtain reliable results from them.

Allelic drop out and stuttering were not detected in any of the 8 loci in any of the regions. However, we detected a signal of null alleles in Sat16 in the central Iowa, northwest Iowa and South Dakota regions and in Sol28 and Sat13 in only the South Dakota region. Heterozygote deficiencies were detected in a similar pattern: Sat16 in central Iowa, northwest Iowa and South Dakota, Sol28 in central Iowa and South Dakota, and Sat13 in only South Dakota (Table 3). Null alleles likely contributed to the observed heterozygote deficiencies across the regions. Heterozygote deficiencies were also detected in Sol28, Sat13 and Sat16 in the global population ($p < 0.0001$).

Diversity, both in terms of alleles and genotypes, was generally lower in central Iowa though the difference was not significant (Table 3). We found a significant probability of linkage between Sol28 and Sat3 after Bonferonni adjustment ($p < 0.001$) when regional populations were assumed, but this linkage was no longer significant ($p = 0.03$) when a global population was assumed and Bonferonni corrections were made. Further, Sol 28 and Sol08 were detected as significantly linked in the global population ($p < 0.0001$). However, as our samples do not appear to consist of a single global population (refer to Bayesian

clustering results) linkage disequilibrium between Sol28 and Sol08 is less likely. Because of the potential linkage disequilibrium between Sol28 and Sat3 when samples were analyzed at the regional level, we conducted analyses both with and without data from Sat3. Results were not significantly affected by this exclusion, so only analyses including Sat3 are presented here.

The four regions, from which samples were collected and hypothesized to be distinct populations, displayed significant global differentiation from one another (Table 4, $F_{ST}$ = 0.05, 95% CI 0.03-0.07). Central Iowa displayed significant differentiation, after Bonferonni correction, from northwest Iowa, South Dakota and Montana ($p < 0.01$ for each comparison) but none of the other regions displayed significant differentiation from each other. Central Iowa, however, did not have a significantly lower inbreeding coefficient ($F_{IS}$ = 0.07, 95% CI -0.05 - 0.20) than northwest Iowa ($F_{IS}$ = 0.08, 95% CI -0.03 - 0.21) or South Dakota ($F_{IS}$ = 0.17, 95% CI 0.03 - 0.31). As null alleles can lead to overestimation of $F_{ST}$, we re-calculated $F_{ST}$ values in the program FreeNA (Chapuis and Estoup 2007) using 10,000 replicates. This program uses the Dempster et al. (1977) method of estimating null allele frequencies to calculate $F_{ST}$ and has been shown to provide unbiased estimates of $F_{ST}$ with low variance (Chapuis and Estoup 2007). The Dempster et al. (1977) method of estimating null alleles uses a maximum likelihood EM algorithm to calculate estimates with incomplete data, in our case the estimates are of $F_{ST}$ and we have incomplete genotypes due to null alleles. FreeNA calculated slightly lower values of $F_{ST}$ between regions with slightly narrower confidence intervals (Table 4). However, the overall patterns remained the same such that significant differentiation was still detected between central Iowa and northwest Iowa, South Dakota, and Montana. Null alleles likely also resulted in an underestimation of our $F_{IS}$ values, but as

these were not significant, adjustment would only increase values and would not result in evidence of excessive inbreeding in any region.

Bayesian clustering analyses conducted in STRUCTURE and GENELAND suggested the jackrabbits sampled from the 4 regions actually constituted 2 genetically discrete populations (Fig. 2), in which central Iowa jackrabbits represented one distinct population and northwest Iowa, South Dakota, and Montana together represented a second population (henceforth referred to as NISD). We re-analyzed the data in both STRUCTURE and GENELAND, first without Montana individuals and second with only Iowa individuals. We removed the Montana samples due to low sample size and analyzed only Iowa samples to assess whether a signal of two populations would still be detected. In both cases, we verified that central Iowa was a genetically distinct population (Table 5).

We tested the discrete populations identified by STRUCTURE and GENELAND for deviations from HWE and the results were consistent with the global and regional tests. Heterozygote deficiencies were identified in Sol28 and Sat16 in both populations and also in Sat13 in the NISD population ($p < 0.001$). Linkage disequilibrium remained significant between Sol28 and Sat3 ($p < 0.001$). We also re-analyzed F statistics using the populations identified by STRUCTURE and GENELAND. Central Iowa showed significant differentiation ($F_{ST} = 0.065$, 95% CI 0.041-0.096, $p = 0.05$) from the NISD population. This value was similar to that calculated in FreeNA adjusted for null alleles ($F_{ST} = 0.060$, 95% CI 0.040-0.087). The central Iowa population did not have a significantly lower inbreeding coefficient ($F_{IS} = 0.065$, 95% CI -0.047-0.197) than the NISD population ($F_{IS} = 0.148$, 95% CI 0.025-0.294).

Individuals identified as potential migrants were not consistent across the three methods of identification (Table 6). We identified 3 potential migrants in the central Iowa population and 6 potential migrants in the NISD population based on assignment tests from STRUCTURE. In Geneclass2, we identified 3 first generation migrants one of which was also assigned as a non-resident. We identified 1 second generation migrant in BAYESASS. Only one jackrabbit, collected in central Iowa, was identified as a migrant in all three methods of population assignment, this individual was also assigned as a non-resident in Geneclass2. A migrant has a genotype with a low probability of occurrence within the population it was sampled, while a non-resident has a genotype with a low probability of having been in the population for more than one generation. The jackrabbit we classified as a central Iowa resident that was assigned as a NISD migrant across all 3 analyses had higher probabilities of migrant assignment than all but 2 individuals in the STRUCTURE method. Migration rates to and from the two populations calculated in BAYESASS were similar, with a rate of 0.038 (95% CI 0.003-0.098) from NISD into central Iowa and 0.011 (95% CI 0.0003-0.040) from central Iowa to NISD. These rates were within the 95% confidence interval for migration rates provided by BAYESASS for data on 2 populations that do not contain enough information to accurately calculate migration rates (0.008-0.325). The 95% CI for our jackrabbit data, however, are much narrower in width and so provide some information that migration rates are low but, given that they overlap with the 95% CI for uninformative data, should be interpreted cautiously.

For our analyses of isolation by distance in the NISD population, a group of 28 South Dakota samples were eliminated from the analyses due to lack of confidence in spatial locations of these animals. We did not find evidence to support an overall pattern of isolation

by distance in the NISD population. We found no significant correlation between genetic distance and linear (Fig 3A, $r < 0.01$, $p = 0.43$) or log transformed geographic distance (Fig. 3B, $r = 0.04$ $p = 0.11$). We detected significant positive spatial autocorrelation among jackrabbits separated by up to 10 km with measures of r (Fig. 4, $r = 0.05$, $p < 0.01$) and Moran's I (Fig.5, $I=0.08$, $p < 0.001$), which together suggest that jackrabbits within 10 km of each other were more genetically similar than expected and that genetic similarity is correlated to the distance separating them. Significant positive spatial autocorrelation among jackrabbits was also detected between 40 km and 70 km using the measure Rousset's a (Fig. 6, $a = 0.18$, $p = 0.02$), signifying jackrabbits separated by 40-70 km are more genetically differentiated than expected. Small sample sizes at some distance classes may have limited our ability to detect spatial autocorrelation at some scales. We did not have adequate samples to evaluate spatial autocorrelation at finer-scale distance classes. We were unable to evaluate whether significant spatial autocorrelation occurred at distances smaller than 10 km or at distances between 10-40 km and 40-70 km.

We found no evidence of a bottleneck having recently occurred in the central Iowa jackrabbit population. One-tailed P values for heterozygote excess across the 8 loci in the population ranged from 0.98 to 1.00. Alleles did not deviate from an L-shaped distribution, across all levels of variance, typical of a population in mutation-drift equilibrium. Together these results suggest that the Central Iowa population did not experience a recent population bottleneck.

# DISCUSSION

The white-tailed jackrabbit population in central Iowa is one of at least two populations still in existence in the state. These populations may be separated by up to 100 km of intervening corn and soybean fields. We had hypothesized that genetic diversity would be significantly lower in white-tailed jackrabbits in Iowa relative to South Dakota due to Iowa's fragmented landscape that is corn-dominated. We detected lower, though non-significant, levels of genetic diversity in the central Iowa jackrabbits than in the northern Iowa, South Dakota and Montana regions. However, we detected significant genetic differentiation between white-tailed jackrabbits in central Iowa and jackrabbits from northwest Iowa, South Dakota and Montana. These findings can potentially be explained by: 1) the spatial distance separating central Iowa from these other areas (100km), 2) barriers to gene flow limiting connectivity between central Iowa and these other areas (e.g., corn-based agriculture, highways), and/or 3) a population bottleneck having occurred in central Iowa.

We did not detect any evidence of a population bottleneck having recently occurred in the central Iowa population. Therefore the lower levels of genetic diversity and significant differentiation of central Iowa white-tailed jackrabbits from northwest Iowa, South Dakota and Montana are likely due to low levels of gene flow rather than a population bottleneck. "Recent" is often referred to as 2Ne-4Ne generations, where Ne is the effective population size. If the central Iowa population experienced a very recent bottleneck event, < 2Ne generations, the signal may not be easily detected (Piry et al. 1999). Estimates of population size, using spotlight-line-transect methods, have indicated potentially significant declines in the central Iowa population in recent years with 43.78 ± 13.37 jackrabbits estimated in 2006 and 18.46 ± 3.75 jackrabbits estimated in 2009 in an area of approximately 0.53 km² (see to

Ch. 2). This decline in population size may be too recent to detect a population bottleneck with current methods or may be due to a more gradual decline of individuals over time as opposed to the rapid loss of individuals that define population bottlenecks.

One potential explanation for the significant genetic differentiation observed between Central Iowa and NISD jackrabbit populations may be that gene flow between these populations may be limited by low dispersal/migration rates due to high mortality rates caused by attempted highway crossings (approximately 50% of Iowa samples collected were road-killed). The spatial gap between the central Iowa and NISD jackrabbit populations is intersected by 2 U.S. Highways (Fig. 1, HWY 30 and HWY 20), built over 70 years ago, that may also serve as barriers to gene flow. Highway 30 is a four lane highway with moderate traffic that runs east to west along the north end of the Research Farm. During telemetry studies (Ch.2), jackrabbits were never observed on the north side of the highway. Gene flow across highways has been documented in the pygmy rabbit, a much smaller (average weight 398-462 g) and likely less mobile Leporid (Estes-Zumpf et al. 2010). However, this species was documented crossing rural 2 lane highways characterized by low levels of traffic. Reduced movement and gene flow across larger, more heavily used roads has been documented in various other species: coyotes (*Canis latrans*) and lynx (*Lynx rufus*) (Riley et al. 2006); grizzly bears, *Ursus arctos* (Proctor et al. 2005); European frog, *Rana temporaria* (Reh and Seitz 1990); bank vole, *Myodes glareolus* (Gerlach and Musolf 2000). In our study, the only individual identified as a migrant by all 3 assignment methods was a road-killed jackrabbit collected on the north side of Highway 30 (Fig. 1B) that we labeled as a central Iowa jackrabbit, but that was identified as an NISD jackrabbit. An explicit

examination of the effects of highways on movement among white-tailed jackrabbit populations remains to be explored.

As our opportunistic sampling approach failed to garner samples between northwest and central Iowa, the central Iowa white-tailed jackrabbit population may be physically separated from the NISD population by about 100 km. Although it is unclear from this study whether remnant white-tailed jackrabbit populations occur in these intervening areas, the bulk of this landscape consists of vast fields of corn. The potential low permeability of this corn landscape may be responsible for reduced gene flow as has been demonstrated in pygmy rabbit (*Brachylagus idahoensis)* populations separated by agricultural fields (Estes-Zumpf et al. 2010). Using both microsatellite and mtDNA data, greater levels of population differentiation were detected between populations of pygmy rabbits separated by increasing areas of agricultural fields than by highways or creeks. It was unclear to what extent these fields were permeable to pygmy rabbits but it was suggested that agriculture might intensify isolation among these populations. European hares have also been shown to alter movement patterns in response to agricultural practices by shifting cores of home ranges following harvest and shifting field types used on a seasonal basis (Chapuis 1990; Marboutin and Aebischer 1996; Reitz and Leoanrd 1994; Tapper and Barnes 1986). Radio-telemetry data suggest white-tailed jackrabbits do not use corn fields in high frequencies, at least before harvest when the corn is tall (Ch. 2 of this study). Given the avoidance of cornfields by jackrabbits, at least for part of the year, genetic differentiation between the remnant central Iowa population of white-tailed jackrabbits and the more continuously distributed animals in northwest Iowa, South Dakota and Montana may be due to expansive corn fields acting as a barrier to gene flow. However, little is known about white-tailed jackrabbit dispersal,

whether timing of dispersal is related to corn harvest, and how these factors may affect gene flow.

Central Iowa jackrabbits currently occur at the eastern periphery of the species range. White-tailed jackrabbits have been extirpated from Missouri and Illinois and are listed as a species of greatest conservation need in Wisconsin and Iowa. As dispersal distances of this species are unknown, it is unclear whether the >100-km distance separating the central Iowa and NISD populations would prevent gene flow between these populations even if corn fields were suitable jackrabbit habitat and highways were not an issue. This possibility is perhaps supported by our detection of significant negative spatial autocorrelation between pairs of jackrabbits separated by 70 km in the NISD population. Low gene flow between central Iowa and NISD jackrabbits may be mitigated by intervening patches of suitable habitat that may serve as stepping stones between the populations, but further investigation into the existence of such patches and jackrabbits populations in intervening areas is necessary.

Our results suggested that recent migration rates were low between central Iowa and NISD jackrabbit populations. Across three methods of population assignments, only 1 individual was consistently identified as a migrant, lending support to our conclusion that contemporary migration appears to be limited between these populations. As the majority of central Iowa samples were collected within 2 km of each other and sample size was small, the samples may not be fully representative of the population, reducing reliability of estimates. However, based on population size estimated over 2006-2009 on the Research Farm in central Iowa (see Ch. 2), we feel confident that we sampled a large and likely representative proportion of the population.

The NISD jackrabbit population displayed weak spatial structure and we did not find evidence of a strong pattern of isolation by distance. Spatial autocorrelation analyses suggested jackrabbits within 10 km were significantly more genetically similar than expected and could not be considered genetically independent. Various Leporid species have been demonstrated to be capable of dispersing 10 km or more. Pygmy rabbits can disperse up to 10-12 km (Estes-Zumpf and Rachlow 2009; Sanchez and Rachlow 2008). European hares have been documented dispersing as far as 17 km (Bray et al. 2007) and snowshoe hares, *Lepus americanus*, as far as 16 km (Gillis and Krebs 1999). A black-tailed jackrabbit, *Lepus californicus*, was recorded covering a distance of 45 km in approximately 4 months (French et al. 1965). Fine-scale spatial structure could not be assessed within 10 km as opportunistic sampling limited the number of animals collected at distance < 10 km. Therefore, we cannot evaluate whether significant positive spatial autocorrelation existed among jackrabbits separated by distances less than 10 km. Further sampling of jackrabbits, at smaller spatial scales (between 0 and 40 km) could provide a more accurate estimate of the scale of spatial autocorrelation within the NISD white-tailed jackrabbit population. This information could give us a better understanding of white-tailed jackrabbit dispersal distance limitations and whether the 100 km that may separate the central and northwest Iowa jackrabbits is too far for migration to connect these populations.

Connectivity of the central Iowa white-tailed jackrabbit population to the NISD population evaluated in our study appears to be limited. Agricultural fields, e.g. corn fields, highways and distance between populations may all play a role in our observations that central Iowa white-tailed jackrabbits are significantly genetically differentiated from animals in northwest Iowa, South Dakota and Montana, where the species is more continuously

distributed. Research into white-tailed jackrabbit dispersal patterns is needed to assess whether they are able to disperse over the distance and through the landscape features separating central Iowa and northwest Iowa, whether and where jackrabbits occur between these two regions, and whether potential suitable habitat patches between these areas exist. The avoidance of unharvested corn fields by jackrabbits has been documented (Ch.2), but the extent to which the agricultural expanse that separates these populations is permeable to white-tailed jackrabbits and whether that permeability varies after harvest of the fields alters structure of the landscape is unknown. With the expected increase in acres planted to corn across the Corn Belt (Heisey 2009), it is essential to identify the degree to which corn itself acts as a barrier to gene flow in white-tailed jackrabbit, and other Iowa wildlife, populations. To answer some of these unknowns, further genetic sampling of white-tailed jackrabbits in central Iowa and northwest Iowa should be conducted with special consideration to the geographic distance and the size and type of landscape features separating individuals.

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A

B

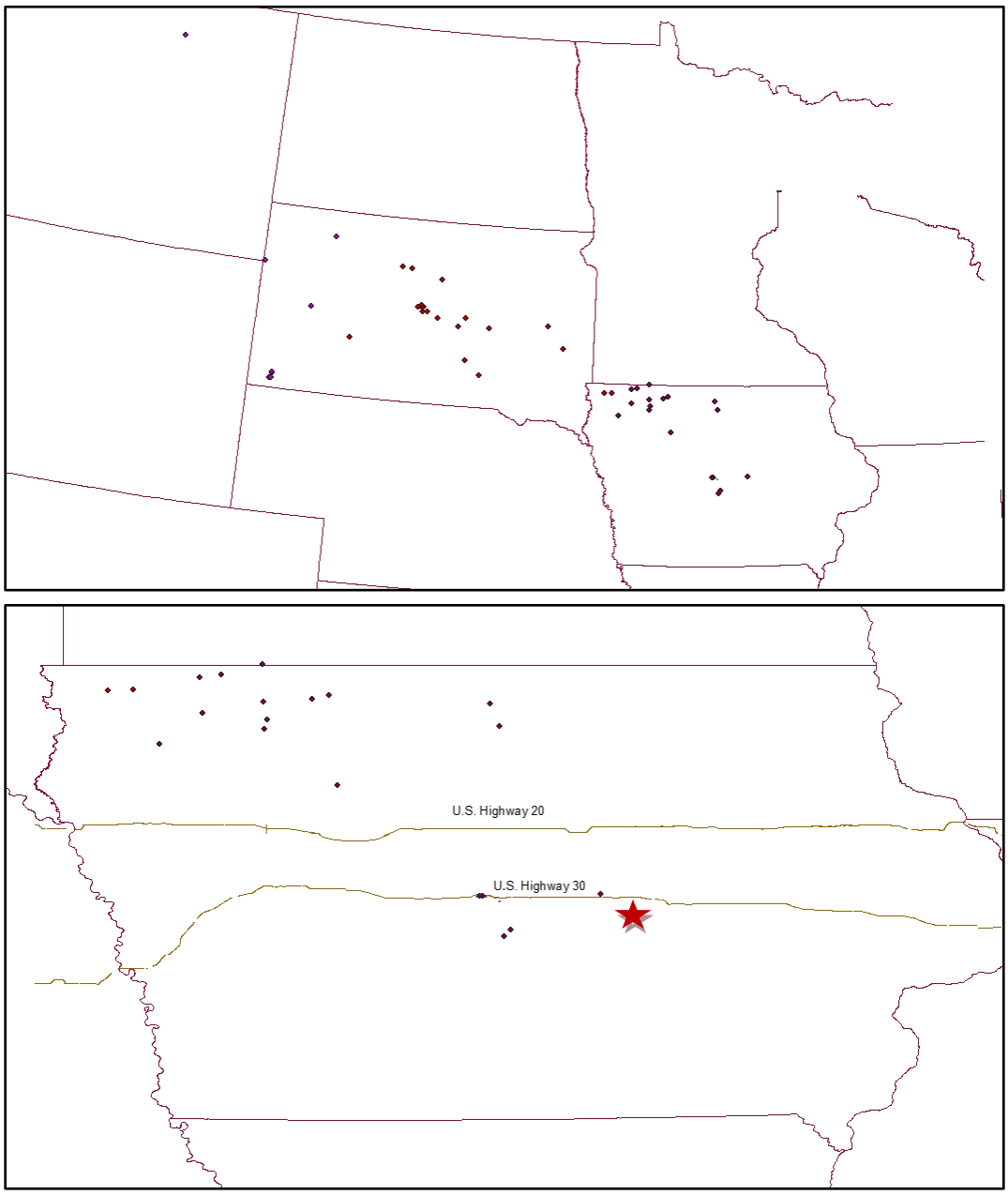



Figure 1. Samples collected from A) across the states of Iowa, South Dakota and Montana are represented with dots. Individual dots may represent more than one jackrabbit sampled, n=28, 18, 57, and 7 in central Iowa, northwest Iowa, South Dakota and Montana, respectively. B) Two major U.S. Highways intersect the spatial gap between central Iowa and northwest Iowa white-tailed jackrabbit populations. The red star indicates the location of sample collection from a jackrabbit identified as a potential migrant from the NISD population to central Iowa across 3 methods of analysis.

Table 1. The number of pair-wise comparisons analyzed within each distance class in the isolation by distance and spatial autocorrelation analyses. A group of 28 samples were removed from the analyses due to lack of confidence in spatial data.

| Distance Class | 10 | 40 | 70 | 100 | 150 | 200 | 300 | 500 | 700 | 1000 | 1450 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| # of pair-wise comparisons | 67 | 57 | 73 | 47 | 58 | 53 | 76 | 280 | 243 | 267 | 157 |

Table 2. Final polymerase chain reaction protocols are given. Reagent volumes (uL) are given per sample and total reaction volumes were 10 uL, except for Sol 30 that was run with a total reaction volume of 13 uL. Primer volumes are given for forward and reverse primers each. All PCRs were run for a total of 30 cycles, preceded by a 3 min step at the denaturation temperature, and 3 min extensions were added as shown below.

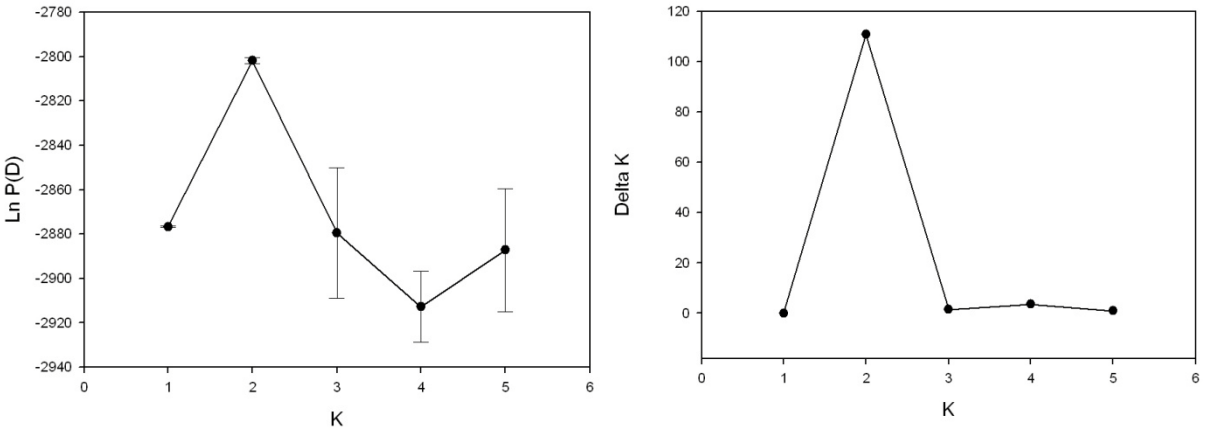
| Locus | Buffer (10 uM) | dNTPs (2mM) | $MgCl_2$ (25 mM) | Primers (10 uM) | Taq (5 units/uL) | $H_2O$ | DNA (20 ng/ul) | Cycle |
|---|---|---|---|---|---|---|---|---|
| Sol08 | 1.1 | 1.1 | 0.5 | 0.075 | 0.2 | 4.95 | 2 | (94℃-30 s/56℃-30 s/72℃-30 s) (72˚C-3 min) |
| Sol28 | 2.0 | 0.75 | --- | 0.2 | 0.2 | 4.15 | 2.5 | (95℃-30 s/58℃-30 s/72℃-30 s) |
| Sol30 | 1.1 | 0.9 | --- | 0.2 | 0.2 | 4.4 | 3 | (95℃-30 s/58℃-30 s/72℃-30 s) |
| Sat2 | 1.1 | 0.9 | --- | 0.1 | 0.2 | 5.6 | 2 | (94℃-30 s/52℃-30 s/72℃-30 s) (72˚C-3 min) |
| Sat3 | 1.1 | 0.9 | 0.2 | 0.15 | 0.2 | 4.8 | 2.5 | (94℃-30 s/70↓60℃-30 s/72℃-30 s)*10, ( 94℃-30 s/60 ℃-30 s/72 ℃-30 s)*20 |
| Sat12 | 1.1 | 0.9 | 0.5 | 0.075 | 0.2 | 4.15 | 3 | (94℃-30 s/50℃-30 s/72℃-30 s) |
| Sat13 | 1.1 | 0.7 | --- | 0.1 | 0.2 | 5.3 | 2.5 | (94℃-30 s/56℃-30 s/72℃-30 s) (72˚C-3 min) |
| Sat16 | 2.0 | 0.9 | 0.2 | 0.075 | 0.2 | 4.05 | 2.5 | (94℃-30 s/54℃-30 s/72℃-30 s) |

Table 3. Measures of genetic diversity across the four regions sampled. Totals for $N_A$ display the sum of distinct alleles across loci with number of private alleles in parentheses. Total observed ($H_O$) and expected ($H_E$) heterozygosities are given for respective regions with SD. Allelic richness ($A_R$) and p-values for HWE tests are also displayed. All loci were within HWE in the Montana region, although this may be due to small sample size. * denotes a significant deviation from HWE after Bonferonni correction with $p<0.001$.

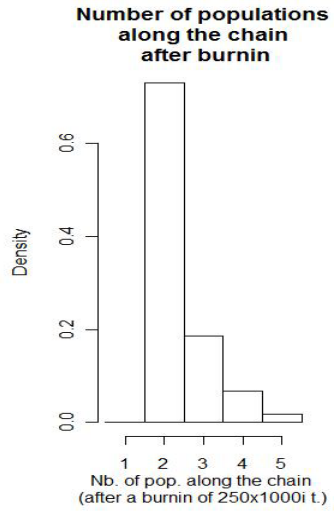
| | Central Iowa (n=28) | | | | | Northwest Iowa (n=18) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | NA | AR | HO | HE | HWE | NA | AR | HO | HE | HWE |
| sol08 | 6 | 4.26 | 0.82 | 0.73 | 0.89 | 5 | 4.17 | 0.61 | 0.74 | 0.03 |
| sol28 | 8 | 4.55 | 0.64 | 0.69 | * | 7 | 5.17 | 0.65 | 0.69 | 0.32 |
| sol30 | 3 | 2.17 | 0.21 | 0.20 | 1.00 | 4 | 2.90 | 0.39 | 0.34 | 1.00 |
| sat02 | 9 | 6.02 | 0.79 | 0.83 | 0.29 | 11 | 7.76 | 0.89 | 0.89 | 0.65 |
| sat03 | 2 | 1.92 | 0.29 | 0.25 | 1.00 | 2 | 2.00 | 0.56 | 0.49 | 0.87 |
| sat12 | 9 | 6.15 | 0.82 | 0.82 | 0.51 | 8 | 5.57 | 0.83 | 0.81 | 0.73 |
| sat13 | 3 | 2.40 | 0.29 | 0.36 | 0.01 | 5 | 4.26 | 0.56 | 0.63 | 0.35 |
| sat16 | 7 | 5.03 | 0.43 | 0.71 | * | 8 | 6.10 | 0.50 | 0.82 | * |
| Total | 47 (2) | 4.06 ± 1.70 | 0.54 ± 0.09 | 0.57 ± 0.09 | | 50 (1) | 4.7 ± 1.82 | 0.62 ± 0.06 | 0.68 ± 0.07 | |
| | South Dakota (n=57) | | | | | Montana (n=7) | | | | |
| | NA | AR | HO | HE | HWE | NA | AR | HO | HE | HWE |
| sol08 | 13 | 6.42 | 0.79 | 0.84 | 0.09 | 5 | 5.00 | 0.86 | 0.78 | 0.88 |
| sol28 | 14 | 6.63 | 0.57 | 0.77 | * | 6 | 6.00 | 0.71 | 0.75 | 0.61 |
| sol30 | 5 | 2.76 | 0.44 | 0.42 | 0.77 | 3 | 3.00 | 0.57 | 0.58 | 0.67 |
| sat02 | 12 | 7.29 | 0.82 | 0.88 | 0.25 | 9 | 9.00 | 0.71 | 0.92 | 0.09 |
| sat03 | 3 | 2.12 | 0.47 | 0.48 | 0.48 | 2 | 2.00 | 0.29 | 0.53 | 0.29 |
| sat12 | 8 | 6.29 | 0.88 | 0.86 | 0.73 | 6 | 6.00 | 0.86 | 0.88 | 0.58 |
| sat13 | 6 | 3.79 | 0.46 | 0.63 | * | 3 | 3.00 | 0.43 | 0.54 | 0.44 |
| sat16 | 10 | 6.10 | 0.35 | 0.84 | * | 5 | 5.00 | 0.43 | 0.66 | 0.12 |
| Total | 71 (16) | 5.17 ± 1.97 | 0.60 ± 0.07 | 0.72 ± 0.06 | | 39 (2) | 4.88 ±2.23 | 0.61 ± 0.08 | 0.71 ± 0.05 | |

Table 4. Pairwise Fst values across the four regions sampled. *denote statistical significance after Bonferroni corrections. Fst values above the diagonal were calculated in FreeNA, adjusting for null alleles and values below the diagonal were calculated in Fstat with 95% CI in parentheses.

| | Central Iowa | Northwest Iowa | South Dakota | Montana |
|---|---|---|---|---|
| Central Iowa | 0 | * 0.058 (0.024-0.098) | * 0.064 (0.045-0.089) | * 0.092 (0.045-0.159) |
| Northwest Iowa | * 0.066 (0.028-0.108) | 0 | 0.006 (-0.002-0.014) | 0.020 (-0.005-0.049) |
| South Dakota | * 0.069 (0.47-0.100) | 0.006 (-0.003-0.014) | 0 | 0.016 (-0.006-0.044) |
| Montana | * 0.097 (0.042-0.171) | 0.018 (-0.007-0.045) | 0.011(-0.014-0.040) | 0 |




A B




C

Figure 2. The A) LN P(D) and B) ΔK values across K = 1-5 depicting optimal K=2 in STRUCTURE. C) The density of MCMC iterations that produce K=1-5. This image was produced by Geneland. Density refers to the proportion of the 100,000 iterations. The 3 figures suggest our data represent 2 genetic populations.

Table 5. The mean LN P(D) values for K = 1-5 when analyses were conducted with all samples across the four regions sampled, without Montana samples, and with only Iowa samples in STRUCTURE.

| K | No Montana | Only Iowa | All Samples |
|---|---|---|---|
| 1 | -2627.87 | -1058.15 | -2876.7 |
| 2 | -2586.87 | -1051.12 | -2801.9 |
| 3 | -2611.80 | -1145.26 | -2879.52 |
| 4 | -2618.63 | -1252.02 | -2912.78 |
| 5 | -2784.45 | -1202.70 | -2887.27 |

Table 6. Potential migrants using the 3 methods of identification are shown. For the STRUCTURE method, posterior probabilities of assignment to the central Iowa and NISD populations are shown. For the Geneclass2 method, the p-values of individuals being assigned as migrants (P migrant) and p-values of individuals being assigned as non- residents (P resident) are shown. For this analysis, individuals 19 and 23 were significantly assigned as migrants, but also had high probabilities of being residents. For the BAYESASS method, posterior probabilities of assignment as residents, first generation migrants (1st gen) and second generation migrants (2nd gen) are shown. Jackrabbit 7 was the only animal consistently identified as a potential migrant.

| ID | Sampling Region | Structure | | Geneclass2 | | Bayesass | | |
|---|---|---|---|---|---|---|---|---|
| | | Central IA | NISD | P migrant | P resident | Resident | 1st gen | 2nd gen |
| 7 | Central Iowa | 0.19 | 0.81 | <0.001 | 0.001 | 11.21 | 32.73 | 56.06 |
| 1 | Central Iowa | 0.41 | 0.59 | | | | | |
| 4 | Northwest Iowa | 0.90 | 0.097 | | | | | |
| 5 | Northwest Iowa | 0.66 | 0.34 | | | | | |
| 19 | Northwest Iowa | | | <0.01 | 0.36 | | | |
| 21 | South Dakota | 0.67 | 0.33 | | | | | |
| 23 | South Dakota | | | 0.02 | 0.78 | | | |
| 30 | Northwest Iowa | 0.86 | 0.14 | | | | | |
| 32 | Central Iowa | 0.49 | 0.51 | | | | | |
| 64 | South Dakota | 0.57 | 0.43 | | | | | |
| 83 | South Dakota | 0.83 | 0.17 | | | | | |

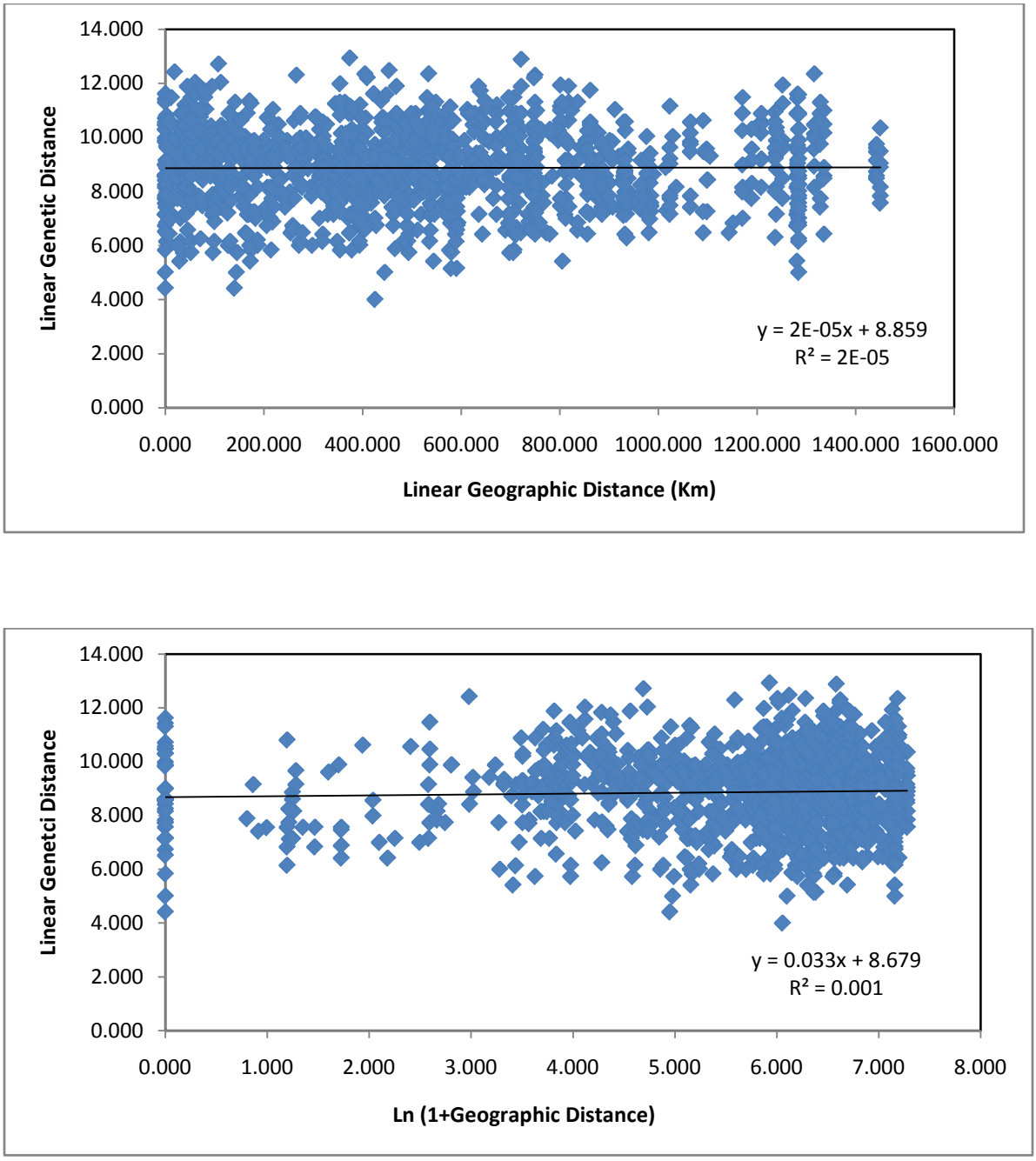



Figure 3. Genetic distance is plotted over A)linear geographic distance and B) Natural log (LN) transformed geographic distance without GSD samples. Equations for trendlines and $R^2$ values resulting from Mantel tests are shown.

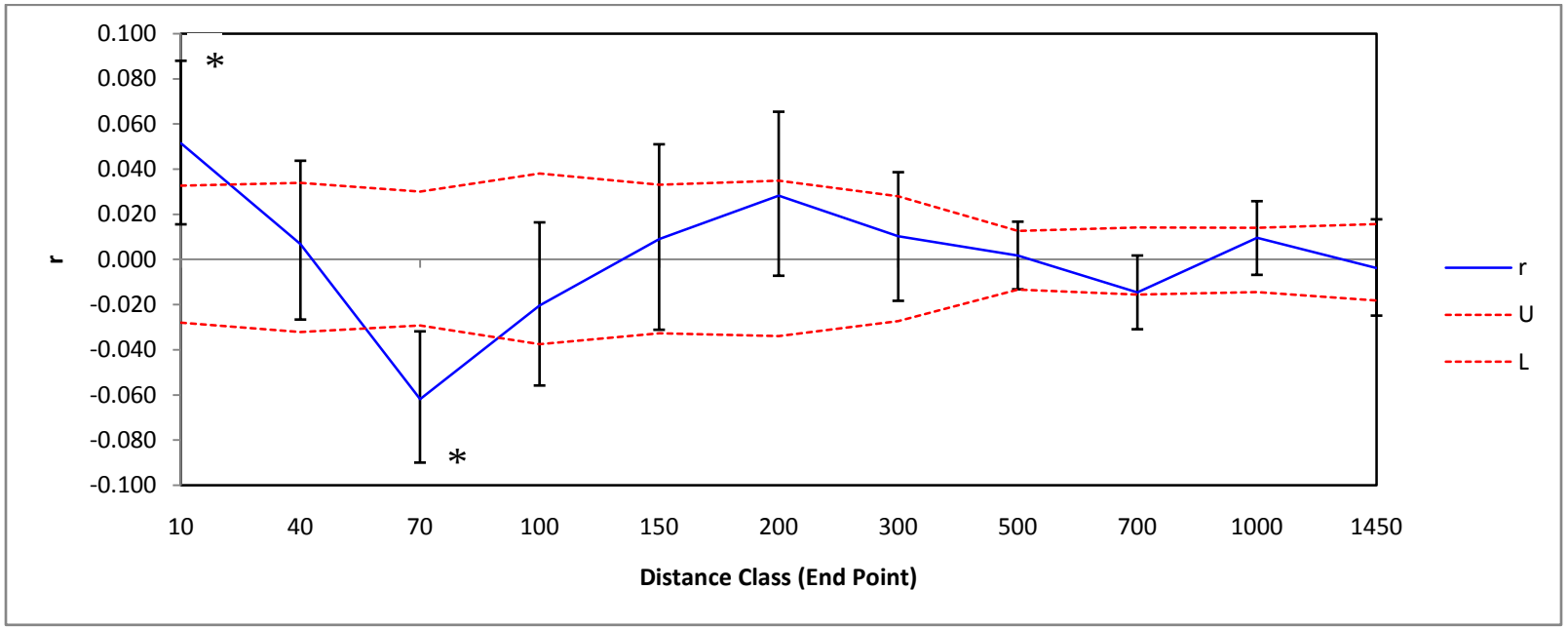



Figure 4. Spatial autocorrelation among white-tailed jackrabbits at discrete distance classses with 95% confidence intervals shown in error bars and upper (U) and lower (L) 95% confidence intervals around the null hypothesis of r = 0 in dotted lines. Significant values are shown with * at 10 km and 70 km.

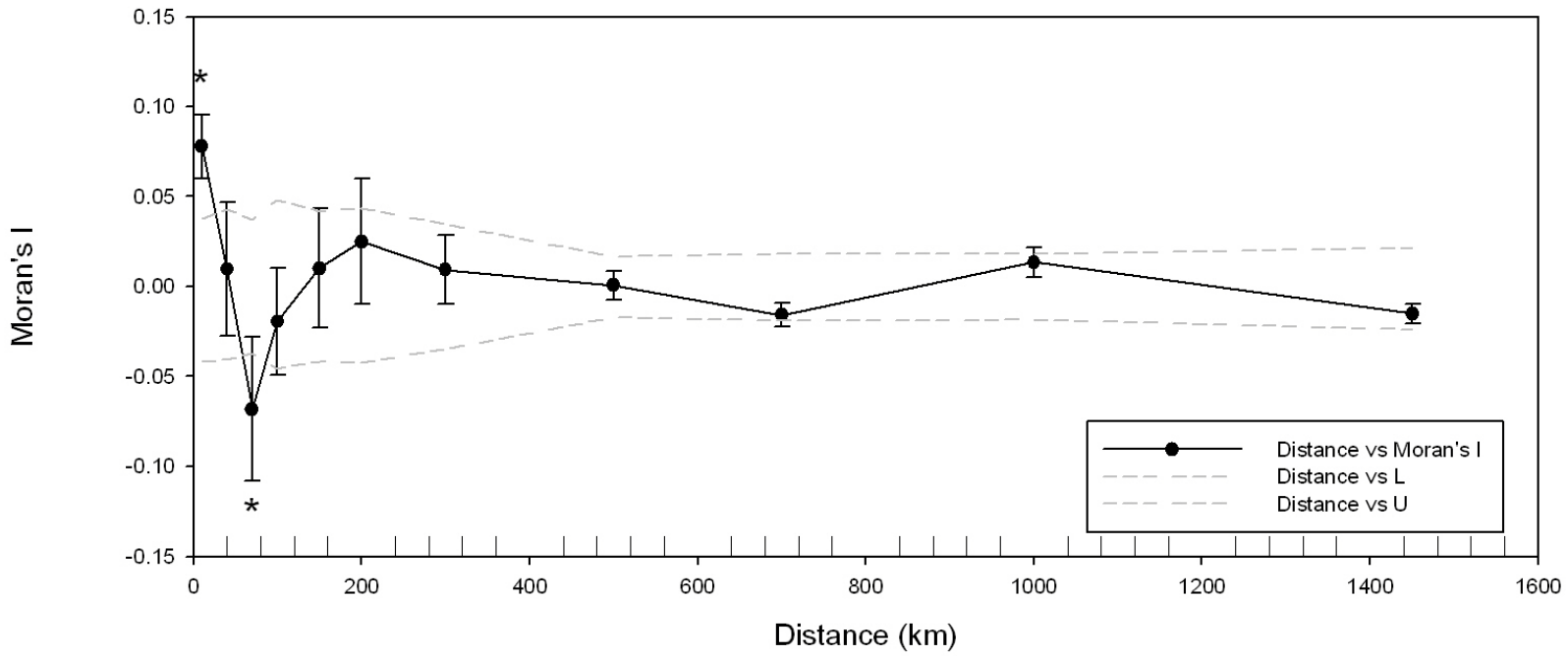



Figure 5. Spatial autocorrelation using Moran's I among white-tailed jackrabbits grouped into discrete distance classes with 95% confidence intervals shown in error bars and upper (U) and lower (L) 95% confidence intervals around the null hypothesis of no relationship between Moran's I and distance based on 20,000 distance permutations. Significant values are shown with * at 10 km and 70 km.

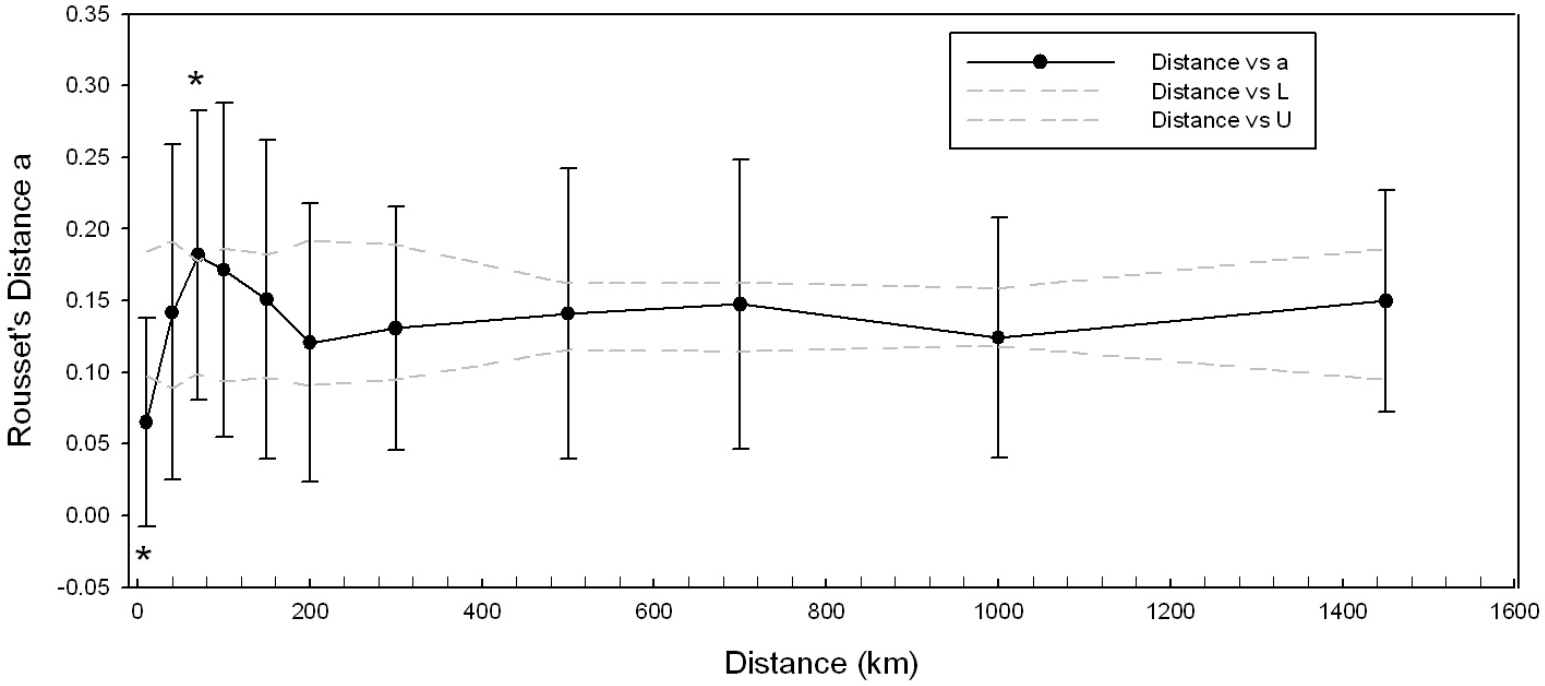



Figure 6. Rousset's Distance 'a' among white-tailed jackrabbits grouped into discrete distance classes with 95% confidence intervals shown in error bars and upper (U) and lower (L) 95% confidence intervals of the null hypothesis of no relationship between a and distance based on 20,000 distance permutations. Significant values are shown with * at 10 km and 70 km.

# CHAPTER 4: GENERAL CONCLUSIONS

White-tailed jackrabbit populations persist in central Iowa and northwest Iowa but these regions are separated by expansive corn fields, highways, and perhaps a distance of over 100 km. My data suggest corn fields are unsuitable habitat for jackrabbits, although there is a temporal aspect in relation to corn harvest. Further, my data suggest that central Iowa jackrabbits constitute a distinct population, which is declining. Agricultural intensification appears to have detrimental effects on central Iowa's white-tailed jackrabbit population by limiting suitable habitat and potentially increasing genetic isolation.

Jackrabbits increased their use of corn fields following harvest in the fall and subsequently decreased use when corn shoots began to gain height in summer the following year. They selected against corn fields in all seasons but the breeding season. Although corn fields appear to be unsuitable jackrabbit habitat, habitat suitability is not as dichotomous as once thought. While habitat was once classified as either strictly suitable or strictly unsuitable (i.e. matrix), more current research is focusing on suitability as a continuum. This range of suitability can vary with landscape composition, including vegetation cover, topography, and water sources and species ecology including habitat requirements and species size (Fahrig and Merriam 1994; Rosenberg et al. 1997; Taylor et al. 1993). Landscape composition can alter the permeability, or ease by which an animal can move through a habitat. Further, the permeability of the "matrix" habitat can vary temporally and this variation may mitigate gene flow between otherwise fragmented populations (Blaum and Wichmann 2007). As agricultural landscapes change on a seasonal basis, movement of jackrabbits through fields may vary seasonally as well, perhaps allowing migration between populations in one season that would be otherwise disconnected in other seasons. Continued

research is needed to establish to what extent corn fields are permeable to white-tailed jackrabbits, whether permeability differs among seasons, and how permeability of corn fields affects the genetic connectivity between these populations.

Using Bayesian clustering methods, we detected two distinct genetic populations of white-tailed jackrabbits among our samples. Central Iowa jackrabbits were identified as one population and northwest Iowa, South Dakota and Montana jackrabbits were identified as belonging to the second population (NISD). The significant differentiation between the two jackrabbit populations may be linked to the potential barriers of expansive corn fields, highways and distance dividing them. However, genetic diversity levels were not significantly different between central Iowa and the NISD population. The lack of significance in genetic diversity may be due to either recent differentiation of the two populations or some level of gene flow maintaining genetic connectivity. Our estimates of migration suggested low levels of successful dispersal occur between these populations. These low migration rates may be explained if corn fields are the main driver in the differentiation of these populations and their permeability increases only with harvest. Harvest reduces corn fields to stubble for approximately 6 months of the year (November-April). Successful migration between populations of jackrabbits during this time may be limited by severe weather as well as physical barriers. Radio-telemetry studies and genetic studies similar to this one with increased sample sizes may give us more insight into the dispersal patterns of this species, decipher whether migration between the two populations is substantial enough to prevent complete isolation of the central Iowa population, and whether corn fields, highways, distance or some combination thereof are acting as significant barriers to gene flow.

We detected a significant decline in size of the white-tailed jackrabbit population on the ISU Research Farm, in central Iowa, over the last four years (2006-2009). Genetic drift and inbreeding are associated with small populations, but these effects may be mitigated by the occasional exchange of individuals with other populations that serve to increase a remnant population's genetic diversity (Hanski and Gaggiotti 2004). However, migration rates between the NISD and central Iowa jackrabbit populations and migration rates appear to be low. If no other populations exist within a white-tailed jackrabbit's maximum dispersal distance, or if no patches of suitable intervening habitat exist through which white-tailed jackrabbits can move between populations, central Iowa white-tailed jackrabbits will be increasingly subject to genetic drift and inbreeding, increasing vulnerability to local extirpation of the population. Again, further sampling is needed to more adequately estimate migration rates between Iowa jackrabbit populations and indentify whether other populations or pockets of jackrabbits exist that were not sampled. The central Iowa population should continue to be monitored in the future to detect any further declines in size. Population size estimates should also be calculated in northwest Iowa to assess whether similar trends of decline are apparent.

Research into white-tailed jackrabbit reproductive biology should also be investigated as it influences population growth. White-tailed jackrabbits may have up to 4 litters in a year (James and Seabloom 1969) but it is unknown how many juvenile jackrabbits survive to reproduce, how many litters they produce each year in Iowa, or their litter size. The start of the breeding season has not been specifically examined in the state. The breeding season can begin as early as February but is likely affected by environmental factors such as timing of snow melt and forage availability (James and Seabloom 1969; Rogowitz 1992). The length

of winters can vary in Iowa and may play a role in the number of litters jackrabbits can produce. Litter size and leveret survival may also be affected by forage availability and quality. Information on white-tailed jackrabbit reproductive biology along with adult survival rates can be used to develop population models.

We have shown that white-tailed jackrabbit movement patterns are affected by agricultural practices. Migration rates between the two identified genetic populations, central Iowa and NISD, are low and agricultural fields are a potential barrier to gene flow between these populations. Anthropogenic alterations of the landscape in Iowa, and other midwestern states, are forecasted to increase by 2016 (Heisey 2009) and many more questions regarding the response of white-tailed jackrabbits to these changes have been raised by my research findings. The extent to which anthropogenic landscape changes may also alter other aspects of ecology, such as reproductive behavior and survival, in jackrabbits and other grassland-adapted wildlife species has yet to be determined.

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# APPENDIX 1

| ID | Location | UTM Zone | UTM Northing | UTM Easting | Latitude | Longitude |
|---|---|---|---|---|---|---|
| 1 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 2 | Lake County, SD  T105N, R51W, S17 | 14N | 4875812 | 650678 | 44.01998558 | -97.11999581 |
| 3 | Hancock County, Garfield Township, T96N,R24W,S26 | 15N | 4772588 | 447524 | 43.10420036 | -93.64490124 |
| 4 | Hancock County,Madison Township, T97N, R24W,S5 | 15N | 4788684 | 442730 | 43.24878358 | -93.70547922 |
| 5 | Dickinson County, T100N, R37W, S11, HWY 86 | 15N | 4819284 | 324401 | 43.50583696 | -95.17224533 |
| 6 | Osceola County, T100N, R39W, S34, 1/2 mile S of Harris | 15N | 4812415 | 302969 | 43.43868825 | -95.43466334 |
| 7 | Marshall County | 15N | 4653002 | 500831 | 42.02905297 | -92.98996134 |
| 8 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 10 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 11 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 12 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 13 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 14 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 15 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 17 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 18 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 19 | Clay County | 15N | 4773175 | 324182 | 43.09088951 | -95.1602076 |
| 20 | Boone | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 21 | Dewey County, SD, 2 miles E of La Plant | 14N | 4999981 | 369793 | 45.14129029 | -100.6561287 |
| 22 | Dewey County, SD, 1 mile W of Whitlock | 14N | 5002444 | 352240 | 45.15999022 | -100.8800041 |
| 23 | Prairie City, SD | 13N | 4983437 | 578815 | 45.00000123 | -104.0000038 |
| 24 | Perkins County, SD, 5 miles W of Bison | 13N | 5044280 | 697995 | 45.52387654 | -102.4646349 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 25 | Potter County, SD, 1/4 mile W of Gettysburg | 14N | 4984721 | 424725 | 45.01194266 | -99.95527953 |
| 26 | O'brien County, Calodonia, Section 5 | 15N | 4763587 | 269180 | 42.98987265 | -95.83131214 |
| 27 | O'brien County, Section 27, Lincoln Township, SW 1/4 | 15N | 4785263 | 292501 | 43.19160694 | -95.55363543 |
| 28 | Dickinson County, Okoboji Township, T98, R37, S36 | 15N | 4792411 | 324640 | 43.26408907 | -95.1606816 |
| 29 | Spencer Airport | 15N | 4779586 | 325898 | 43.14897625 | -95.14114987 |
| 30 | Pocahontas County | 15N | 4732161 | 362065 | 42.729562 | -94.68489051 |
| 31 | Emmet County, sec 27, R-34W T-98N | 15N | 4793627 | 350141 | 43.28054495 | -94.84697824 |
| 32 | Polk Co Ankeny TRS81-23-30 SW | 15N | 4627584 | 452674 | 41.79870602 | -93.56965557 |
| 33 | Polk County, Crocker Township, Sec 11, T-80N R-24W | 15N | 4622558 | 449127 | 41.75321965 | -93.61191754 |
| 39 | Spencer Airport | 15N | 4779586 | 325898 | 43.14897625 | -95.14114987 |
| 40 | Spencer Airport | 15N | 4779586 | 325898 | 43.14897625 | -95.14114987 |
| 41 | Montana | 13N | 5357645 | 377037 | 48.35999015 | -106.6600055 |
| 42 | Montana | 13N | 5357645 | 377037 | 48.35999015 | -106.6600055 |
| 43 | Montana | 13N | 5357645 | 377037 | 48.35999015 | -106.6600055 |
| 44 | Montana | 13N | 5357645 | 377037 | 48.35999015 | -106.6600055 |
| 45 | Montana | 13N | 5357645 | 377037 | 48.35999015 | -106.6600055 |
| 46 | Montana | 13N | 5357645 | 377037 | 48.35999015 | -106.6600055 |
| 47 | Montana | 13N | 5357645 | 377037 | 48.35999015 | -106.6600055 |
| 48 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 49 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 50 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 52 | central SD | 14N | 4915667 | 421149 | 44.38999613 | -99.9900038 |
| 53 | Eastern SD | 14N | 4914078 | 620308 | 44.36999545 | -97.49000451 |
| 55 | Eastern SD | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 56 | Eastern SD | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 59 | Eastern, SD | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 60 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 61 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 62 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 63 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 64 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
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| 67 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 68 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
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| 71 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
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| 74 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 75 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 76 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 77 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 78 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 79 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 80 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 81 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 82 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 83 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 85 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |
| 86 | Brule City, SD N 43. 74525, W 99.30231 | 14N | 4843623 | 475659 | 43.7452468 | -99.30231116 |

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|---|---|---|---|---|---|---|
| 87 | Brule County, SD, N 43.52055, W 98.98059 | 14N | 4818624 | 501569 | 43.5205517 | -98.98058574 |
| 88 | Sully/HughesCounty Line, SD, 196 St., N 44. 54801, W 100.32361 | 14N | 4933594 | 394862 | 44.54800696 | -100.3236126 |
| 89 | Hughes County, SD, 201 St and 291 Ave, N 44.47587, W 100. 33637 | 14N | 4925597 | 393718 | 44.47586411 | -100.3363648 |
| 90 | Hughes County, SD, 201 St and 296 Ave, N 44.47305, W 100. 23024 | 14N | 4925152 | 402153 | 44.47305119 | -100.2302463 |
| 91 | Hughes County,SD,Greg Goose Rd. and 197 St., N 44. 52850, W100.35057 | 14N | 4931462 | 392685 | 44.52849754 | -100.3505683 |
| 92 | Sully County, SD, 194th St and 288 Ave., N 44. 57355, W 100.36449 | 14N | 4936484 | 391662 | 44.57354288 | -100.364495 |
| 93 | CharlesMit County,SD T99N, R66W, S10, SW 1/4 | 14N | 4915667 | 421149 | 44.38999613 | -99.9900038 |
| 94 | Hughes County, SD W1/2 Sec 24, T 111N, R 74 W | 14N | 4915667 | 421149 | 44.38999613 | -99.9900038 |
| 95 | Hughes County, SD SW1/4 Sec 6, T 112N, R 75 W | 14N | 4915667 | 421149 | 44.38999613 | -99.9900038 |
| 96 | SD, Sec 15, T111N, R71W, Hyde County | 14N | 4918546 | 472057 | 44.41966737 | -99.35101255 |
| 97 | SD, Sec 31, T110N, R66W, Hand County | 14N | 4904186 | 514938 | 44.29077096 | -98.81276367 |
| 98 | SD, Sec 4, T112 N, R80W, Hughes County | 14N | 4932953 | 383833 | 44.54054355 | -100.4622725 |
| 99 | SD, Sec 5, T109N, R72W, Hyde County | 14N | 4902383 | 459143 | 44.27354458 | -99.51196125 |
| 100 | Ag Farm (formerly 110) | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 101 | Emmet County, High Lake Township, 1/4 mi SW of Wallingford, IA on 250th St | 15N | 4796189 | 358616 | 43.3052449 | -94.74323332 |
| 102 | Osceola County, Highway 60 and 140th St | 15N | 4810954 | 291645 | 43.42247461 | -95.5738988 |
| 103 | Clay County, Spencer Airport | 15N | 4779586 | 325898 | 43.14897625 | -95.14114987 |
| 104 | Lyon County, HWY A34 1 mi W of George | 14N | 4803218 | 743079 | 43.34242487 | -96.00111147 |
| 105 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 106 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 107 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 108 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 109 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 110 | Jackson County, SD N 43.94394 W 101.91125 | 14N | 4869767 | 266370 | 43.94387419 | -101.9112451 |
| 111 | SW of Edgemont, SD Fall River County N 43 15 42 W 103 58 31.9 | 13N | 4778913 | 615193 | 43.1541972 | -103.5831952 |
| 112 | Meade County, SD N 44. 37275 W 102.84350 | 13N | 4915536 | 671810 | 44.37272734 | -102.843504 |

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|---|---|---|---|---|---|---|
| 113 | NW of Edgemont, SD Fall River County N 43 23 03.4 W 103 53 44.1 | 13N | 4787437 | 619011 | 43.23033732 | -103.5344156 |
| 114 | SW of Edgemont, SD Fall River County N 43 15 37 W 103 52 39.8 | 13N | 4778941 | 620009 | 43.1536999 | -103.5239741 |
| 115 | SW of Edgemont, SD N 43 15 36.5 W 103 52 39.9 | 13N | 4778935 | 620008 | 43.15364604 | -103.5239877 |
| 116 | Sruther Fall, SD River County, Ardmore Rd. | 13N | 4789641 | 620143 | 43.24999749 | -103.5199996 |
| 117 | Lyon County, T98NR45W?, S4 | 14N | 4802256 | 729700 | 43.33799514 | -96.16636816 |
| 363 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 942 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 948 | SE of Ag Farm | 15N | 4651815 | 437963 | 42.01591683 | -93.7492642 |
| 958 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |
| 962 | Ag Farm | 15N | 4652018 | 436449 | 42.01762418 | -93.76757026 |

# APPENDIX 2

| ID | Region | Sol 08 | | Sol 28 | | Sol 33 | | Sat 02 | | Sat 03 | | Sat 12 | | Sat 13 | | Sat 16 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Central | 115 | 117 | 153 | 157 | 215 | 215 | 251 | 257 | 133 | 133 | 107 | 131 | 122 | 122 | 106 | 106 |
| 2 | SD | 107 | 113 | 153 | 157 | 215 | 215 | 243 | 255 | 133 | 133 | 119 | 135 | 114 | 116 | 090 | 090 |
| 3 | NW | 113 | 113 | 153 | 153 | 215 | 217 | 251 | 257 | 127 | 133 | 123 | 131 | 116 | 116 | 090 | 094 |
| 4 | NW | 107 | 113 | 153 | 157 | 215 | 215 | 251 | 251 | 127 | 133 | 123 | 123 | 116 | 116 | 100 | 116 |
| 5 | NW | 107 | 107 | 153 | 153 | 215 | 215 | 239 | 245 | 127 | 133 | 111 | 123 | 116 | 116 | 098 | 098 |
| 6 | NW | 107 | 115 | 171 | 171 | 211 | 215 | 245 | 263 | 133 | 133 | 111 | 111 | 116 | 116 | 090 | 116 |
| 7 | Central | 105 | 107 | 153 | 183 | 215 | 215 | 249 | 255 | 127 | 133 | 131 | 135 | 116 | 116 | 090 | 100 |
| 8 | Central | 107 | 107 | 153 | 157 | 215 | 215 | 251 | 259 | 133 | 133 | 115 | 123 | 116 | 116 | 100 | 100 |
| 10 | Central | 115 | 117 | 153 | 187 | 215 | 215 | 249 | 251 | 133 | 133 | 107 | 123 | 116 | 116 | 102 | 102 |
| 11 | Central | 107 | 117 | 153 | 157 | 215 | 215 | 251 | 261 | 133 | 133 | 111 | 123 | 116 | 116 | 102 | 102 |
| 12 | Central | 113 | 117 | 153 | 153 | 215 | 215 | 245 | 251 | 127 | 133 | 119 | 123 | 116 | 116 | 096 | 102 |
| 13 | Central | 107 | 115 | 153 | 157 | 213 | 215 | 247 | 255 | 133 | 133 | 111 | 123 | 116 | 118 | 102 | 116 |
| 14 | Central | 107 | 115 | 153 | 183 | 215 | 215 | 251 | 255 | 127 | 133 | 107 | 123 | 116 | 118 | 098 | 102 |
| 15 | Central | 107 | 117 | 153 | 157 | 215 | 215 | 245 | 251 | 133 | 133 | 111 | 123 | 116 | 116 | 102 | 102 |
| 17 | Central | 107 | 115 | 161 | 161 | 215 | 217 | 255 | 255 | 133 | 133 | 119 | 123 | 116 | 118 | 096 | 102 |
| 18 | Central | 115 | 117 | 157 | 157 | 215 | 215 | 255 | 261 | 133 | 133 | 123 | 131 | 116 | 116 | 102 | 102 |
| 19 | NW | 113 | 115 | 159 | 171 | 215 | 217 | 247 | 257 | 127 | 133 | 107 | 111 | 118 | 120 | 090 | 098 |
| 20 | Central | 113 | 115 | 153 | 153 | 215 | 215 | 251 | 251 | 127 | 133 | 107 | 123 | 116 | 116 | 102 | 102 |
| 21 | SD | 107 | 107 | 153 | 157 | 215 | 215 | 251 | 251 | 133 | 133 | 119 | 127 | 116 | 120 | 106 | 106 |
| 22 | SD | 113 | 113 | 153 | 153 | 211 | 217 | 251 | 257 | 133 | 133 | 107 | 115 | 116 | 118 | 090 | 102 |
| 23 | SD | 113 | 127 | 153 | 169 | 215 | 215 | 239 | 253 | 127 | 133 | 111 | 115 | 116 | 116 | 090 | 116 |
| 24 | SD | 115 | 135 | 171 | 183 | 215 | 215 | 255 | 257 | 133 | 133 | 111 | 123 | 116 | 116 | 090 | 100 |
| 25 | SD | 133 | 133 | 183 | 183 | 215 | 217 | 239 | 251 | 133 | 133 | 119 | 123 | 116 | 122 | 096 | 096 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 26 | NW | 113 | 113 | 153 | 155 | 215 | 215 | 253 | 255 | 133 | 133 | 119 | 123 | 116 | 122 | 090 | 090 |
| 27 | NW | 107 | 113 | 153 | 153 | 213 | 215 | 251 | 253 | 133 | 133 | 111 | 123 | 114 | 116 | 090 | 096 |
| 28 | NW | 107 | 115 | 000 | 000 | 215 | 215 | 245 | 255 | 127 | 133 | 111 | 111 | 116 | 120 | 090 | 090 |
| 29 | NW | 113 | 115 | 153 | 159 | 215 | 217 | 251 | 251 | 127 | 133 | 111 | 115 | 114 | 116 | 098 | 098 |
| 30 | NW | 107 | 113 | 153 | 153 | 215 | 215 | 243 | 253 | 133 | 133 | 119 | 123 | 116 | 118 | 102 | 102 |
| 31 | NW | 115 | 115 | 153 | 157 | 215 | 215 | 247 | 263 | 127 | 133 | 123 | 127 | 118 | 122 | 090 | 116 |
| 32 | Central | 107 | 117 | 151 | 151 | 215 | 215 | 259 | 259 | 127 | 133 | 111 | 119 | 116 | 118 | 098 | 098 |
| 33 | Central | 115 | 115 | 153 | 153 | 215 | 217 | 251 | 255 | 127 | 133 | 119 | 123 | 116 | 118 | 098 | 102 |
| 39 | NW | 107 | 109 | 153 | 183 | 215 | 215 | 253 | 255 | 127 | 127 | 119 | 127 | 116 | 116 | 096 | 108 |
| 40 | NW | 107 | 115 | 153 | 183 | 215 | 217 | 255 | 263 | 127 | 127 | 111 | 123 | 116 | 116 | 090 | 098 |
| 41 | Montana | 107 | 115 | 153 | 153 | 215 | 215 | 263 | 265 | 133 | 133 | 115 | 123 | 116 | 120 | 098 | 098 |
| 42 | Montana | 105 | 113 | 153 | 153 | 215 | 219 | 241 | 257 | 127 | 127 | 111 | 127 | 116 | 118 | 100 | 110 |
| 43 | Montana | 107 | 117 | 155 | 159 | 215 | 217 | 249 | 259 | 127 | 133 | 111 | 119 | 116 | 120 | 098 | 098 |
| 44 | Montana | 105 | 107 | 155 | 157 | 217 | 217 | 259 | 259 | 133 | 133 | 115 | 119 | 116 | 116 | 100 | 100 |
| 45 | Montana | 105 | 107 | 153 | 183 | 215 | 215 | 257 | 257 | 127 | 133 | 119 | 123 | 120 | 120 | 098 | 098 |
| 46 | Montana | 115 | 117 | 153 | 161 | 215 | 217 | 251 | 255 | 127 | 127 | 111 | 135 | 116 | 116 | 096 | 098 |
| 47 | Montana | 107 | 107 | 153 | 161 | 215 | 217 | 239 | 263 | 133 | 133 | 135 | 135 | 116 | 116 | 090 | 098 |
| 48 | Central | 113 | 115 | 153 | 171 | 215 | 215 | 251 | 261 | 133 | 133 | 123 | 123 | 116 | 116 | 098 | 102 |
| 49 | Central | 107 | 131 | 153 | 171 | 215 | 215 | 259 | 261 | 133 | 133 | 123 | 123 | 116 | 116 | 102 | 102 |
| 50 | Central | 107 | 115 | 157 | 157 | 215 | 215 | 255 | 259 | 133 | 133 | 115 | 119 | 116 | 116 | 096 | 102 |
| 52 | SD | 107 | 117 | 153 | 153 | 215 | 215 | 247 | 257 | 133 | 133 | 111 | 119 | 116 | 118 | 090 | 100 |
| 53 | SD | 107 | 107 | 153 | 153 | 215 | 217 | 249 | 249 | 127 | 133 | 123 | 127 | 116 | 120 | 090 | 090 |
| 55 | SD | 113 | 113 | 153 | 157 | 215 | 215 | 249 | 249 | 133 | 133 | 107 | 115 | 116 | 120 | 096 | 096 |
| 56 | SD | 107 | 107 | 153 | 153 | 215 | 217 | 249 | 249 | 127 | 133 | 123 | 127 | 116 | 120 | 090 | 090 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 59 | SD | 113 | 113 | 153 | 157 | 215 | 215 | 249 | 249 | 133 | 133 | 107 | 115 | 116 | 120 | 096 | 096 |
| 60 | SD | 105 | 109 | 159 | 159 | 215 | 215 | 239 | 263 | 133 | 133 | 111 | 123 | 114 | 118 | 098 | 100 |
| 61 | SD | 115 | 133 | 163 | 163 | 215 | 217 | 247 | 251 | 133 | 133 | 119 | 127 | 120 | 120 | 106 | 106 |
| 62 | SD | 105 | 115 | 153 | 169 | 215 | 217 | 247 | 247 | 133 | 133 | 111 | 131 | 120 | 120 | 102 | 102 |
| 63 | SD | 107 | 109 | 151 | 151 | 215 | 215 | 243 | 255 | 127 | 127 | 115 | 119 | 116 | 116 | 102 | 106 |
| 64 | SD | 107 | 107 | 153 | 185 | 215 | 215 | 247 | 251 | 133 | 133 | 115 | 135 | 116 | 116 | 106 | 106 |
| 65 | SD | 105 | 113 | 153 | 169 | 215 | 219 | 251 | 257 | 127 | 133 | 111 | 135 | 116 | 120 | 090 | 090 |
| 66 | SD | 105 | 115 | 000 | 171 | 215 | 215 | 251 | 257 | 133 | 133 | 115 | 127 | 116 | 116 | 090 | 090 |
| 67 | SD | 113 | 117 | 159 | 159 | 215 | 215 | 255 | 255 | 127 | 133 | 115 | 119 | 116 | 118 | 096 | 106 |
| 68 | SD | 105 | 113 | 151 | 153 | 215 | 215 | 255 | 255 | 127 | 133 | 119 | 127 | 116 | 118 | 090 | 090 |
| 69 | SD | 107 | 113 | 153 | 177 | 215 | 215 | 249 | 255 | 127 | 133 | 115 | 115 | 120 | 122 | 096 | 106 |
| 70 | SD | 103 | 107 | 000 | 181 | 215 | 217 | 249 | 251 | 127 | 135 | 107 | 115 | 116 | 118 | 096 | 106 |
| 71 | SD | 111 | 115 | 155 | 155 | 215 | 215 | 247 | 249 | 133 | 133 | 107 | 127 | 116 | 122 | 100 | 100 |
| 72 | SD | 107 | 131 | 185 | 185 | 211 | 217 | 253 | 259 | 127 | 133 | 123 | 123 | 116 | 118 | 096 | 098 |
| 73 | SD | 107 | 107 | 153 | 185 | 215 | 217 | 255 | 255 | 127 | 127 | 119 | 127 | 116 | 116 | 090 | 090 |
| 74 | SD | 105 | 113 | 000 | 185 | 215 | 215 | 249 | 257 | 127 | 127 | 111 | 111 | 116 | 116 | 096 | 096 |
| 75 | SD | 115 | 131 | 153 | 185 | 215 | 217 | 253 | 255 | 133 | 133 | 107 | 127 | 116 | 118 | 090 | 098 |
| 76 | SD | 115 | 119 | 157 | 171 | 215 | 217 | 239 | 251 | 133 | 133 | 115 | 119 | 116 | 116 | 100 | 100 |
| 77 | SD | 115 | 131 | 155 | 155 | 215 | 215 | 247 | 253 | 127 | 133 | 115 | 115 | 120 | 120 | 108 | 108 |
| 78 | SD | 105 | 113 | 153 | 175 | 215 | 217 | 247 | 251 | 127 | 133 | 115 | 123 | 116 | 120 | 098 | 098 |
| 79 | SD | 115 | 119 | 157 | 173 | 215 | 217 | 239 | 253 | 133 | 133 | 115 | 119 | 116 | 116 | 106 | 106 |
| 80 | SD | 105 | 117 | 185 | 185 | 213 | 215 | 239 | 249 | 127 | 127 | 111 | 123 | 116 | 116 | 098 | 098 |
| 81 | SD | 107 | 115 | 153 | 153 | 215 | 215 | 239 | 249 | 127 | 127 | 111 | 111 | 116 | 118 | 106 | 108 |
| 82 | SD | 107 | 117 | 153 | 185 | 215 | 215 | 241 | 247 | 127 | 133 | 123 | 131 | 114 | 118 | 098 | 098 |
| 83 | SD | 107 | 115 | 153 | 157 | 215 | 217 | 249 | 255 | 133 | 133 | 111 | 123 | 116 | 116 | 096 | 098 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 85 | SD | 115 | 115 | 153 | 153 | 215 | 217 | 249 | 253 | 127 | 133 | 107 | 119 | 116 | 116 | 098 | 098 |
| 86 | SD | 113 | 117 | 177 | 181 | 217 | 217 | 253 | 257 | 127 | 133 | 119 | 127 | 116 | 116 | 100 | 100 |
| 87 | SD | 107 | 113 | 153 | 155 | 215 | 215 | 255 | 263 | 127 | 133 | 115 | 131 | 120 | 120 | 106 | 106 |
| 88 | SD | 113 | 115 | 157 | 181 | 215 | 217 | 257 | 263 | 127 | 127 | 119 | 131 | 116 | 116 | 090 | 100 |
| 89 | SD | 115 | 117 | 153 | 181 | 215 | 215 | 251 | 259 | 127 | 133 | 107 | 119 | 116 | 116 | 096 | 096 |
| 90 | SD | 113 | 113 | 153 | 169 | 215 | 215 | 247 | 255 | 127 | 133 | 119 | 123 | 120 | 120 | 106 | 106 |
| 91 | SD | 105 | 107 | 153 | 157 | 215 | 215 | 249 | 259 | 127 | 127 | 119 | 127 | 116 | 116 | 106 | 106 |
| 92 | SD | 105 | 113 | 159 | 159 | 215 | 215 | 243 | 255 | 127 | 133 | 107 | 119 | 116 | 120 | 098 | 098 |
| 93 | SD | 107 | 113 | 153 | 153 | 215 | 215 | 251 | 253 | 127 | 133 | 115 | 115 | 116 | 116 | 090 | 090 |
| 94 | SD | 105 | 133 | 153 | 171 | 211 | 217 | 251 | 253 | 127 | 133 | 119 | 123 | 116 | 118 | 096 | 098 |
| 95 | SD | 105 | 115 | 153 | 153 | 211 | 215 | 251 | 253 | 127 | 133 | 119 | 123 | 118 | 118 | 098 | 098 |
| 96 | SD | 107 | 111 | 171 | 171 | 215 | 215 | 251 | 259 | 133 | 133 | 111 | 127 | 120 | 120 | 106 | 106 |
| 97 | SD | 105 | 107 | 153 | 157 | 215 | 215 | 249 | 255 | 133 | 133 | 111 | 119 | 116 | 116 | 104 | 104 |
| 98 | SD | 107 | 113 | 153 | 153 | 215 | 215 | 247 | 255 | 127 | 133 | 111 | 119 | 116 | 120 | 094 | 094 |
| 99 | SD | 105 | 115 | 153 | 153 | 215 | 217 | 251 | 251 | 127 | 127 | 111 | 123 | 124 | 124 | 100 | 100 |
| 100 | Central | 107 | 107 | 153 | 157 | 213 | 215 | 251 | 261 | 133 | 133 | 123 | 123 | 116 | 118 | 102 | 102 |
| 101 | NW | 115 | 115 | 153 | 159 | 215 | 215 | 257 | 259 | 133 | 133 | 119 | 123 | 118 | 120 | 094 | 094 |
| 102 | NW | 113 | 113 | 153 | 153 | 215 | 215 | 239 | 261 | 133 | 133 | 119 | 135 | 116 | 120 | 090 | 094 |
| 103 | NW | 113 | 115 | 171 | 179 | 215 | 215 | 251 | 255 | 127 | 133 | 111 | 119 | 114 | 116 | 098 | 098 |
| 104 | NW | 105 | 105 | 157 | 159 | 215 | 215 | 251 | 255 | 127 | 133 | 107 | 119 | 116 | 116 | 100 | 100 |
| 105 | Central | 107 | 115 | 157 | 157 | 213 | 215 | 255 | 255 | 133 | 133 | 111 | 115 | 118 | 118 | 098 | 098 |
| 106 | Central | 107 | 117 | 157 | 157 | 215 | 217 | 255 | 259 | 133 | 133 | 119 | 137 | 116 | 116 | 102 | 106 |
| 107 | Central | 107 | 107 | 153 | 157 | 215 | 215 | 247 | 255 | 133 | 133 | 115 | 123 | 116 | 116 | 098 | 106 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 108 | Central | 107 | 117 | 157 | 157 | 215 | 215 | 253 | 259 | 133 | 133 | 131 | 135 | 116 | 118 | 106 | 106 |
| 109 | Central | 107 | 117 | 153 | 157 | 215 | 215 | 259 | 261 | 133 | 133 | 111 | 111 | 116 | 118 | 106 | 106 |
| 110 | SD | 105 | 115 | 169 | 177 | 215 | 217 | 247 | 253 | 127 | 133 | 107 | 115 | 120 | 120 | 090 | 090 |
| 111 | SD | 105 | 115 | 153 | 153 | 215 | 215 | 247 | 253 | 127 | 133 | 111 | 111 | 120 | 120 | 098 | 106 |
| 112 | SD | 113 | 119 | 153 | 157 | 215 | 217 | 243 | 259 | 127 | 133 | 111 | 131 | 118 | 118 | 096 | 106 |
| 113 | SD | 113 | 117 | 153 | 185 | 211 | 215 | 247 | 251 | 133 | 133 | 107 | 119 | 116 | 118 | 090 | 090 |
| 114 | SD | 109 | 113 | 153 | 185 | 215 | 215 | 247 | 267 | 127 | 133 | 107 | 127 | 116 | 116 | 096 | 100 |
| 115 | SD | 107 | 115 | 159 | 185 | 215 | 215 | 247 | 251 | 127 | 133 | 107 | 115 | 116 | 000 | 100 | 104 |
| 116 | SD | 115 | 115 | 153 | 153 | 211 | 215 | 247 | 257 | 133 | 133 | 111 | 131 | 116 | 120 | 096 | 098 |
| 117 | NW | 105 | 113 | 153 | 157 | 211 | 215 | 253 | 257 | 127 | 133 | 107 | 119 | 118 | 118 | 098 | 098 |
| 363 | Central | 107 | 115 | 153 | 157 | 215 | 215 | 255 | 255 | 133 | 133 | 111 | 123 | 116 | 116 | 116 | 116 |
| 942 | Central | 107 | 117 | 153 | 183 | 215 | 215 | 261 | 261 | 127 | 133 | 107 | 107 | 116 | 116 | 102 | 116 |
| 948 | Central | 117 | 117 | 153 | 185 | 215 | 215 | 249 | 253 | 127 | 133 | 107 | 123 | 116 | 116 | 102 | 102 |
| 958 | Central | 115 | 131 | 157 | 171 | 215 | 215 | 255 | 261 | 133 | 133 | 127 | 131 | 116 | 116 | 102 | 116 |
| 962 | Central | 107 | 115 | 153 | 157 | 215 | 215 | 251 | 261 | 133 | 133 | 107 | 131 | 116 | 116 | 102 | 102 |