POPULATION GENETIC ANALYSES OF THE BAIRD'S POCKET GOPHER, *GEOMYS BREVICEPS*

A Thesis

by

SARAH RAE WELBORN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2012

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

Co-Chairs of Committee,	Jessica E. Light
	Gary Voelker
Committee Member,	William J. Murphy
Head of Department,	John B. Carey

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ABSTRACT

Population Genetic Analyses of the Baird's Pocket Gopher, *Geomys breviceps*.

(August 2012)

Sarah Rae Welborn, B.S. Texas A&M University

Co-Chairs of Advisory Committee, Dr. Jessica E. Light
Dr. Gary Voelker

The Baird's pocket gopher (*Geomys breviceps*) is a solitary, fossorial rodent found throughout areas of Texas, Arkansas, Oklahoma, and Louisiana. Research focusing on the population genetics of pocket gophers and other species with limited vagility and isolated populations is lacking. Through the use of mitochondrial and microsatellite data, a series of population genetic analyses were completed to better understand the population structure and gene flow among a series of *G. breviceps* localities. Pocket gophers were captured from five localities in the Brazos Valley and used in this study. Due to the lack of microsatellite loci available for *G. breviceps*, 10 loci were created for use in this study. Overall estimates from the population genetic analyses showed high levels of gene flow amongst nearby localities with decreasing levels as distance between localities increased. Findings suggest that 2-3 localities located within 2 km of each other function as one genetic cluster thus showing 3-4 total genetic clusters total in this study. Results also suggest that the Baird's pocket gopher is capable of moving at least 2 km, but further analyses should be completed to better understand dispersal distance.

DEDICATION

I dedicate this thesis to my mom and dad. Thank you for always being not only the most inspirational and wonderful parents any woman could ask for but also for your continual love, support, friendship, and guidance. I love y'all.

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I would like to thank my committee chair, Dr. J.E. Light, and my committee members, Dr. G. Voelker and Dr. W.J. Murphy for their guidance, support and wonderful advice throughout the course of this research.

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Finally, thanks to my family for all of their continual love and support throughout my entire life. Thank you for always making me smile and laugh even when I thought it just was not my day and for always believing in me. Thanks also go to R. Bay for his love, support and laughter and for all the joy and happiness he brings to my life. We are each other's lucky charms. I love you all. Thank you all with all my heart.

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CHAPTER I

INTRODUCTION

Pocket gophers are a group of solitary and fossorial rodents classified in the family Geomyidae. There are six genera and approximately 40 species of pocket gophers distributed throughout North and Central America (Merrit 2010). As a group, pocket gophers are highly modified morphologically for a fossorial lifestyle (Sulentich *et al.* 1991). For example, the forelimbs (both carpals and metacarpals) of pocket gophers are often shortened (Stein 2000). Previous research has found that there is a link between an underground lifestyle and an overall decrease in size of many characteristics of fossorial mammals (Nevo 1979). Senses such as sight are not as essential for fossorial mammals due to the lack of visibility underground, thus causing the reduction of the eyes over time (Merrit 2010). Although hearing is an important sense for underground life, external ears are often reduced to decrease friction (Merrit 2010; Stein 2000). In contrast to the previously stated reductions, pocket gophers have increased muscle mass as well as large, long claws for digging at the anterior end of their bodies (pocket gophers use their claws to loosen and break up the soil; Stein 2000).

The anatomical modifications of pocket gophers enable them to dig elaborate and narrow burrow systems below the surface of the soil (Sulentich *et al.* 1991). Pocket gophers spend the majority of their lives (lifespans of some species have been recorded as up to 3 years in males and 4.75 years in females; Howard & Childs 1959) within these

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This thesis follows the style of *Molecular Ecology*.

tunnel systems building new tunnels and sealing tunnels that are no longer in use (Sulentich *et al.* 1991). Previous studies have recorded that pocket gophers can burrow up to 12.8 meters per day in some cases (Andersen 1987). Burrows are occupied by one gopher (except for when rearing young) and often defended due to the energy costs required to create them (Buffenstein 2000; Busch *et al.* 2000). By spending the majority of their lives underground, fossorial mammals are able to maintain a closed system within their burrows. Factors such as temperature and humidity are predictable in a closed system, thus enabling fossorial mammals to use these factors as an alarm for intruders or predators (Merrit 2010; Nevo 1979).

Pocket gophers are herbivores and their diet consists of roots (21 - 60% of the diet, depending upon the species) and native plants found within and around the burrow (Busch *et al.* 2000). Due to competition for these resources, pocket gophers are often highly territorial, which adds to their solitary nature (Smolen *et al.* 1980). In comparison to other fossorial mammals, pocket gophers are unique in possessing fur-lined cheek pouches. These external pouches extend from the mouth to the shoulder and are utilized in the transportation of food and nesting material in and around the burrow system (Merrit 2010). Pocket gophers store food in caches within the burrow system (Sulentich *et al.* 1991) and depending upon the plant diversity and the overall health of the surrounding environment, these caches can hold a wide variety of plant species.

Many fossorial species are well known for their negative economic impacts. Pocket gophers are generally seen as a nuisance to gardens, yards, and pastures. In agriculture, they are considered to be detrimental when raising livestock and growing

crops. Holes and soft spots in the soil associated with pocket gopher burrow systems are known to cause leg injuries in horses and cattle (Case & Jasch 1994). Pocket gophers, especially when in high abundance, can damage gardens and some crops due to their mounds and feeding behavior (Case & Jasch 1994). So well known are the unfavorable aspects of pocket gophers that many papers have been published with the sole purpose to educate the reader on how to eliminate or remove pocket gophers (due to the depth and complexity of their burrows, a variety of methods such as placing chemicals in the soil, baiting with poisoned food, and using explosions within the burrow system have been recommended; Proulx 1997; Ramey *et al.* 2002; Sterner *et al.* 1999).

However, pocket gophers and other fossorial mammals do have positive economic impacts. Fossorial mammals are known to aerate the soil, promote colonizing plant growth, increase water penetration, increase amounts of helpful nutrients such as phosphorus, and enhance overall plant diversity (Hole 1981; Sherrod *et al.* 2005). Aerated soil helps to promote a healthy natural ground environment by allowing better penetration of water and nutrients and promoting plant growth. The combination of these benefits helps to promote a healthier natural environment at a basic level. Therefore, although fossorial mammals can have many negative impacts, their overall effect on the natural environment can be quite positive.

Due to their level of specialization and overall morphology, pocket gophers have relatively low vagility outside of their burrow systems (Nevo 1979; Patton *et al.* 1972). Previous studies have found that general activity is confined to the burrow system, with above ground activity restricted to short foraging excursions (Teipner *et al.* 1983). When

moving to a new burrow system is necessary, adult pocket gophers often will do so by moving through other individuals' burrow systems to avoid above ground exposure (Howard & Childs 1959). Juveniles and subadults are the primary dispersers and often disperse either early or late in the breeding season from their mother's burrows (Busch *et al.* 2000; Williams & Cameron 1984). Juvenile dispersal is often restricted to above ground movement and driven by competition with adult pocket gophers (Busch *et al.* 2000; Howard & Childs 1959).

Pocket gophers' inability to move well outside of the burrow system can therefore translate to reduced dispersal capabilities, isolated populations, and limited gene flow among populations (Burt & Dowler 1999; Hafner *et al.* 1983). For example, King (2010) found a maximum dispersal distance of 46 m for *Geomys breviceps*. Dispersal distances in some species of *Thomomys* are known to range from 100 m to 500 m per year depending upon the population's respective soil type and terrain (Daly & Patton 1990; Hafner *et al.* 1983; Hafner *et al.* 1998; Smith *et al.* 1983; Vaughan 1963). Evidence of long distance dispersal within pocket gophers is lacking due most likely to their fossorial nature (Hafner *et al.* 1998). Low vagility is also believed to result in the development and persistence of isolated populations (Burt & Dowler 1999) and can subsequently reduce heterozygosity (Williams & Baker 1976). Interestingly, less genetic variation has been observed in pocket gophers compared to their above-ground counterparts (Merrit 2010; Meshriy *et al.* 2011).

Despite the unique characteristics and reduced genetic diversity of solitary species with isolated populations, little is known about the population genetics of these

species (Hambuch & Lacey 2000; Lacey 2001). The field of molecular population genetics can help to investigate gene flow, migration rates, dispersal distances, effective population sizes (Burt & Dowler 1999), and much more in these unique lineages. A population genetic study of a solitary species can provide an informative comparison to studies that often focus on more social and widespread species, thus increasing our understanding about population-level processes on a whole.

This study uses mitochondrial and microsatellite data to investigate population genetics among a series of populations of the Baird's pocket gopher, *Geomys breviceps*, found in and around Brazos County, Texas. The Baird's pocket gopher is found throughout the eastern portion of Texas as well as Arkansas, Oklahoma, and the western portion of Louisiana (Schmidly 2004; Sulentich *et al.* 1991). *Geomys breviceps* prefers habitats that are open and contain sandy soils, while generally avoiding clay-based soils (Schmidly 2004). The diet of *G. breviceps* consists mainly of roots, stems and leaves (Sulentich *et al.* 1991). Due to their preference for early succession forbs, *G. breviceps* is known to increase in concentration in areas of Texas where farmlands have been overgrazed (Cameron 2000). The Baird's pocket gopher reproduces within the burrow system from February through August, with an average number of 1.31-1.70 litters per year and 2.66 young per litter (Busch *et al.* 2000; Sulentich *et al.* 1991). Poor climate and/or lack of a quality food source can result in a decrease in reproductivity and affect levels of activity in subsequent years (Busch *et al.* 2000). Common predators of *G. breviceps* include king snakes, great horned owls, red-tailed hawks, weasels, skunks, and coyotes (Busch *et al.* 2000). Morphologically, *G. breviceps* is almost identical to co-

distributed *Geomys* species, *G. attwateri* and *G. bursarius*. However, these species can be differentiated based on genetic and karyotypic data (Burt & Dowler 1999; Dowler 1989; Schmidly 2004). *G. breviceps* has a diploid number of 74 and a fundamental number of 72 while *G. attwateri* has a diploid number of 70 and a fundamental number of 72 or 74 (Burt & Dowler 1999; Dowler 1989). Previous research focusing on *G. breviceps* has been limited to hybridization studies (with *G. attwateri*; e.g., Dowler 1989) and site fidelity and population structure (King 2010). In her study, King (2010) focused on the spatial distribution and movement patterns in a series of *G. breviceps* individuals in Arkansas. To date, nothing is known about the population genetics within and among populations of *G. breviceps*.

Herein, molecular data will be used to investigate population genetics of *G. breviceps*. Because no microsatellite loci have been developed for *Geomys* (the only pocket gopher loci developed to date are for *Thomomys*; Steinberg 1999), microsatellite libraries were built, and primers identified and characterized. Mitochondrial and microsatellite data were analyzed to examine gene flow and population demography among several *G. breviceps* localities. Due to the solitary, fossorial nature of pocket gophers, the expectation is that there will be little gene flow among populations, with decreasing gene flow as distance between localities increases (Hafner *et al.* 1983). Determining the population genetic structure of *G. breviceps* populations may help to elucidate the role of morphological and behavioral modifications on the population structure and gene flow within a solitary and territorial species.

CHAPTER II

PRIMER NOTE*

Pocket gophers (Rodentia: Geomyidae) are solitary, fossorial New World rodents, and are highly modified for an underground life. Modifications include fur lined cheek pouches for food transportation, increased muscle mass at the anterior ends of their bodies, small eyes, reduced ears, and elongated claws for digging. Pocket gophers spend the majority of their lives in their burrow systems (Sulentich *et al.* 1991), have low vagility (Sudman *et al.* 2006), and are often distributed in isolated populations (Patton *et al.* 1972). These characteristics are relatively rare among mammals and little is known about the population genetics of solitary species with isolated populations (Lacey 2001). Thus, designing microsatellite loci for pocket gopher species may provide markers that can be used to gain a better understanding of population dynamics in rare, solitary species with low vagility. Herein, I develop microsatellites for the Baird's pocket gopher, *Geomys breviceps*, and determine the utility of these loci across several pocket gopher species. The primers developed here have been published in Welborn *et al.* (2012).

DNA was extracted from a total of 40 *G. breviceps* specimens using the DNeasy Tissue Kit (QIAGEN Inc.; Valencia, California). Following extraction, DNA was digested with the restriction enzymes *RsaI* and *HaeIII* (New England Biolabs) to produce DNA fragments of more desirable sizes with blunt ends. The digested DNA was run on an agarose gel and fragments of 500 to 1000 base pairs were dissected. This size

*Reprinted with permission from "Characterization of 10 polymorphic loci on the Baird's pocket gopher (*Geomys breviceps*) and cross amplification in other gopher species" by Welborn, SR, Renshaw, MA, and Light, JE, 2012. *Conservation Genetics Resources*, **4**, 467 – 469, Copyright [2012] by Springer Science and Business Media.

range was chosen to maximize the total possible microsatellites recovered. The dissected material was purified using a DNA gel extraction kit (QIAGEN Inc.; Valencia, California), and adaptors were attached to the blunt ends on each fragment using T4 DNA ligase (Promega). DNA fragments were hybridized with di, tri, and tetra oligonucleotide probes, incubated with streptavidin-coated magnetic M-280 Dynabeads (Invitrogen), and thoroughly rinsed. This enriched DNA was increased in quantity though PCR amplification using the following protocol: initial denaturation at 95°C for 2 min, 35 cycles with denaturation of 95°C for 20 s, annealing at 60°C for 20 s, and an extension step at 72°C for 1.5 min, followed by an additional extension step at 72°C for 20 min. PCR products were cleaned with a PCR purification kit (QIAGEN Inc.; Valencia, California). Cleaned products were ligated into pCR®2.1 TOPO® vector (Invitrogen) and transformed into *Escherichia coli* (One Shot® TOP10 Chemically Competent Cells, Invitrogen). Cells were dispersed onto X-Gal/LB/agar/IPTG plates treated with ampicillin and incubated overnight at 37°C. Incubation allowed for growth of the transformed *E. coli* cells. Positive clones (appeared as white on the plates) were picked using sterilized toothpicks and placed into 96-well culture plates filled with LB (treated with ampicillin). Plates were covered and incubated overnight at 37°C to increase culture density. Subsamples of the library were placed into new 96 well culture plates filled with LB (with ampicillin), incubated overnight, frozen in a -80°C freezer and sent to the University of Florida DNA Sequencing Core Laboratory (Gainesville, Florida) to be sequenced using ABI Prism BigDye Terminator cycle sequencing protocols (Applied Biosystems, Foster City, California).

Sequences were screened for microsatellites, and 35 primer pairs were developed using Primer3 (http://frodo.wi.mit.edu/primer3/) and tested for amplification and polymorphisms. PCR amplifications were performed in 10 μL reactions containing 3.7 μL Emerald Master Mix (Takara Bio Inc.), 4.25 μL water, 0.5 μL fluorescently dye-labeled "tail" primer (56-FAM; 5'-GCCTCGTTTATCAGATGTGGA; 10 μM), 0.05 μL forward primer with additional "tail" sequence (Integrated DNA Technologies; 10 μM), 0.5 μL reverse primer (10 μM), and 1 μL DNA. All polymerase chain reactions of microsatellite loci were accomplished as described by Karlsson *et al.* (2008). PCR products were loaded onto a polyacrylamide gel and run using an ABI Prism 377 DNA Sequencer (Biosystematics Center, College Station, Texas). Results were analyzed using the Genescan® 400 ROX-Size Standard (Applied Biosystems) and Genescan 3.1.2 (Applied Biosystems). Allele sizes were determined using the Genotyper® software, version 2.5 (Applied Biosystems). Of the originally developed 35 primer pairs, 10 amplified successfully and were polymorphic (Table 1), thus suitable for population genetic analyses.

Variability of the 10 polymorphic loci was assessed in 40 *G. breviceps* specimens from 4 different populations (10 pocket gophers per population) found in the western portion of College Station, Texas. All specimens are housed in the Texas Cooperative Wildlife Collection at Texas A&M University (specimen voucher information such as museum numbers and collection localities is available upon request). I estimated the number of alleles per locus (N_A) across all 40 individuals, while expected heterozygosity (H_E), observed heterozygosity (H_O), probability of deviation from the expectations of

Table 1 - Summary data for 10 polymorphic microsatellite loci characterized for the Baird's pocket gopher, *Geomys breviceps*. Standard population genetic measurements were determined for 4 populations. Primer sequences are listed with forward on top and reverse on bottom. Size represents clone size in base pairs (bp) of the allele in the sequenced clone. Abbreviations are as follows: number of individuals assayed (N), number of alleles per locus (N_A), size range in base pairs of alleles (Range), range of expected heterozygosity (H_E), range of observed heterozygosity (H_O), and the range of the probability of deviation from the expectations of Hardy-Weinberg equilibrium (P_{HW}).

Locus	Primer Sequence (5'-3')	Repeat Motif	Size (bp)	N	N_A	Range	H_E	H_O	P_{HW}
Gbr06	CTCATCGCTGGGGAGAGA	$(CA)_{15}$	221	40	10	234 – 288	0.689 – 0.789	0.600 – 1.000	0.052 – 0.503
	CTTGGATCTGGGGATCCTTT								
Gbr09	TGGCTCAAGTGAGAGCATCA	$(CA)_{18}$	214	40	11	210 – 252	0.616 – 0.857	0.700 – 1.000	0.027 – 0.633
	GGAGGAGGAACAAGCAATCA								
Gbr10	TAGTGCATGCTCTGGCTTTG	$(CA)_{19}$	235	40	11	216 – 282	0.552 – 0.847	0.500 – 0.700	0.103 – 1.000
	AAATGCCCTCCAGAAGGAAC								
Gbr14	GGACCTGGTGACACTGGTTT	$(GT)_{14}$	203	40	5	201 – 308	0.526 – 0.668	0.600 – 1.000	0.006 – 1.000
	TTCTTATGCACCCCCTTTCA								
Gbr15	CTCTCCCTCAGCTCAGCAGT	$(GT)_{14}$	212	40	14	222 – 258	0.789 – 0.800	0.700 – 0.900	0.127 – 0.928
	GTGTCCAGCCCAGTTATGCT								
Gbr25	CCTGGGAGACTAGCATGAGG	$(GT)_{27}$	227	40	16	237 – 260	0.621 – 0.842	0.600 – 0.800	0.212 – 0.593
	CACAAGAAAGCCAGAAGTGC								

Table 1 – Continued.

Locus	Primer Sequence (5'-3')	Repeat Motif	Size (bp)	N	N_A	Range	H_E	H_O	P_{HW}
Gbr26	TGGAATCACCAACAAGCAGA	$(CA)_{20}$	240	40	16	250 – 274	0.763 – 0.873	0.300 – 0.900	0.005 – 0.469
	TAACAGGTGTGAGGCGACAG								
Gbr27	TGATGACACGCTGACTTTCC	$(GT)_{10}$	229	40	5	243 – 258	0.189 – 0.532	0.200 – 0.700	0.479 – 1.000
	TGGAGGTGTAGCTCAAGTGG								
Gbr33	GTGGTAGTGGTGGTGTTTGC	$(AAGG)_{14}$	227	40	12	228 – 280	0.689 – 0.821	0.300 – 0.800	0.009 – 0.884
	ACACTGGAGTGTCTCATGTGG								
Gbr36	CCACCAGAGAAATCAAAGAAG	$(AGGC)_4$	173	40	2	193 – 197	0.100 – 0.505	0.100 – 0.800	0.172 – 1.000
	AGCCACTGCTCAACTTCAGG								

Hardy-Weinberg equilibrium (P_{HW}), and linkage disequilibrium were estimated on a per population basis (Arlequin v 3.5.1.2 (Excoffier *et al.* 2005)). Bonferroni correction (Rice 1989) was used when calculating both Hardy-Weinberg and linkage disequilibrium.

The number of alleles per locus ranged from 2 to 16 and observed heterozygosity ranged from 0.100 to 1.000 (Table 1). Following Bonferroni correction, none of the loci deviated significantly from Hardy-Weinberg expectations. The loci Gbr10, Gbr15 and Gbr27 showed signs of linkage disequilibrium and only Gbr36 showed signs of null alleles (GENEPOP v4.0; (Rousset 2008)). Loci that show signs of linkage disequilibrium are thought to be appearing in populations either more or less often than would normally be expected. When a locus exhibits signs of null alleles, it means that the locus can no longer be detected due to some form of mutation. Loci showing signs of linkage disequilibrium and null alleles need to be used cautiously for population level analyses. Based upon the results reported in Table 1, the 10 loci designed here appear to be suitable for more extensive population level analyses.

Microsatellite loci that have been developed for a specific species can be used in other closely related species. Therefore the 10 polymorphic microsatellite loci designed here, as well as 4 previously described *Thomomys mazama* loci (Steinberg 1999), were tested for amplification in 18 additional pocket gopher species (Table 2). The *Geomys* primers had fairly high amplification rates in the majority of the species tested, except for *T. bottae* and *T. talpoides* (Table 2). The Steinberg (1999) primers also successfully amplified in all species except *G. texensis* (Table 2). These 14 microsatellite loci can be

Table 2 – Success amplifying *Geomys breviceps* (designed here) and *Thomomys mazama* (Steinberg 1999) microsatellite loci across 18 pocket gopher species. Successful amplification (plus signs; failed amplifications are indicated by minus signs) was determined by generation of PCR products of the expected size (determined by agarose gel electrophoresis). The 4 *Thomomys* primers (Steinberg 1999) all amplified and were polymorphic for *G. breviceps*.

	Gbr06	**Gbr09**	**Gbr10**	**Gbr14**	**Gbr15**	**Gbr25**	**Gbr26**	**Gbr27**	**Gbr33**	**Gbr36**	**Tm1**	**Tm2**	**Tm6**	**Tm7**
Geomys attwateri	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Geomys bursarius	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Geomys personatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Geomys pinetus	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Geomys texensis	+	+	-	+	+	-	+	+	+	+	-	+	-	-
Cratogeomys castanops	+	+	-	-	+	+	+	+	+	+	+	+	-	+
Cratogeomys fumosus	-	+	-	+	+	-	+	+	+	+	+	+	+	+
Cratogeomys perotensis	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Cratogeomys planiceps	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Orthogeomys grandis	+	+	-	+	+	-	+	-	+	+	+	+	+	+
Orthogeomys hispidus	+	+	-	+	+	-	+	+	+	+	+	+	+	+
Orthogeomys underwoodi	+	+	-	+	+	+	-	+	-	+	+	-	+	+
Pappogeomys bulleri	-	+	-	+	+	+	+	+	+	+	+	+	+	+
Thomomys atrovarius	+	+	+	-	+	+	+	+	-	+	+	+	+	+
Thomomys bottae	-	-	-	+	-	-	+	-	+	-	+	+	+	+
Thomomys talpoides	+	+	-	+	+	+	-	-	-	+	+	+	+	+
Thomomys umbrinus	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zygogeomys trichopus	+	+	+	+	+	+	+	+	+	-	+	+	+	-

useful in future analyses investigating population dynamics of *G. breviceps* as well as other pocket gopher species.

CHAPTER III

POPULATION GENETIC ANALYSIS AND CONCLUSION

Introduction

Pocket gophers belonging to the genus *Geomys* have been the subject of a variety of research studies focusing on phylogenetics, systematics, morphology, hybridization, cospeciation, and site fidelity and population structure (e.g., Burt & Dowler 1999; Chambers *et al.* 2009; Demastes & Hafner 1993; Dowler 1989; King 2010; Sudman *et al.* 2006). The Baird's pocket gopher (*G. breviceps*) is distributed across parts of Texas, Arkansas, Oklahoma, and the western portion of Louisiana (Schmidly 2004; Sulentich *et al.* 1991). Although previous research on *G. breviceps* primarily has focused on hybridization with a co-distributed species, *G. attwateri* (Burt & Dowler 1999; Dowler 1989), little is known about the population structure and genetics of this, and other, solitary and fossorial species (Hambuch & Lacey 2000). Since dispersal distances and gene flow among pocket gopher populations are relatively unknown (e.g., Burt & Dowler 1999; Daly & Patton 1990; Hafner *et al.* 1983; Hafner *et al.* 1998; Hambuch & Lacey 2000; Merrit 2010; Meshriy *et al.* 2011; Smith *et al.* 1983; Vaughan 1963), the goal of this study is to provide a preliminary investigation of gene flow among populations of *G. breviceps* using mitochondrial and microsatellite data.

Materials and Methods

Sampling – Fifty specimens of *G. breviceps* were collected from five localities (10 samples per locality) in Brazos and Grimes Counties, Texas (Figure 1; Table 3). All

specimens were collected according to procedures approved by the Texas A&M University Animal Care and Use Committee and the American Society of Mammalogists (Sikes *et al.* 2011). Collected specimens were thoroughly brushed for ectoparasites and tissues were taken and frozen for subsequent molecular work. All specimens are installed in the Texas Cooperative Wildlife Collection at Texas A&M University (Table 3). Distances among *G. breviceps* populations varied from approximately 0.75 to 58.68 km (Table 4).

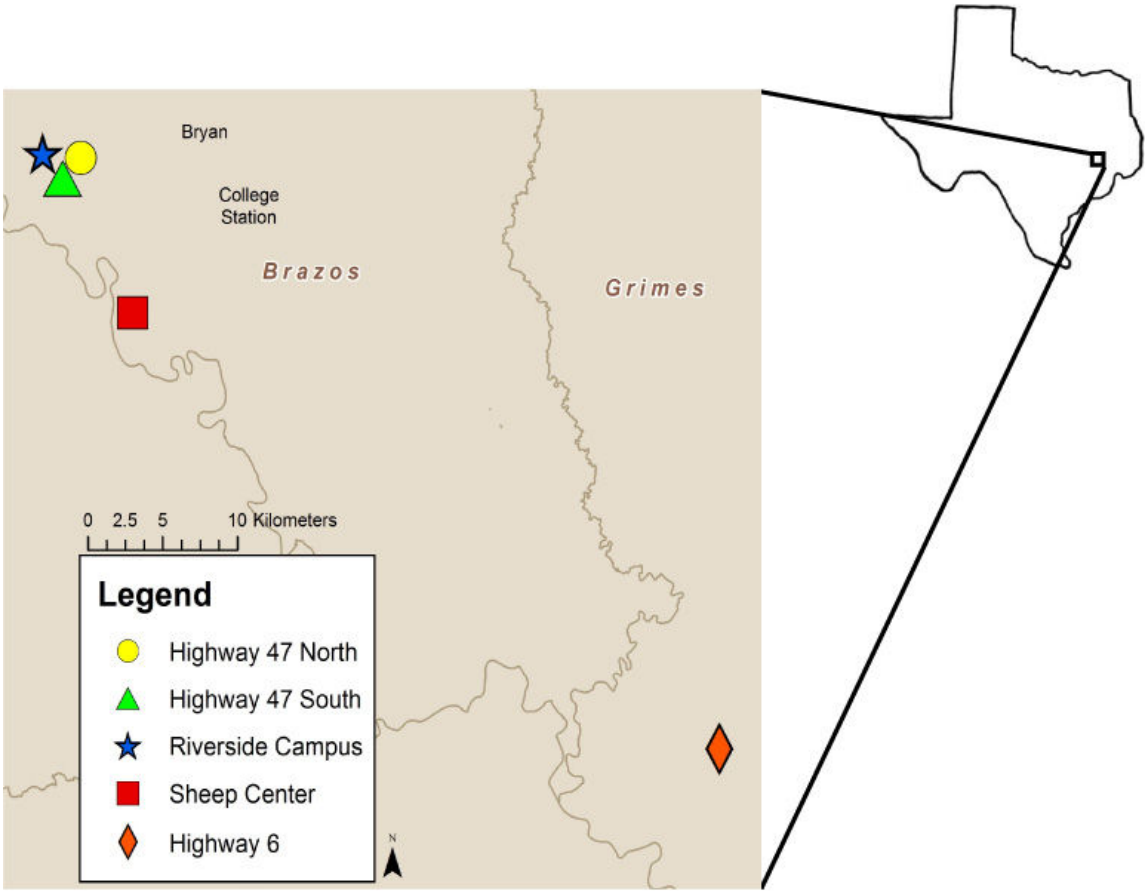


Figure 1 – Localities of the *Geomys breviceps* sampled in this study. Exact locality information is available in Table 3.

Table 3 – List of 50 *Geomys breviceps* specimens and collection numbers according to localities depicted in Figure 1. The Highway 47 localities are separated by a small stretch of highway. Preparation number abbreviations are Jessica E. Light (JEL), Ben D. Marks (BDM), and Sarah R. Welborn (SRW; Texas Cooperative Wildlife Collection numbers pending).

Locality	Preparation Numbers
Highway 47 North	
Texas: Brazos Co., Bryan, Highway 47N 0.7 mi S of Texas Highway 21 exit 30°38.132 N 96°26.859 W	SRW 9, 10, 14-16; JEL 283, 286-288; BDM 1547
Highway 47 South	
Texas: Brazos Co., Bryan, Highway 47S 0.7 mi S of Texas Highway 21 exit 30°38.092 N 96°26.885 W	SRW 3-6; JEL 297, 298, 304-306; BDM 1550
Riverside Campus	
Texas: Brazos Co., Bryan, Texas A&M University Riverside Campus front pasture 30°38.453 N 96°27.722 W	SRW 7, 8, 11-13, 19; JEL 319-322
Sheep Center	
Texas: Brazos Co., College Station, Texas A&M University Sheep Center pastures 30°33.760 N 96°24.548 W	SRW 17, 18, 20-23; JEL 327-330
Highway 6	
Texas: Grimes Co., Navasota, Roadside next to Faith Outreach Center 30°21.485 N 96°03.716 W	SRW 25-34

Table 4 – Rounded distances (shown in kilometers) among the five localities of *Geomys breviceps* used in this study. The Highway 47 localities are separated by a small stretch of highway. Distances were obtained using ArcMap10 (ESRI 2011).

	Highway 47 North	Highway 47 South	Riverside Campus	Sheep Center	Highway 6
Highway 47 North	-				
Highway 47 South	0.750	-			
Riverside Campus	1.850	1.600	-		
Sheep Center	9.975	9.790	11.300	-	
Highway 6	56.550	56.730	58.680	48.102	-

Laboratory Methods – Pocket gopher DNA was extracted from all tissues using a DNeasy Tissue Kit (QIAGEN Inc.; Valencia, California) following the manufacturer's instructions. Portions of two mitochondrial genes were amplified and sequenced: cytochrome oxidase *c* subunit I (COI; 1469 base pairs [bp]) and NADH dehydrogenase 2 (ND2; 827 bp). COI and ND2 were amplified using the primers COI5285 and COI6929 (Spradling *et al.* 2004) and L5219ND2 and H6315ND2 (Sorenson *et al.* 1999), respectively. Polymerase chain reaction (PCR) amplifications were conducted in 25 µL reactions containing 12 µL of water, 10 µL EmeraldAmp®MAX PCR Master Mix (Takara Bio Inc), 1 µL each of the forward and reverse primers, and 1 µL of DNA. Double-stranded PCR amplifications for COI were performed with an initial denaturation of 95°C for 5 min followed by 30 cycles of 95°C (1 min), 49°C (1 min), and 72°C (2 min), and a final extension of 72°C for 5 min. Double-stranded PCR amplifications for ND2 were performed with an initial denaturation of 94°C for 5 min, followed by 30 cycles of 94°C (30 s), 50°C (30 s), and 72°C (90 s), and a final extension of 72°C (5 min). Amplified products were purified using EXOSap-IT (USB Corporation). All sequencing reactions were performed at the University of Florida DNA Sequencing Core Laboratory (following Light & Reed 2009) using the primers listed above as well as the following internal primers for COI: Mco-173f, Mco-1480r and Mco-1345r (Hafner *et al.* 2007), and Gco1F1, Gco1R1 and CO1-570F (Spradling *et al.* 2004). Sequences were edited using Sequencher 4.9 (Gene Codes Corporation, Madison, Wisconsin), and aligned by eye. Se-AL v2.0a11 (Rambaut 1996) was used to

remove primer sequences in reference to translated protein sequences. All sequences will be deposited in GenBank.

Fourteen polymorphic microsatellite loci previously identified in Chapter 2 and Welborn *et al.* (2012) were genotyped for all pocket gophers in each locality. PCR amplifications for each locus were performed following Chapter 2 and Welborn *et al.* (2012). These PCRs included an initial denaturation of 95°C for 3 min, 2 cycles of 95°C (30 s), 58°C (45 s), and 72°C (1 min), 2 cycles of 95°C (30 s), 55°C (45 s), and 72°C (1 min), 2 cycles of 95°C (30 s), 52°C (45 s), and 72°C (1 min), 34 cycles of 95°C (30 s), 50°C (45 s), and 72°C (1 min), and a final extension step of 72°C (10 min). PCR products were loaded onto a polyacrylamide gel and run using an ABI Prism 377 DNA Sequencer (Biosystematics Center, College Station, Texas) to separate and visualize amplification products. Genescan 3.1.2 (Applied Biosystems) was utilized to visualize the gel for analysis and data were imported into GenoTyper 2.5 (Applied Biosystems) for allele-calling. Microsatellite allele scores also were confirmed by eye.

Mitochondrial Analyses – Pairwise distances (uncorrected *p* distances) of mitochondrial data were calculated in PAUP v 4.0 (Swofford 2003) for each gene individually as well as the combined two-gene data set. Population structure also was assessed using an analysis of molecular variance (AMOVA) in Arlequin v. 3.5 (Excoffier *et al.* 2005). Φ statistics (F-statistic analogs) were used to account for varying levels of genetic distance among haplotypes. Pairwise estimation of Φ_{CT} (degree of differentiation among all populations) and Φ_{ST} (degree of differentiation within

populations) were determined with each population predefined by locality and using 10,000 randomization replicates to assess significance.

Number of haplotypes and haplotype diversity was determined using DNAsp, v. 5.10.01 (Rozas *et al.* 2003). Haplotype networks were constructed to visualize relationships among the localities using the program TCS version 1.21 (Clement *et al.* 2000). TCS was used to produce a statistical parsimony network for COI, ND2, and the combined two-gene data set. For all analyses, haplotype connectivity was set to a 95% parsimony criterion (with the assumption of equal weighting among mutations) and all gaps were treated as missing data.

Isolation by distance (IBD) was determined using the program IBDWS v 3.23 (Jensen *et al.* 2005) to test for a correlation between genetic and geographic distances. In all IBD analyses, genetic distance (Φ_{ST}) was used along with map distances obtained from ArcMap10 (ESRI 2011). Analyses were run for 10,000 randomizations and significance was determined statistically through the use of the Mantel test. All IBD analyses were run with the individual COI and ND2 data sets as well as the combined two-gene data set.

Microsatellite Analyses – Microsatellite data were organized per locality and loci and input files were formatted using the program Convert v. 1.31 (Glaubitz 2004). Observed heterozygosity, expected heterozygosity, and Hardy-Weinberg equilibrium were determined with Genepop v. 4.1 (Rousset 2008) and Arlequin v. 3.5 (Excoffier *et al.* 2005). Number of alleles and allelic richness were calculated for each locality in FSTAT v. 2.9.3.2 (Goudet 1995). F_{ST} statistics were calculated for each locality using

Arlequin v. 3.5 (Excoffier *et al.* 2005). Population structure also was assessed using an analysis of molecular variance (AMOVA; Excoffier *et al.* 2005). Each population was predefined by locality and significance was determined by using 10,000 randomization replicates.

The Bayesian-based program Structure 2.2.1 (Pritchard *et al.* 2000) was used to determine the most likely clusters of genetic variation from a predefined *K* (number of clusters as defined by the user). The data were input with an admixture model and 5 runs were performed for clusters $K = 1 - 5$. Each run was completed with Markov chain-Monte Carlo repetitions with a burn-in of 10,000 followed by 100,000 repetition steps (Evanno *et al.* 2005). Structure Harvester v 0.6 (Earl & vonHoldt 2011) was used to determine the ΔK, mean ln Prob(Data) (Evanno *et al.* 2005), and the most likely number of clusters (*K*).

Isolation by distance (IBD) also was determined for microsatellite data using the program IBDWS v 3.23 (Jensen *et al.* 2005) to test for a correlation between genetic and geographic distances. In all IBD analyses, genetic distances (F_{ST}) were used along with map distances obtained from ArcMap10 (ESRI 2011). Analyses were run for 10,000 randomizations and significance was determined statistically through the use of the Mantel test.

Migrate-N v 3.0.3 (Beerli & Felsenstein 1999) was used to estimate levels of gene flow among localities. Initial runs were completed to estimate priors for average long term mutation scaled migration (M) and theta (Θ). The final run was run twice at different starting points with 1 long chain to better verify convergence. Burn-in was set

to 10,000 and was then followed by 500,000 repetitions. A heated chain scheme was used to thoroughly search through parameter space.

Isolation and migration among the five *G. breviceps* localities were examined in IMa (Hey & Nielsen 2007). IMa is based on Markov chain-Monte Carlo simulations of gene genealogies using a Metropolis-Coupled version of the Metropolis-Hastings criterion (Hey & Rasmus 2004). In comparison to Migrate, use of this criterion allows for better mixing of chains by using multiple heated chains to search the parameter space. Chosen final runs consisted of a burn-in of 1,000,000 followed by 90,000 generations with geometric heating and 50 chains. Each run was run twice at different starting seeds to ensure convergence.

Results

Mitochondrial Genetic Variation – For the combined two-gene data set, pairwise distances showed high levels of similarity among localities (Table 5). Uncorrected *p* distances within localities were small ranging from 0.0013 to 0.0088. When analyzed individually, uncorrected *p* distances for COI and ND2 showed comparable levels of genetic similarity as the combined two-gene data set (results available upon request).

Results from AMOVA analyses of the combined two-gene data set showed significant signs of population structure among the five *G. breviceps* localities (Table 6). Initial AMOVAs were run to look at variation among localities that were less than 2 km apart: Highway 47 North and Highway 47 South as well as Highway 47 North, Highway 47 South, and the Riverside Campus localities (Table 4). For the Highway 47 North and South comparisons, there were high levels of variation within populations (91%) and

Table 5 – Uncorrected *p* distances compared among localities of *Geomys breviceps* from the combined two-gene mtDNA data set.

	Highway 47 North	**Highway 47 South**	**Riverside Campus**	**Sheep Center**	**Highway 6**
Highway 47 North	-				
Highway 47 South	0.0082	-			
Riverside Campus	0.0099	0.0078	-		
Sheep Center	0.0090	0.0070	0.0067	-	
Highway 6	0.0118	0.0112	0.0136	0.0125	-

Table 6 – AMOVA among five, four, and three *Geomys breviceps* localities using the two-gene mitochondrial data set (see text for explanation of population assignment). Significance of variance component (*P*) was tested by permutation according to Excoffier *et al.* (2005).

Source of Variation	**Variance Components**	**% of Variation**	**Fixation Index**	***P* value**
All 5 Localities				
Among Populations	3.964	37.095	Φ_{CT} =0.165	$P < 0.0001$
Within Populations	6.723	62.905	Φ_{ST} = 0.827	$P < 0.0001$
4 Localities (Highway 47 North and Highway 47 South grouped)				
Among Populations	4.649	40.148	Φ_{CT} = 0.172	$P < 0.0001$
Within Populations	6.930	59.852	Φ_{ST} = 0.825	$P < 0.0001$
3 Localities (Highway 47 North, Highway 47 South and Riverside Campus grouped)				
Among Populations	5.446	40.885	Φ_{CT} = 0.182	$P < 0.0001$
Within Populations	7.874	59.115	Φ_{ST} = 0.818	$P < 0.0001$

low and nonsignificant levels of variation among populations, suggesting that these two localities can be grouped together. For the Highway 47 North, Highway 47 South and Riverside comparison, although there again were high levels of variation within populations (82%), variation among populations was significant, indicating population structure. To be conservative in the assessment of population structure, AMOVAs were run with all 5 localities, 4 localities (grouping Highway 47 North and Highway 47 South together) and 3 localities (grouping Highway 47 North, Highway 47 South, and Riverside Campus together). For all comparisons, pairwise estimations of Φ_{CT} and Φ_{ST} were significant with the majority of the variation within the populations (variation between populations increased when populations less than 2 km apart were grouped together; Table 6). AMOVAs for both the COI and ND2 data sets were similar to those of the combined two-gene data set (results available upon request).

Number of haplotypes (and haplotype diversity) for COI, ND2 and combined two-gene data sets were 17 (0.9143), 16 (0.92), and 30 (0.9673), respectively. All 3 haplotype networks show extensive haplotype sharing among Highway 47 North, Highway 47 South, and Riverside localities (Figure 2). Individuals from the Sheep Center locality were represented by 4 or 6 haplotypes (depending on the data set), with only one instance of haplotype sharing in the ND2 data set. Individuals from the Highway 6 locality were represented by 2 or 3 haplotypes (depending on the data set), with no evidence of haplotype sharing with the other localities (Figure 2).

IBD analyses showed a significant relationship between genetic and geographic distance in both the individual gene and combined data sets. Correlation coefficients (r)

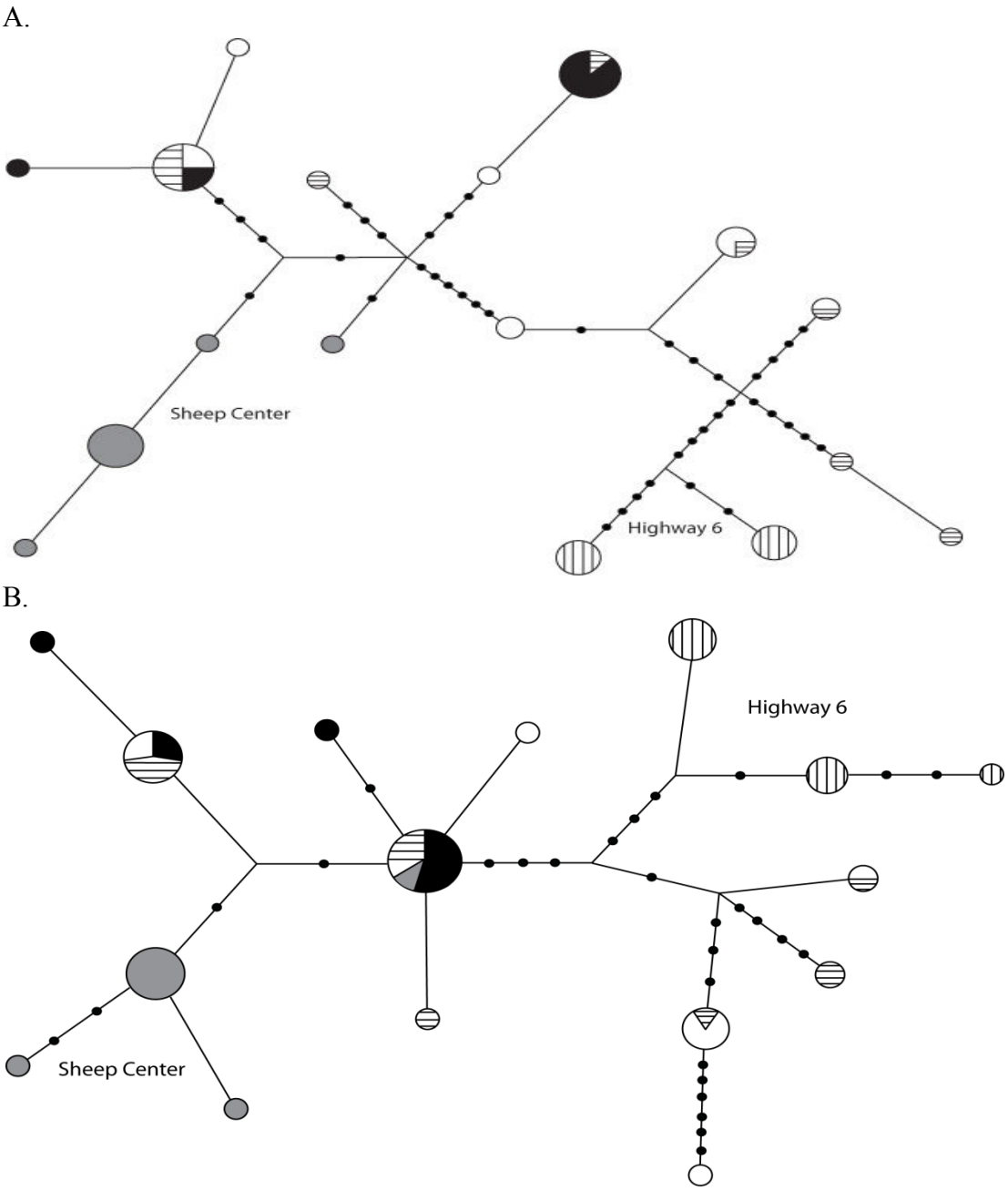


Figure 2 – Statistical parsimony haplotype networks inferred from mitochondrial data among 5 localities of *Geomys breviceps* using TCS 1.21 (Clement *et al.* 2000). Each connection represents a mutation step with all inferred haplotypes shown as small black circles. Observed haplotpes are shown as larger circles proportional to the number of specimens. Localities are represented by different colors and patterns: Highway 47 North in white, Highway 47 South in horizontal stripes, Riverside Campus in black, Sheep Center in grey, and Highway 6 in vertical stripes. A) COI data set. B) ND2 data set. C) Combined two-gene data set.

C.

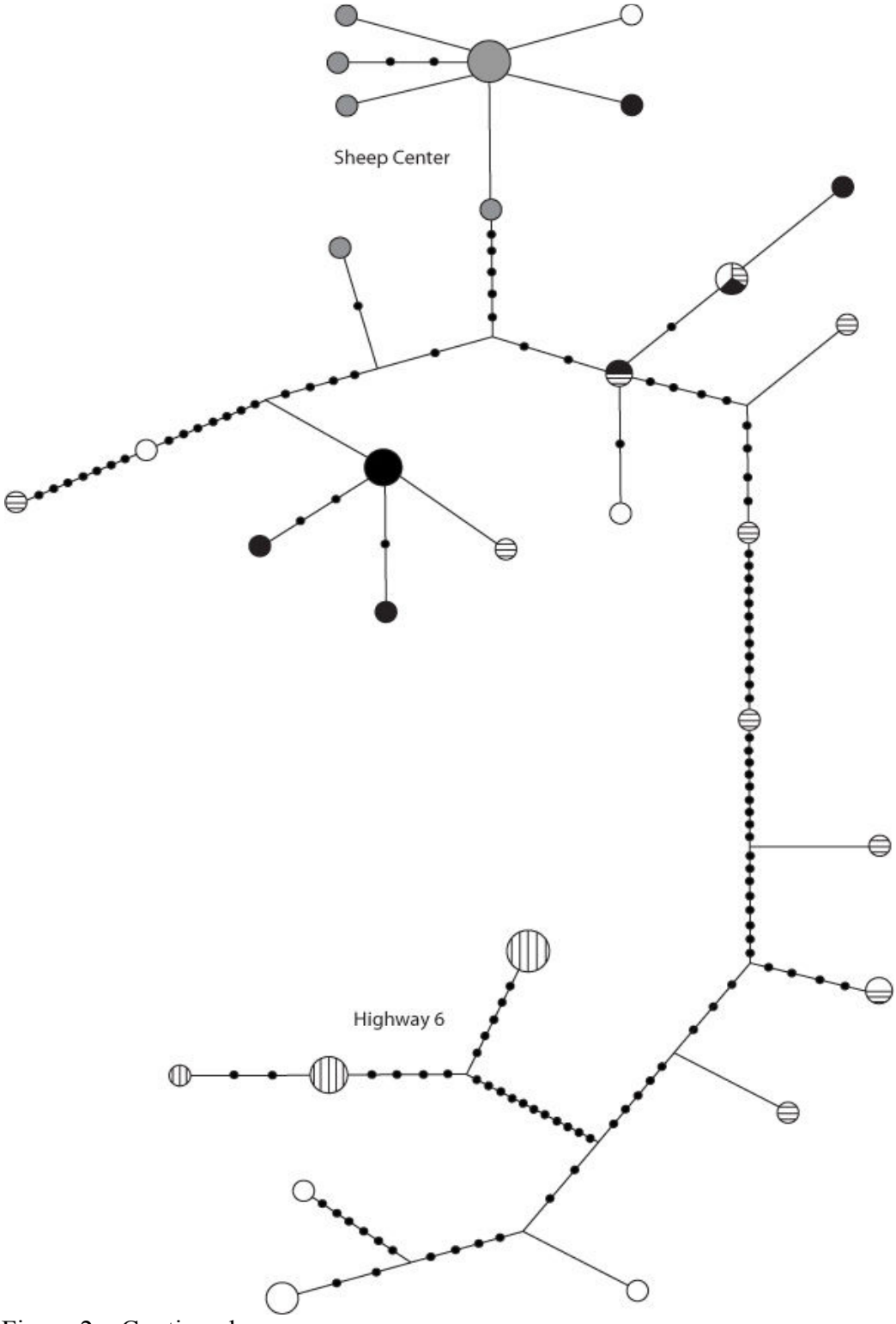


Figure 2 – Continued.

and their corresponding significance values for COI, ND2, and the combined two-gene data set were 0.6985 ($P = 0.008$), 0.7793 ($P = 0.039$), and 0.7906 ($P = 0.008$), respectively.

Microsatellite Genetic Variation – General summary data from the microsatellite data (Table 7) indicate that locus Tm6 was monomorphic for Riverside Campus and loci Tm2 and Tm6 were monomorphic for Sheep Center. Individuals from Highway 47 North, Highway 47 South and Sheep Center showed signs of significant deviation from Hardy-Weinberg equilibrium at locus Gbr26. Individuals from Highway 6 also showed signs of significant deviation from Hardy-Weinberg equilibrium at loci Tm1 and Tm6. Results from preliminary Structure and AMOVA analyses (available upon request) did not differ if run with or without the loci deviating from Hardy-Weinberg equilibrium, therefore all loci were included in all subsequent analyses. Similar analyses were also run with and without the loci showing signs on null alleles and linkage disequilibrium seen in Welborn *et al.* (2012). The results did not differ and thus these loci were included in the subsequent analyses as well. The most polymorphic loci were Gbr26 and Tm7 with 17 alleles, the least polymorphic locus was Gbr36 with 2 alleles, and allelic richness ranged from 2 to 10 (Table 7). For future microsatellite analyses, all Gbr loci and Tm 7 proved to be informative and will be useful for future studies.

Results from AMOVA analyses showed significant variation among populations and individuals of the five *G. breviceps* localities (Table 8). Similar to the mitochondrial analyses, initial AMOVAs were run to look at variation among localities that were less

Table 7 – Summary statistics for 14 microsatellites from five localities of *Geomys breviceps*. Abbreviations are as follows: number of alleles (N_A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), and the probability of conforming to Hardy-Weinberg equilibrium (P_{HW}). Bolded numbers represent loci that deviate from Hardy-Weinberg equilibrium (see text).

Locality	**Gbr06**	**Gbr09**	**Gbr10**	**Gbr14**	**Gbr15**	**Gbr25**	**Gbr26**
Highway 47 North							
N_A	6	4	6	3	8	10	8
A_R	6	4	6	3	8	10	8
H_O	0.900	0.800	0.800	0.600	0.900	0.800	0.700
H_E	0.811	0.700	0.821	0.574	0.816	0.842	0.805
P_{HW}	0.081	0.284	0.195	1.000	0.809	0.220	**0.004**
Highway 47 South							
N_A	4	6	7	3	7	8	8
A_R	4	6	7	3	7	8	8
H_O	1.000	1.000	0.700	0.900	0.700	0.600	0.300
H_E	0.763	0.821	0.847	0.668	0.795	0.805	0.836
P_{HW}	0.502	0.366	0.127	0.010	0.134	0.382	**0.001**
Riverside Campus							
N_A	4	7	4	3	6	5	8
A_R	4	7	4	3	6	5	8
H_O	0.900	0.900	0.500	0.900	0.800	0.800	0.900
H_E	0.689	0.858	0.668	0.647	0.789	0.621	0.874
P_{HW}	0.054	0.612	0.117	0.068	0.507	0.348	0.475
Sheep Center							
N_A	5	6	4	3	5	4	9
A_R	5	6	4	3	5	4	9
H_O	0.600	1.000	0.700	1.000	0.800	0.700	0.500
H_E	0.742	0.847	0.553	0.611	0.789	0.774	0.816
P_{HW}	0.273	0.029	1.000	0.011	0.927	0.563	**0.005**
Highway 6							
N_A	3	5	3	2	4	5	5
A_R	3	5	3	2	4	5	5
H_O	0.700	0.800	0.400	1.000	0.300	0.700	0.600
H_E	0.668	0.726	0.352	0.526	0.616	0.805	0.663
P_{HW}	0.182	0.241	1.000	0.010	0.010	0.212	0.387

Table 7 – Continued.

Population	Gbr27	Gbr33	Gbr36	Tm1	Tm2	Tm6	Tm7
Highway 47 North							
N_A	2	4	2	5	2	2	8
A_R	2	4	2	5	2	2	8
H_O	0.200	0.300	0.100	0.700	0.100	0.600	0.700
H_E	0.189	0.689	0.100	0.716	0.100	0.505	0.842
P_{HW}	1.000	0.020	1.000	0.949	1.000	1.000	0.010
Highway 47 South							
N_A	3	6	2	7	2	2	7
A_R	3	6	2	7	2	2	7
H_O	0.700	0.700	0.400	0.900	0.100	0.100	0.600
H_E	0.532	0.779	0.337	0.821	0.100	0.479	0.774
P_{HW}	0.505	0.658	1.000	0.392	1.000	0.022	0.206
Riverside Campus							
N_A	3	6	2	5	2	1	4
A_R	3	6	2	5	2	1	4
H_O	0.300	0.800	0.200	1.000	0.200	-	0.400
H_E	0.279	0.821	0.189	0.805	0.189	-	0.695
P_{HW}	1.000	0.887	1.000	0.125	1.000	-	0.031
Sheep Center							
N_A	3	6	2	7	1	1	10
A_R	3	6	2	7	1	1	10
H_O	0.300	0.600	0.800	1.000	-	-	0.800
H_E	0.479	0.763	0.505	0.842	-	-	0.868
P_{HW}	0.479	0.050	0.173	0.027	-	-	0.531
Highway 6							
N_A	2	4	2	7	2	4	6
A_R	2	4	2	7	2	4	6
H_O	0.300	0.500	1.000	1.000	0.200	0.100	0.900
H_E	0.395	0.432	0.526	0.853	0.189	0.647	0.737
P_{HW}	0.480	1.000	0.010	**0.001**	1.000	**0.001**	0.017

Table 8 – AMOVA among five, four, and three *Geomys breviceps* localities using microsatellite data (see text for explanation of locality assignment). Significance of variance component (*P*) was tested by permutation according to Excoffier *et al.* (2005).

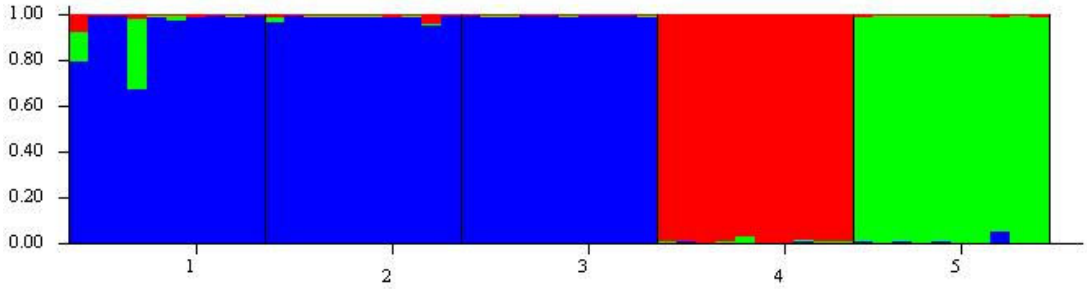
Source of Variation	Variance Components	% of Variation	Fixation Index	*P* value
All 5 Localities				
Within Populations	0.732	14.612	$F_{IS} = 0.000$	$P < 0.5578$
Among Populations	0.001	0.001	$F_{ST} = 0.146$	$P < 0.0001$
Among Individuals	4.280	85.388	$F_{IT} = 0.144$	$P < 0.0001$
4 Localities (Highway 47 North and Highway 47 South grouped)				
Within Populations	0.782	15.407	$F_{IS} = 0.003$	$P < 0.4821$
Among Populations	0.013	0.251	$F_{ST} = 0.154$	$P < 0.0001$
Among Individuals	4.280	84.342	$F_{IT} = 0.157$	$P < 0.0001$
3 Localities (Highway 47 North, Highway 47 South and Riverside Campus grouped)				
Within Populations	0.794	15.254	$F_{IS} = 0.030$	$P < 0.1000$
Among Populations	0.133	2.558	$F_{ST} = 0.153$	$P < 0.0001$
Among Individuals	4.280	82.188	$F_{IT} = 0.178$	$P < 0.0001$

than 2 km apart: Highway 47 North and Highway 47 South as well as Highway 47 North, Highway 47 South, and the Riverside Campus populations. These initial runs showed high levels of variation within populations (93% for both; i.e., low levels of variation among populations). As in the mitochondrial analyses, variation among the Highway 47 North and Highway 47 South populations was not significant, suggesting that these localities can be grouped together. In contrast, variation among the Highway 47 North, Highway 47 South and Riverside populations was significant, indicating population structure. However, to be conservative in the assessment of population structure AMOVAs were run using all 5 localities, 4 localities (grouping Highway 47 North and Highway 47 South together) and 3 localities (grouping Highway 47 North, Highway 47 South, and Riverside Campus together). Regardless of how the localities were grouped, the majority of the variation remained among populations (F_{ST}) and among individuals (F_{IT}) where estimations were significant. Variations within population (F_{IS}) estimations were not significant (Table 8).

A *K* of 3 was determined to be the most likely set of genetic clusters in Structure and Structure Harvester with a Δ ln Prob(Data) = 192.86. In this analysis, Highway 47 North, Highway 47 South and Riverside Campus were clustered together to form one defined group (Figure 3A). A *K* of 4 was also likely (Figure 3B), but did not score as well as 3 clusters with Δ ln Prob(Data) = 60.19. A *K* of 1 and a *K* of 5 were determined to be the least likely.

IBD analyses showed a significant relationship between genetic and geographic distances. The correlation coefficient (r) for the microsatellite dataset was 0.7655, with a significance value (*P*) of 0.008.

A.



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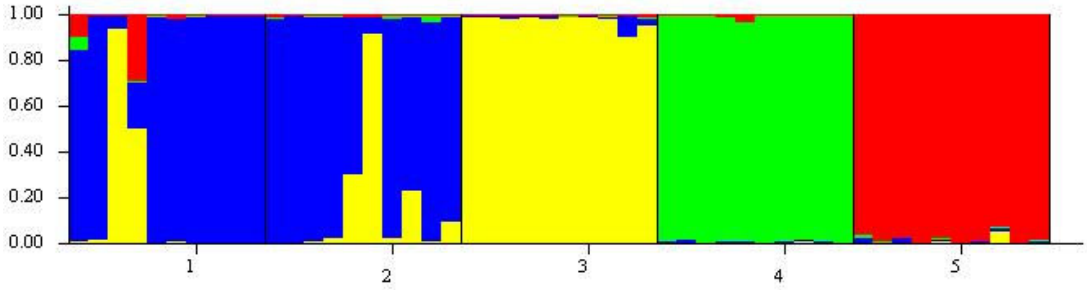

Figure 3 – Bar plot produced by Structure utilizing microsatellite data from all five *Geomys breviceps* localities. The Highway 47 North, Highway 47 South and Riverside Campus localities are grouped together suggesting 3 genetic population clusters. A) $K = 3$. B) $K = 4$.

Migrate-N estimations of levels of gene flow were estimated for groupings as presented in AMOVA analyses above: all 5 localities, 4 localities (grouping Highway 47 North and Highway 47 South together), and 3 localities (grouping Highway 47 North, Highway 47 South, and Riverside Campus together). Migrate-N results for all locality

groupings were similar, with results from the 3 locality data set presented below (other results are available upon request). Estimates of average long term mutation scaled migration (M) were moderate ranging from 0.241 to 0.3117 (Table 9) and thetas (Θ) ranged from 2.201 to 3.925 (Table 10) for the Highway 47 North, Highway 47 South and Riverside Campus grouping, Sheep Center, and Highway 6. When gene flow was estimated among 4 or 5 localities, estimates of average long term mutation scaled migration were moderate when examined among localities less than 2 km apart (ranging from 0.398 to 0.464). These estimates increased to 0.520 to 0.701 when comparing only Highway 47 North, Highway 47 South and Riverside Campus, and to 0.693 to 0.732 when comparing only Highway 47 North to Highway 47 South, indicating that some or all of these localities are functioning as one population. It is important to note that in these comparisons of localities less than 2 km apart, the confidence intervals were extremely large and overlapping which makes interpretation of average long term mutation scaled migration unclear.

The program IMa (Hey & Nielsen 2007) also was utilized to further investigate levels of migration. However, after continually altering parameters to obtain adequate mixing of Markov chains in the MCMC mode portion of the analysis, stable estimates of most of the parameters were not obtained. Without adequate mixing, parameter estimates are unreliable, thus Load-trees mode analyses were not performed.

Table 9 – Estimated average long term mutation scaled migration (M) results from Migrate-N comparing 3 localities of *Geomys breviceps* (grouping Highway 47 North, Highway 47 South and Riverside Campus together, as represented by NSR). 95% confidence intervals (95% CI) also are shown.

Population Comparison	M	95% CI
NSR to Sheep Center	0.289	0.104 – 0.490
NSR to Highway 6	0.317	0.111 – 0.553
Sheep Center to NSR	0.299	0.129 – 0.483
Sheep Center to Highway 6	0.282	0.098 – 0.486
Highway 6 to NSR	0.276	0.111 – 0.456
Highway 6 to Sheep Center	0.241	0.071 – 0.426

Table 10 – Theta (Θ) results from Migrate-N comparing 3 localities of *Geomys breviceps* (grouping Highway 47 North, Highway 47 South and Riverside Campus together, as represented by NSR). 95% confidence intervals (95% CI) also are shown.

Population	Θ	95% CI
NSR	3.925	3.012 – 4.872
Sheep Center	3.147	2.028 – 4.380
Highway 6	2.201	1.440 – 3.012

Discussion

Molecular population genetics can be used to investigate gene flow, migration rates, dispersal distances, effective population sizes, and much more. Unfortunately, these studies focusing on a solitary species with low vagility, such as the Baird's pocket gopher, are rare (e.g., Hambuch & Lacey 2000). Analysis of molecular data using modern analytical programs can elucidate the role that morphological and behavioral modifications have on population structure and gene flow within solitary and territorial species. This study found that both mitochondrial and microsatellite data were informative when trying to better understand population processes in *G. breviceps*.

For both mitochondrial and microsatellite data, there are high levels of gene flow among nearby populations (less than 2 km apart), with decreasing gene flow with increasing distance (after approximately 9 km for this study; Tables 5-8). Given their proximity, the findings presented here indicate that Highway 47 North, Highway 47 South and most likely Riverside Campus act as one functioning population, or genetic cluster (Tables 5-8; Figures 2 and 3). Higher estimates of average long term mutation scaled migration (M) amongst Highway 47 North, Highway 47 South and Riverside Campus help support the grouping of these localities. Although migration rates cannot be determined due to large confidence intervals, there does appear to be some migration occurring among all the localities (Table 9). From these results we can suggest that there are 3-4 genetic clusters; with Sheep Center and Highway 6 each being a separate cluster and Highway 47 North, Highway 47 South and possibly Riverside Campus being a genetic cluster.

The results of this study can help to provide insight regarding movement and dispersal of the Baird's pocket gopher. The high levels of gene flow among the Highway 47 localities and Riverside Campus suggest that the highway that separates these localities does not hinder their movement. The soil under highways is often extremely compacted and rocky and could hinder burrowing (Griscom *et al.* 2010), suggesting that above-ground movement would be necessary. Highway 47 (which separates Highway 47 North and Highway 47 South) was constructed in 1987, supporting above ground movement amongst these localities for at least 20 years (Estridge 2008). Even with limited mobility above the soil due to their morphological adaptations for a fossorial

lifestyle (Merrit 2010; Stein 2000; Sulentich *et al.* 1991), the results presented herein support that *G. breviceps* is able to move distances of at least 2 km above ground. Further studies examining additional localities will be necessary to determine the largest possible distance *G. breviceps* can move above ground. These analyses also support that levels of gene flow are reduced when comparing the Highway 6 population to the other 4 populations which are separated by over 48 km. However, the evidence that there is some gene flow occurring among all 5 localities suggests that *G. breviceps* may be capable to moving large distances or that there was once a much large, continuous population of *G. breviceps* throughout the Brazos valley.

Isolation by distance analyses can be used to investigate evidence of sex-biased dispersal when comparing mitochondrial and nuclear DNA. High levels of isolation by distance would suggest that dispersal would be infrequent; whereas low levels of isolation by distance would suggest just the opposite for either females or males depending on the data examined. Significant levels of isolation by distance are seen here in both mitochondrial and microsatellite data sets, with high correlations between geographic and genetic distances. This suggests that as geographic distance increases, genetic differentiation among populations also increases, supporting decreasing levels of dispersal at farther distances. Unfortunately with significant results for both mitochondrial and microsatellite analyses, these data cannot be used to determine if *G. breviceps* undergoes male or female biased dispersal.

In their examination of *G. attwateri,* Williams and Cameron (1984) found that the majority of the dispersing individuals were juveniles caught in above-ground traps.

Above-ground dispersal by juveniles also was seen in *Thomomys* (Howard & Childs 1959), with dispersal distances up to 500 m depending the upon surrounding environment (Daly & Patton 1990; Hafner *et al.* 1983; Hafner *et al.* 1998; Smith *et al.* 1983; Vaughan 1963). In King's study (2010) focusing on site fidelity and population structure, she found high site fidelity in that population of *G. breviceps* and that juveniles moved the largest distances (up to 46 m, although maximum dispersal distance is unknown due to the limited geographic scale of this study). Although this study cannot address dispersal specifically, results indicate that *G. breviceps* may be capable of dispersing large distances. Further studies will be necessary to better understand dispersal distances and who is doing the dispersing (males versus females and juveniles versus adults).

Conclusion

Overall, a general relationship between distance and level of gene flow is seen in this study of the Baird's pocket gopher: as the distance among localities increases, levels of gene flow decrease. Results from this research can aid in better understanding levels of gene flow within *G. breviceps*, and possibly be useful in understanding population processes in fossorial mammals. Future research should aim to include more localities as well as more sampling at each locality. Similar research should also be conducted on other pocket gopher species as well as other fossorial species. This would allow for a better understanding of population structure in such species as well as the process of speciation, gene flow and the dispersal of both solitary and social fossorial species.

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