Population genetic structure of the Baird's pocket gopher, *Geomys breviceps*, in eastern Texas

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Pocket gophers are a group of solitary and fossorial rodents classified in the family Geomyidae. There are 6 genera and approximately 40 species of pocket gophers distributed throughout North and Central America (Merrit 2010). Pocket gophers belonging to the genus *Geomys* have been the subject of a variety of research studies focusing on phylogenetics, systematics, morphology, hybridization, cospeciation, site fidelity, and population structure (e.g., Dowler 1989, Demastes and Hafner 1993, Burt and Dowler 1999, Sudman et al. 2006, Chambers et al. 2009, King 2010). The Baird’s pocket gopher (*Geomys breviceps*), is common throughout the Brazos Valley region of Texas, and its larger distribution includes eastern Texas, Arkansas, Oklahoma, and western Louisiana (Sulentich et al. 1991, Schmidly 2004). Similar to other pocket gophers, *G. breviceps* is highly modified morphologically for a fossorial lifestyle (Sulentich et al. 1991, Merrit 2010). Morphological specializations for digging include large ever-growing incisors and increased muscle mass and large, long claws at the anterior end of their bodies (Stein 2000). These anatomical modifications enable pocket gophers to dig elaborate and narrow burrow systems below the surface of the soil (Sulentich et al. 1991). Within these burrow systems, pocket gophers spend the majority of their lives building new tunnels and sealing tunnels that are no longer in use (Howard and Childs 1959, Sulentich et al. 1991).

Due to their level of specialization and overall morphology, *G. breviceps* and other pocket gopher species have relatively low vagility outside of their burrow systems ( Patton et al. 1972, Nevo 1979, Patton and Feder 1981). Previous studies of pocket gophers have found that general activity is confined to the burrow system, with aboveground activity...
restricted to dispersal events and short foraging excursions (Howard and Childs 1959, Teipner et al. 1983, Connior and Risch 2010). The inability of pocket gophers to move well outside of the burrow system can translate to reduced dispersal capabilities, isolated populations, small effective population sizes, and limited gene flow among populations (Patton and Feder 1981, Hafner et al. 1983, Williams and Cameron 1984, Hafner et al. 1998, Burt and Dowler 1999, Connior and Risch 2010), and can result in the development and persistence of isolated populations (Burt and Dowler 1999), which can subsequently reduce heterozygosity (Williams and Baker 1976).

Despite the unique morphological adaptations and reduced genetic diversity of solitary species with isolated populations, little is known about pocket gopher population genetics. Herein, we use mitochondrial and microsatellite data to investigate population genetics among a series of localities of the Baird’s pocket gopher found in and around the Brazos Valley, Texas. Determining the population genetic structure of *G. breviceps* populations may help to elucidate the role of morphological and behavioral modifications in structuring populations and gene flow within a solitary and territorial species.

**METHODS**

Fifty specimens of *G. breviceps* were collected from 5 localities (10 specimens per locality) in Brazos and Grimes counties, Texas (Appendix 1). The 5 localities (Highway 47 North, Highway 47 South, Riverside Campus, Sheep Center, and Highway 6) were separated by distances ranging between 0.75 km and 58.68 km (Appendix 2). Notably, *G. breviceps* hybridizes with the distantly related Attwater’s pocket gopher (*Geomys attwateri*) west of the Brazos River in Burleson and Milam counties, Texas (Honeycutt and Schmidly 1979, Tucker and Schmidly 1981, Dowler 1989, Burt and Dowler 1999). All specimens included in this study were located east of the Brazos River, ruling out the possibly of sampling *G. attwateri* or hybrids. Furthermore, all molecular data gathered as part of this study match unambiguously to *G. breviceps* (i.e., GenBank BLAST searches and unpublished data). 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). All collected specimens were deposited in the Biodiversity Research and Teaching Collections at Texas A&M University (Appendix 1).

Pocket gopher DNA was extracted from all tissues by using a DNeasy Tissue Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer’s instructions. Portions of 2 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 and Reed 2009) using the primers listed above and 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, WI) and aligned by eye. Se-Al v2.0a11 (Rambaut 1996) was used to remove primer sequences in reference to translated protein sequences. All sequences were submitted to GenBank (GenBank accession numbers KF542692–KF542741 for COI and KF542742–KF542791 for ND2).

All mitochondrial analyses were performed on each gene individually and the combined 2-gene data set (COI and ND2). Pairwise distances (uncorrected p distances) of mitochondrial data were calculated in PAUP* version...
4.0b10 (Swofford 2002). Number of haplotypes and haplotype diversity was determined using DNAsp, v. 5.10.01 (Rozas et al. 2003). Haplotype networks were constructed using the program TCS version 1.21 to visualize relationships among the localities (Clement et al. 2000). In TCS, 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. Population structure was assessed using an analysis of molecular variance (AMOVA) in Arlequin v. 3.5 (Excoffier et al. 2005). F statistics (F-statistic analogs) were used to account for varying levels of genetic distance among haplotypes, and pairwise estimation of F_CT (degree of differentiation among all populations) and F_ST (degree of differentiation within populations) were determined using 10,000 randomization replicates to assess significance, with each population predefined by locality (Appendix 1). 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 (F_ST) was used along with distances obtained from ArcMap10 (ESRI 2011). Analyses were run for 10,000 randomizations, and significance was determined statistically through use of a Mantel test.

Ten polymorphic microsatellite loci (Gbr06, Gbr09, Gbr10, Gbr14, Gbr15, Gbr25, Gbr26, Gbr27, Gbr33, Gbr36), previously identified in Welborn et al. (2012), and 4 polymorphic microsatellite loci (Tm1, Tm2, Tm6, Tm7), previously identified in Steinberg (1999), were genotyped for all pocket gophers at each locality. PCR amplifications for each locus were performed following 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, TX) 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 allelcalling. Microsatellite allele scores also were confirmed by eye.

Microsatellite data were organized per locality, and locus 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 AMOVA (Excoffier et al. 2005), in which each population was predefined by locality and significance was determined using 10,000 randomization replicates.

Spatial genetic analyses, using pairwise geographic and genetic distances, were performed using the genetic spatial autocorrelation option (Peakall et al. 2003, Banks and Peakall 2012) in GenAlEx 6.4 (Peakall and Smouse 2006, 2012). The genetic spatial autocorrelation option uses pairwise comparisons to estimate r, an autocorrelation coefficient, for specified distance classes. Given the distances among the 5 collection localities (Appendix 2), distance classes were estimated at 2-km intervals up to 60 km to determine the geographic distances for which spatial autocorrelation is significant (Cullingham et al. 2008). Permutation and bootstrapping (999 iterations) were used to test the hypothesis of no spatial structure. Analyses were conducted for males and females (n = 50), females only (n = 33), and males only (n = 17) to test for sex-biased philopatry.

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 and vonHoldt 2012) 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) was also determined for microsatellite data using the program IBDWS v 3.23 (Jensen et al. 2005) as described above, except genetic distances (F_ST) were used to test for a correlation between genetic and geographic distances. Migrate-N v 3.0.3 (Beerli and Felsenstein 1999) was used to estimate levels of gene flow among localities. Initial runs were completed to estimate
priors for $M$ (mutation-scaled migration rate) and $\theta$ (theta). The final run was performed twice at different starting points with one 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.

Results

Results for the individual mitochondrial gene (COI and ND2) and the combined 2-gene data set were similar. Only the results for the combined 2-gene data set are presented here; individual gene results are available upon request. Pairwise distances of the combined 2-gene data set showed high levels of similarity among localities, ranging from 0.007 to 0.012. Uncorrected $p$ distances within localities were small, ranging from 0.007 to 0.001. Number of haplotypes (and haplotype diversity) for the combined 2-gene data set was 20 (0.9257) and the haplotype network showed extensive haplotype sharing among the Highway 47 North, Highway 47 South, and Riverside localities. The Sheep Center and Highway 6 localities were represented by 2 and 4 haplotypes, respectively (haplotype network available on Figshare: http://dx.doi.org/10.6084/m9.figshare.769352). Initial AMOVA analyses were run to examine variation among localities that were <2 km apart (Appendix 2): Highway 47 North and Highway 47 South, as well as Highway 47 North, Highway 47 South, and the Riverside Campus localities. For the Highway 47 North and South comparisons, there were high levels of variation within populations (100%) and low and nonsignificant levels of variation among populations, suggesting that these 2 localities can be grouped together. For the Highway 47 North, Highway 47 South, and Riverside comparison, although there were high levels of variation within populations (86%), variation between Riverside Campus and both Highway 47 populations was significant, indicating population structure. To be conservative in the assessment of population structure, AMOVA analyses 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). Results from AMOVA analyses of the combined 2-gene data set showed significant signs of population structure among the 5 $G.\ breviceps$ localities (Table 1). For all comparisons, pairwise estimations of $\Phi_{CT}$ were significant, with more variation apparent among the populations (variation among populations increased when populations <2 km apart were grouped together; Table 1). IBD analyses showed a significant relationship between genetic and geographic distances (correlation coefficient $r = 0.5713, P = 0.008$).

General summary data from the microsatellite data show that all 14 loci were polymorphic except locus Tm6 at Riverside Campus and loci Tm2 and Tm6 at Sheep Center (summary statistics available on Figshare: http://dx.doi.org/10.6084/m9.figshare.769352). The most polymorphic loci were Gbr26 and Tm7, with 17 alleles, and the least polymorphic locus was Gbr36, with 2 alleles. The number of alleles per locus and allelic richness ranged from 2 to 10. Observed heterozygosity was lowest for Highway 47 North ($H_{O} = 0.100$ at Gbr36 and Tm2), Highway 47 South ($H_{O} = 0.100$ at Tm2), and Highway 6 ($H_{O} = 0.100$ at Tm6), and expected heterozygosity was lowest for Highway 47 North ($H_{E} = 0.100$ at Gbr36 and Tm2). 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 did not differ when run with or without the loci deviating from Hardy–Weinberg equilibrium (results available upon request); therefore, all loci were included in all subsequent analyses. Similar analyses were run with and without loci that showed signs of null alleles and linkage disequilibrium in Welborn et al. (2012). Results did not differ, and thus these loci were included in the subsequent analyses.

Results from AMOVA analyses of the microsatellite data showed significant variation among populations and individuals of the 5 $G.\ breviceps$ localities (Table 1). Similar to the mitochondrial analyses, initial AMOVA analyses were run to examine variation among localities that were <2 km apart: Highway 47 North and Highway 47 South, as well as Highway 47 North, Highway 47 South, and the Riverside...
Campus localities. These initial runs showed high levels of variation within populations (93% for both comparisons). As in the mitochondrial analyses, variation among the Highway 47 North and Highway 47 South localities was not significant, suggesting that these localities can be grouped together. In contrast, variation among the Highway 47 North, Highway 47 South, and Riverside localities was significant, indicating population structure. However, to be conservative in the assessment of population structure, AMOVA analyses were run using all 5 localities, 4 localities, and 3 localities (see mitochondrial genetic variation in Table 1). Regardless of how the localities were grouped, there was significant variation among populations \( F_{ST} \) and among individuals \( F_{IT} \), whereas variation within populations \( F_{IS} \) was not significant (Table 1).

A significant positive spatial autocorrelation was found for all samples and all data sets (females plus males, females only, and males only) collected at distances of 2 km. For distances >10 km, significant negative spatial autocorrelations were found for most data sets. The only exceptions were nonsignificant and positive (but close to zero at 0.002) autocorrelations for males at 10 km, nonsignificant and positive (but close to zero at 0.018) autocorrelations for females at 42 km, and nonsignificant yet negative autocorrelations for females plus males at 42 km.

A \( K \) of 3 was the most likely set of microsatellite genetic clusters in Structure and Structure Harvester, with a \( \Delta \ln \text{Prob(Data)} \) of 192.86. In this analysis, Highway 47 North, Highway 47 South, and Riverside Campus were clustered together to form one defined group (Fig. 1A). A \( K \) of 4 also was likely (Fig. 1B) but did not score as well as 3 clusters, with a \( \Delta \ln \text{Prob(Data)} \) of 60.19. A \( K \) of 1 and a \( K \) of 5 were the least likely. IBD analyses showed a significant relationship between genetic and geographic distances (correlation coefficient \( r = 0.7655, P = 0.008 \)). Migrate-N estimations of levels of gene flow were performed for groupings as presented in AMOVA analyses above: all 5 localities, 4 localities, and 3 localities; all analyses produced similar results (Table 2). Estimates of \( M \) (mutation-scaled migration rate) ranged from 0.241 to 0.317, and estimates of \( \theta \) (theta) ranged from 2.021 to 3.925 (Table 2). Estimates of \( M \) were moderate when examined among localities <2 km apart (range 0.398–0.464). These estimates increased to 0.520–0.701 when comparing
only Highway 47 North, Highway 47 South, and Riverside Campus, and to 0.693–0.732 when comparing only Highway 47 North to Highway 47 South. These high M estimates indicate that some or all of these localities may be functioning as one population. However, it is important to note that the confidence intervals were extremely large and overlapping in the comparison of localities that were <2 km apart, making interpretation of M difficult.

**DISCUSSION**

In general, population genetic studies focusing on a solitary species with low vagility, such as the Baird’s pocket gopher, are rare (e.g., Hambuch and Lacey 2000, Connior and Risch 2010, Lopes and De Freitas 2012, Mapelli et al. 2012). This study found that both mitochondrial and microsatellite data were informative in understanding population processes across a series of localities of *Geomys breviceps* in the Brazos Valley of eastern Texas. Our findings support high levels of gene flow among nearby populations (<2 km apart), with decreasing gene flow as distance increases (after approximately 9 km; Tables 1 and 2). These results indicate that Highway 47 North, Highway 47 South, and Riverside Campus are functioning as 1–2 populations or genetic clusters, and
Sheep Center and Highway 6 are each functioning as separate clusters (Tables 1, 2; Fig. 1).

The results of this study provide insight regarding movement and dispersal of the Baird’s pocket gopher. The high levels of gene flow and significantly positive spatial autocorrelations among the Highway 47 localities and Riverside Campus (distances of ~2 km) suggest that the highway that separates these localities does not hinder movement of pocket gophers. The soils under highways are often extremely compacted and rocky, and could possibly hinder burrowing (Griscom et al. 2010), suggesting that aboveground movement would be necessary. Highway 47 (which separates the Highway 47 localities) was constructed in 1987 and has driven aboveground movement among these localities for at least 20 years (Estridge 2008). Even with limited mobility above the soil due to its morphological adaptations for a fossorial lifestyle (Sulentich et al. 1991, Stein 2000, Merrit 2010), *G. breviceps* is able to move distances of at least 2 km above ground. Genetic spatial autocorrelation analyses, however, indicate that dispersal distances >10 km are unlikely. In contrast, population genetic analyses support the conclusion that there is some gene flow occurring among all 5 localities (although levels of gene flow are reduced when comparing the Highway 6 population to the other 4 populations, which are separated by over 48 km). The evidence for gene flow suggests that that *G. breviceps* may be capable of moving large distances (albeit rarely) or that there was once a much larger, continuous population throughout the Brazos Valley. Further studies examining additional localities will be necessary to determine the largest possible distance *G. breviceps* can move above ground.

IBD 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 is infrequent; whereas low levels of isolation by distance would suggest the opposite for either females or males depending on the genetic marker examined. Significant levels of IBD 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. Genetic spatial autocorrelation analyses can also be used to assess sex-biased dispersal. However, results reported herein were similar regardless of the gender analyzed, supporting a lack of sex-biased dispersal in *G. breviceps*.

In their examination of *G. attwateri* (a pocket gopher species located near and often hybridizing with *G. breviceps*), Williams and Cameron (1984) found that the majority of dispersing individuals were juveniles caught in aboveground traps. Aboveground dispersal by juveniles also has been observed in the pocket gopher genus *Thomomys* (Howard and Childs 1959), with dispersal distances up to 500 m, depending upon surrounding environment (Vaughan 1963, Hafner et al. 1983, Smith et al. 1983, Daly and Patton 1990, Hafner et al. 1998). In a recent study focusing on *G. breviceps* site fidelity and population structure, King (2010) found high site fidelity and that juveniles moved the largest distances (up to 46 m; maximum dispersal distance is unknown due to the limited geographic scale of the study). Based on these previous findings, future studies using juveniles (as well as adult males and females) from a variety of distance classes will be necessary to better understand dispersal distances in the Baird’s pocket gopher.

**Conclusion**

Overall, a general relationship between distance and level of gene flow is seen in this study of the Baird’s pocket gopher in eastern Texas: 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 can possibly be useful in understanding population processes in other fossorial species. Additional research including more localities, samples per locality, and other species can facilitate a better understanding of population structure and the processes of speciation, gene flow, and dispersal in both solitary and social fossorial species.

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LITERATURE CITED


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**APPENDIX 1.** *Geomys breviceps* specimens examined in this study listed by locality and museum acronym. All specimens are deposited in the Biodiversity Research and Teaching Collections (TCWC) at Texas A&M University.


**Sheep Center** – Texas: Brazos Co., College Station, Texas A&M University Sheep Center pastures, 30°33.760’N, 96°24.548’W (TCWC 60859–60862, 60864–60887, 61194, 61195).


**APPENDIX 2.** Rounded distances (km) between the 5 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).

<table>
<thead>
<tr>
<th>Highway 47 North</th>
<th>Highway 47 South</th>
<th>Riverside Campus</th>
<th>Sheep Center</th>
<th>Highway 6</th>
</tr>
</thead>
<tbody>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
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<td>—</td>
<td>9.975</td>
<td>9.790</td>
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<td>9.790</td>
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<tr>
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