<u>Project Title</u>: **Dispersal and Genetic Structure of an Arkansas endemic subspecies, the Ozark Pocket Gopher (*Geomys bursarius ozarkensis*).**

<u>Project Summary</u>:

In this pre-proposal I present a plan to learn about the dispersal and genetic structure of the Ozark pocket gopher (*Geomys bursarius ozarkensis*) in order to guide management and conservation of this endemic subspecies in Arkansas. The Arkansas Wildlife Action Plan lists this subspecies as critically imperiled and calls for study of its current distribution, dispersal and to collect DNA samples. A previous study by Connior (2009) provided better understanding of the distribution of Ozark pocket gophers, capture methods, survival, and home range size. This study seeks to broaden that understanding by emphasizing detection of dispersal through capture/mark/recapture and use of drift fences. I plan to collect genetic samples to examine intrapopulation variation in microsatellite DNA in order to estimate gene flow, past dispersal distances and population expansion. Microsatellite DNA has been used to identify the genetic structure of populations within numerous taxa and a study on a closely related species of pocket gopher, Baird's pocket gopher (*G. breviceps*) (Welborn and Light 2014) can be used as a model for this study. This study will provide needed information for the successful management of the Ozark pocket gopher.

<u>Project Leader</u>:

Thomas E. Nupp, Ph.D.
Fisheries and Wildlife Science Program Director and Professor of Wildlife Science
127 McEver Building
Arkansas Tech University
1701 N. Boulder Ave.
Russellville, AR 72801
(479)968-0313

<u>Project Partner</u>:

Geoffrey Ecker, Ph.D.
Assistant Professor of Biology
10 McEver Building
Arkansas Tech University
1701 N. Boulder Ave.
Russellville, AR 72801
(479)968-0312

<u>Project Budget</u>:

Arkansas SWG request = $101,298.00 (for two year study)
Matching funds provided = $54,546.00 (from Arkansas Tech University)
Total Project funds - $155,844.00

Need:

The Ozark pocket gopher (*Geomys bursarius ozarkensis*) is a critically imperiled subspecies of the plains pocket gopher as documented in the Arkansas Wildlife Action Plan (AGFC 2017). It is found only in one or perhaps two counties within the upper White River floodplain in North-Central Arkansas (Elrod et al. 2000, Connior and Risch 2010). Because of the relict, rare (<3500 individuals, Kershen 2004), and isolated nature of this subspecies, conservation data on its current population size, dispersal potential, and population genetic structure is important for establishing its current status and identifying future conservation issues.

Among the critical pieces of information for the future conservation of Ozark pocket gophers is a better understanding of the current population extent and size, its potential for dispersal into new suitable habitats, and the population's genetic structure. Gaining knowledge of the population's genetic structure is particularly important because the population has been isolated for as long as 1.4 million years (Elrod et al. 2000) and is further subdivided into more-or –less isolated fields and pastures. The extent to which gophers move among fields, which oftentimes would require above ground movements, remains a vital question to their conservation. Connior and Risch (2010) documented one example of an adult male Ozark pocket gopher dispersing above ground during the spring breeding season and then staying in the new burrow system for the duration of their study. Connior (2009) did not capture gophers in the late summer and fall which corresponds to the likely population peak and perhaps the peak of dispersal of subadult/juvenile males, the most common dispersers (Williams and Cameron 1984). Williams and Cameron (1984) did note that dispersal of Attwater's pocket gopher (*G. attwateri*) peaked in May in their south Texas study, corresponding to the end of the breeding season. Breeding continued through June in Missouri (Pitts and Choat 1997) and Connior (2009) hypothesized that dispersal may peak in fall in Arkansas.

Collecting genetic data on Ozark pocket gophers has the potential to provide highly beneficial data for the conservation of this subspecies. Because the potential habitat for the Ozark pocket gopher is highly fragmented (Kershen 2004) and the population size is small it is possible that the population exhibits measurable intrapopulation genetic divergence (see Patton and Yang 1977). Genetic structure within populations can reveal isolation of alleles and hence imply the frequency and numbers of dispersal events. Recent techniques in the examination of variability in microsatellite DNA have demonstrated utility of this technique for explaining the biogeographical history of populations while providing details on the current status of populations, whether expanding or contracting. This technique has been applied to a variety of species ranging from humans (Kimmel et al. 1998) to bats (Storz and Beaumont 2002) and Allegheny woodrats (Castleberry et al. 2002). In particular, Castleberry et al. (2002) used the technique to demonstrate that genetic distinctness of 19 subpopulations of Allegheny woodrats (*Neotoma magister*) has resulted from relatively recent colonization and subsequent isolation by distance between rock outcrops. Similarly, an examination of the structure of Ozark pocket gopher populations could provide information on the status of the entire population (expanding or contracting) or information that could prove useful for determining what parts of the population may be the most genetically distinct. Lastly this genetic analysis could provide data on the history of dispersal events that distribute alleles between isolated populations.

Purpose and Objectives:

The objectives of this study are to:

1. Determine locations of active Ozark pocket gopher colonies.
2. Measure dispersal of Ozark pocket gophers through use of mark/release/recapture and drift fences.
3. Collect samples suitable for examining genetic structure of Ozark pocket gopher populations.
4. Assess population genetic structure and evidence for dispersal history based on variation among microsatellite loci.

Location:

Ozark pocket gophers are known to occur in as many as 51 fields and pastures in Izard County, Arkansas (Kershen 2004). As most if not all known locations of Ozark pocket gophers occur on private land I plan to approach willing landowners and select among potential fields based on their proximity to each other and the availability of adjacent fields where dispersal might be observed.

Approach:

Field Capture Methods - Fields containing Ozark pocket gophers will be located and landowners contacted for permission to use their fields for field research. Fields used by Connior (2009) will provide a starting point for the selection of potential study sites. Fields will be selected for trapping and installation of drift fences based on their proximity to one another and the availability of potential habitat nearby. Pocket gophers will be captured by placing traps within existing burrow systems. Connior and Risch (2009) had good success with a relatively simple homemade box trap and I will follow their recommendations. Traps will be placed within active burrow systems near soil pushups. All captured gophers will be handled following the guidelines of the American Society of Mammalogists (Sikes et al. 2011), marked using a Passive Integrated Transponder (PIT) tag (see Schooley et al 1993) and other basic morphological data will be collected including sex, mass, and reproductive condition (size of testes, lactation, pubic symphysis condition etc.). Recaptures of gophers can provide evidence of dispersal activity particularly if the gopher is captured in an adjacent field. Trapping will focus on the summer, fall, and possibly winter seasons to maximize the potential for encountering dispersal events. All captured gophers will have an ear punch or blood sample taken for later genetics work.

Measuring Dispersal with Drift Fences - The goal of drift fences is to capture dispersing pocket gophers. Thus each field chosen for livetrapping will also have a drift fence along part of the field where likely dispersers would choose to travel above ground to get to some adjacent habitat. Drift fences will be constructed of Visqueen or polypropylene silt fence with openings every 5-8 m where a five-gallon bucket will be buried such that it’s opening at ground level. Pocket gophers encountering the drift fence will travel along it and fall into the bucket. Connior (2009) did not capture any gophers in drift fences operated at two sites from March to August. My study will focus on the summer and fall seasons where dispersal events may be more likely. Even so it is expected that dispersal events may be rare given that intervening habitat between study fields may be inhospitable for gophers.

Genetic Data - Because genetic structure of a population is indicative of past population isolation and can reveal rates of dispersal between populations, all captured pocket gophers will have a genetic sample recorded for future use. Most likely this will consist of a small punch of ear tissue or a blood sample. Tissue samples will be preserved in a 95% ethanol solution and refrigerated until they can be processed. I expect to collect and process between 80 and 100 samples in order to characterize the population. Welborn et al. (2012) characterized 10 polymorphic microsatellite markers for use in describing genetic structure of Baird's pocket gopher (*B. breviceps*) and determined that 9 of those 10 microsatellites were successfully amplified in *B. bursarius*. This provides a starting point for choosing primers for identifying microsatellites that will be useful in describing the genetic structure of the Ozark pocket gopher. We will also explore the usefulness of 4 microsatellites identified by Steinberg (1999) for *Thomomys spp.* We will extract microsatellite DNA so that it can be amplified and used in further tests. Researchers have used a variety of statistical procedures to analyze the microsatellite data to estimate gene flow, estimated dispersal distances, population expansion, and numerous other statistics (see Castleberry 2000, Storz and Beaumont 2002, Welborn and Light 2014). Lastly, as populations of gophers are more or less isolated, genetic structure of the populations could provide information on the genetic variability contained within those subpopulations and hence some indication of their future viability.

Expected Results and Benefits:

At the conclusion of this study we will:

1. Describe capture locations of Ozark pocket gophers and provide recommendations for future livetrapping methodology and trap placement.
2. Document dispersal events of Ozark pocket gophers.
3. Provide information on the current status of Ozark pocket gophers.
4. Provide data on the genic diversity and stability of the Ozark pocket gopher population.
5. Produce presentations/publications of these research results.

Budget:

Nupp-AGFC-SWG Request	AGFC Share	ATU Share	
Graduate Assistant Stipend (2 years)	26,400		
Graduate Tuition (18 hours per year		10,224	
Field Assistant (Extra Labor)	10,000		
Field Assistant (NWS)	6,000		
Professional Services (Nupp Summer Payment)	8,000	33,340	PI Salary (approximately 20% ef year)
Benefits	2,681	8,532	PI Benefits
Supplies	10,217	2,450	Supplies
Travel	10,000		
Subtotal - Direct Costs	73,298		
Indirect (38.20% MTDC)	28,000		
Total AGFC-SWG Request	**101,298**	**54,546**	**Total ATU Match**
AGFC Share	**65.00%**	**35.00%**	**Total ATU Share**
Total Project Cost		**155,844**	

Qualificatios:

Thomas E. Nupp holds a Ph.D. from Purdue University and the rank of Professor of Wildlife Science and Fisheries and Wildlife Science Program Director at Arkansas Tech University. He is an experienced mammalogist, having taught Mammalogy at ATU for over 20 years and conducting numerous mammal-related studies. He is a long-standing member of the American Society of Mammalogists and The Wildlife Society. In addition he has served as past-president, president, and secretary/treasurer of the Arkansas Chapter of The Wildlife Society. He has produced numerous technical reports and papers for the US Fish and Wildlife Service, Army Corps of Engineers and Arkansas Game and Fish Commission.

Geoffrey Ecker holds a Ph.D. from the University of Connecticut and the rank of Assistant Professor of Biology and Curator of the Herbarium at Arkansas Tech University. He has published several papers examining the genetics and ecology of switchgrass (*Panicum virgatum*). He has expertise in population genetics, field botany, plant ecology, lichenometry, agronomy, and forest ecology.

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