| U.S. Department of Agriculture<br>**Work Unit Description AD-416**<br>U.S. Dept. of Agriculture, State Agricultural Experiment Stations and Other Institutions | | | Date (Month/Day/Year)<br><br>09/25/2012 |
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| 1. Accession No. | Agency Identifiers | 5. Work Unit/Project No. | 6. Status |
| | 2. NIFA | 3. LA.B | LAB94169 | A = New Project |

**7. Title**
**Genetic Variation in Longleaf Pine Associated Species**

| 8. Performing Organization 1001 - 2010<br>School of Renewable Natural Resources<br>Agricultural Experiment Sta, Louisiana State Univ | 9. Cooperating Departments within State Performing Institution |
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12. Investigator Name(s) Last Name and Intitials)

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| 14. Project Type<br>McIntire-Stennis | 15. Contract/Grant/Agreement No. | 16. Amount | 17. FY |
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| 18. Award Date (Month/Day/Year) | 19. Start Date (Month/Day/Year)<br><br>10/01/2012 | 20. Termination Date (Month/Day/Year)<br><br>09/30/2016 |
|---|---|---|

Goals/Objectives/Expected Outputs

1. Assess genetic population structure in Louisiana Bachman's Sparrows 2. Examine genetic variation at Mhc loci in Gopher Tortoises

Methods

Bachman's Sparrows: Bachman's sparrows will be sampled east and west of the Mississippi. Birds will be sampled in habitat fragments (30 birds per fragment) that vary in size (ha) and type (longleaf forest or clearcut). Capture locations will be marked by GPS. Phylogenetic differences between birds on the east and west sides of the Mississippi will be examined by using two mitochondrial loci (ND2 and ND3) and one nuclear locus (TGFB2 intron 5) (DaCosta et al. 2009; Sorenson et al. 1999). Sequences for individuals will be analyzed in PAUP* (v. 4 (Swofford 1998) with a maximum likelihood gene tree. An analysis of molecular variance (AMOVA) will be used to test for significant genetic differences (Excoffier et al. 1992), and the program MIGRATE (Beerli & Felsenstein 2001) will be used to estimate gene flow (4Nem) between populations on opposite sides of the Mississippi River. Analyses with sequence data will be supplemented with analyses using microsatellite data (see below). Genetic variation and structure of Bachman's Sparrow in habitat fragments will be calculated using expected and observed heterozygosity, and allelic richness. Data analysis will include calculations of population subdivision (Fst), inbreeding (Fis), effective population size, isolation-by-distance Mantel tests, and implementation of the program STRUCTURE (Pritchard et al. 2000). Gopher Tortoises: Mhc primers specific to gopher tortoises will be developed by screening primers developed in closely related species. Initial sequences will be elongated with vectorette PCR (Arnold & Hodgson 1991). Allelic diversity within populations will be estimated by the number of alleles per locus, allelic richness, observed and expected heterozygosity. Genetic variation between populations will be estimated with $\Phi ST$ values (Excoffier & Lischer 2010). Sequence diversity within and between populations will be estimated using Nei's standard genetic distance (Nei 1987; Tamura et al. 2011), and haplotype diversity and nucleotide diversity ($\pi$) (Librado & Rozas 2009). To determine the relationship between forest fragment size and MHC variation, regression analysis will be used. Forest fragment size estimated with GIS information for each individual tortoise population will serve as the predictor variable. Expected heterozygosity and allelic richness for each tortoise population will be the response variables for allelic-based analyses, while nucleotide diversity ($\pi$) and haplotype diversity will be the response variables for sequence-based analyses. To determine the relationship between forest fragment isolation and MHC variation, two different approaches will be used. The first approach will test for isolation by distance using Mantel's test (Mantel 1967), the second will use Analysis of Molecular Variance to determine the extent of spatial genetic variation.

23. Non-Technical Summary

Longleaf pine forest once dominated the southeast, but as a consequence of fire suppression, timber harvesting, and habitat fragmentation less than 3% remains and all of it is highly fragmented (Allen et al. 2006). Unsurprisingly, the loss and fragmentation of longleaf pine habitat has caused associated species to decline and become fragmented in their distributions. Species dependent on longleaf pine habitat include several species of birds (e.g. Bachman's Sparrow), reptiles (e.g. Gopher Tortoise), and plants (e.g. pitcher plants, orchids), many of which are species of concern (Dunning 2006; Ennen et al. 2010; Koopman & Carstens 2010; McCoy et al. 2007; Tucker et al. 2004). Plans to recover these species will be improved by genetic studies, which can be used to estimate adaptive potential, connectivity among populations, and inbreeding, an outcome of small population size that can reduce reproductive success and survivorship (Allendorf & Luikart 2007). In order to assist species recovery plans, I propose to examine levels of genetic variation, gene flow, and inbreeding for a panel of species occupying longleaf pine fragments. The results of these studies can be used to help formulate appropriate and effective management strategies for species as well as their habitat.

24. Keywords

Genetic analyses; Longleaf pine; Bachman's Sparrow; Gopher tortoise

**** **The Original signed document is on file at this institution.** ****

| Signature | Title | Date |
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| Dept:<br>Admin: | Associate Director | 9/23/12 |