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GENETIC STRUCTURE OF NORTHERN BOBWHITES IN NORTHEAST MISSISSIPPI AND SOUTHWEST TENNESSEE

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ABSTRACT

Precipitous declines in northern bobwhite (*Colinus virginianus*) populations across most of the natural range may increase susceptibility to genetic isolation, restrict gene flow among subpopulations, and exacerbate vulnerability to catastrophic stochastic processes. We characterized the level of genetic variability of 223 individual bobwhites representing 4 disjunct populations in northeast Mississippi and southwest Tennessee in 2002. Analyses at 8 microsatellite loci suggested observed heterozygosity was lower than expected but showed no significant heterozygosity excess. Estimates of F_{IS} coefficients were positive in each subpopulation, but low overall, suggesting only minor loss in heterozygosity over the entire population. Gene diversity was high and genetic differentiation within and among subpopulations and isolation by distance effects were minimal, suggesting adequate levels of gene flow. We suggest, despite population losses, gene flow is maintained among subpopulations, which may reflect the bobwhite's ability to disperse successfully in the agricultural landscape in this region. Maintenance of gene flow across seemingly inhospitable landscapes suggests focal area management directives may enhance population sustainability. Greater understanding of the genetic structure of northern bobwhite populations on larger geographic scales and across the species' range is paramount to population recovery.

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Key words: *Colinus virginianus*, genetic structure, Mississippi, northern bobwhite, population genetics, Tennessee

INTRODUCTION

Northern bobwhites are distributed across a variety of habitat types in the eastern United States and northern Mexico (Brennan 1999). Bobwhites exhibit limited mobility compared to most avian species, which may increase susceptibility to population isolation from habitat loss (Roseberry 1993). Population isolation may simultaneously increase susceptibility to natural stochastic processes (Roseberry 1993) and genetic drift resulting from limited gene flow (Ellsworth et al. 1989). Bobwhite populations, as measured by the North American Breeding Bird Survey (BBS), have steadily declined by 3.8% annually in the United States and 5.0–5.3% annually in Mississippi and Tennessee since 1966 (Sauer et al. 2011). Declines in BBS counts presumptively reflect both a reduction in average number of birds detected per point and an increasing frequency of points where individuals were absent, which may indicate increasing population isolation.

The bobwhite decline has raised concern for the overall abundance and long-term viability of many local populations (Vance 1976, Roseberry et al. 1979, Exum et al. 1982, Brennan 1991, Fies et al. 1992). Consequently, a number of studies have been conducted on the dynamics and viability of bobwhite populations (Guthery et al. 2000, Fies et al. 2002, Thogmartin 2002, Sandercock et al. 2008). However, standard population viability analyses may not be applicable to fragmented populations because important parameters such as inbreeding levels within and gene flow among fragmented populations are not well understood (Roseberry 1993).

There is a paucity of studies characterizing patterns of genetic variability within and among subpopulations of bobwhite (Ellsworth et al. 1989, Wehland 2006). Precipitous population declines and potential for reduced gene flow as habitats become increasingly inhospitable suggest a critical need for a comprehensive understanding of genetic relationships among bobwhite subpopulations. We evaluated patterns of polymorphism at 8 microsatellite loci in bobwhite in 4 presumably disjunct locations in north and east Mississippi and southwest Tennessee to examine the extent of genetic differentiation, substructur-

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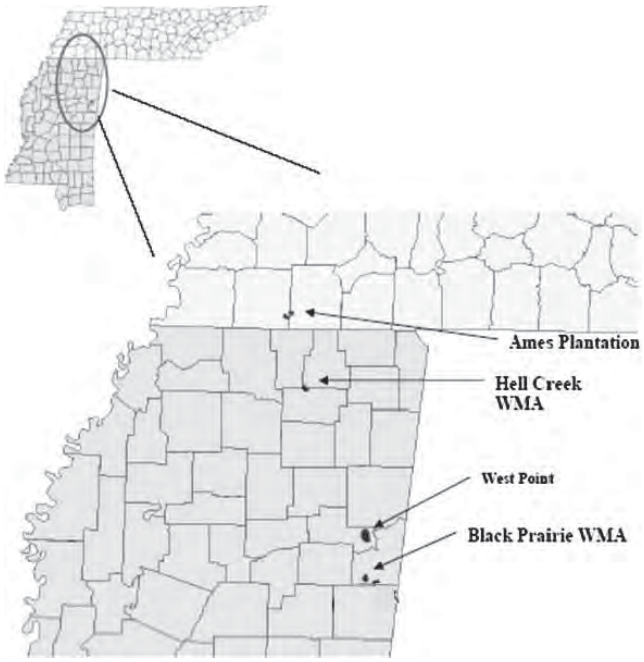

Fig. 1. Geographic locations of 4 northern bobwhite subpopulations in northeast Mississippi and southwest Tennessee sampled for analyses of population genetic structure in 2002.

ing, and variability within and among subpopulations in this region.

STUDY AREA

We sampled wild bobwhite populations at 3 locations in northern Mississippi (West Point Unit [WPU; 4,963.75 ha], Black Prairie Wildlife Management Area [BPWMA; 1,831.24 ha], Hell Creek Wildlife Management Area [HCWMA; 937.56 ha]) and one location in southwest Tennessee (Ames Plantation [AP; 5,478.09 ha]) from February to June 2002 (Fig. 1). Sampling area estimates were based on home range sizes from radio-marked bobwhites for WPU and BPWMA, and area under habitat management for HCWMA and AP. Distance among sites ranged from 32.27 km (WPU to BPWMA) to 202.63 km (BPWMA to AP). The region represents historic portions of the Black Belt Prairie range and is dominated by a mix of row-crop agriculture, exotic forage, interspersed with pine (*Pinus* spp.) plantations and other forest fragments with limited urban development.

METHODS

We obtained 223 samples of juvenile and adult body feathers from bobwhites captured in baited walk-in funnel traps at 4 locations (WPU, $n = 39$; BPWMA, $n = 65$; HCWMA, $n = 39$; AP, $n = 70$). All individuals were banded upon sampling, and recaptures were not resampled. We included samples from only a single individual where multiple individuals were captured in a single trap to diminish possible confounding effects of related individuals in genetic analysis. Bobwhite subpopulations at each study site were representative samples of the wild regional population, and study areas had not previously been the subject of captive bobwhite relocations as of 2002. Bird handling and tissue sampling followed procedures in the Mississippi State University Institutional Animal Care and Use Committee protocol (01–051).

Table 1. Locus identity, annealing temperatures (°C), and accession numbers for each locus used to examine population genetic structure of northern bobwhites in Mississippi and Tennessee, 2002.

Locus	Annealing temperature	Accession number
LEI 142	66	X83257
LEI 160	66	X85523
LEI 70	63	X82869
LEI 197	63	Z83776
LEI 31[b]	63	X83980
LLSD3	54	X99053
NBGP8[a]	57	AY522965
NBGP9	57	AY522966
NBGP10	57	AY522967
NBGP11	57	AY522968
NBGP12N[a]	57	AY522970
NBGP15[b]	57	AY522980

[a] Loci removed due to significant deviations from HW equilibrium.
[b] Loci removed due to excessive failed assays.

Genetic Analysis

Feather samples from each individual were stored on site in dry envelopes. The tips of each feather rachis were later transferred to 500 µl 70% ethanol. DNA was extracted from feather samples using a Qiagen DNeasy Tissue Extraction Kit (Qiagen Inc.,Valencia, CA, USA) combined with Dithiothreitol (DTT) to aid in breakdown of the keratinized feather shaft. Twelve di- and tetranucleotide microsatellite markers (LEI142, LEI160, LEI70, LEI197, LEI31, LLSD3 [Fok and Parkin 2001]; NBGP8, NBGP9, NBGP10, NBGP11, NBGP12N, NBGP15 [Schable et al. 2004]; Table 1) were amplified in 10 µl polymerase chain reactions (PCR) containing DNA template, Takara Ex-Taq DNA polymerase, 10X PCR buffer (containing 20 mM Mg^{+2}), 2.5 mM each dNTP (pH 7∼9), and 10 mM each fluorescent-labeled primer (Invitrogen Inc., Grand Island, NY, USA). PCR reactions were conducted with an initial denaturation of 5 min at 95 °C, followed by 40 cycles of 95 °C for 30 sec, 30 sec at the locus-specific optimized annealing temperature (Table 1), and 72 °C for 30 sec. Cycling was followed by a final extension period of 20 min at 72 °C. Products were identified and sized with 0.5 µl 400 bp standard following amplification by capillary electrophoresis on a Beckman-Coulter CEQ 8000XL DNA Sequencer (Beckman-Coulter Inc., Brea, CA, USA). Fragments representing pairs of alleles at each locus (i.e., genotypes) were generated for each individual in a subpopulation, which allowed for examination of detailed genetic structure within and among subpopulations.

Table 2. Size range (Bp), % failed assays (%), number of alleles (Na), average frequency of private alleles (F_p), observed heterozygosity (H), within population gene diversity (H_S), and overall gene diversity (H_T) per locus for northern bobwhite in Mississippi and Tennessee, 2002.

Locus	Bp	%	N_a	F_p	H	H_S	H_T
LEI 70	138–168	19.64	10	0.014	0.640	0.812	0.815
LLSD3	136–180	30.36	13	0	0.253	0.631	0.679
LEI 197	112–150	26.79	12	0.053	0.665	0.780	0.785
LEI 160	174–200	15.48	9	0.010	0.591	0.880	0.882
LEI 142	102–126	16.67	11	0.068	0.600	0.866	0.882
NBGP9	142–190	10.71	17	0.014	0.488	0.563	0.567
NBGP10	136–254	8.93	29	0.018	0.643	0.769	0.783
NBGP11	128–146	11.31	7	0.020	0.694	0.848	0.865

Statistical Analysis

Allele and genotype frequencies, and observed and expected numbers of heterozygotes in each subpopulation were calculated using Program GENEPOP Version 1.2 (Raymond and Rousset 1995). This program was also used to analyze genotypic deviations from Hardy-Weinberg (HW) equilibrium and linkage equilibrium. We estimated observed heterozygosity (H_O) for each locus over all subpopulations (Nei 1978), within subpopulation gene diversity (H_S), overall gene diversity (H_T), number of alleles, allele frequency, and allelic richness as implemented in Program FSTAT Version 2.9.3 (Goudet 2001). We used Weir and Cockerham's (1984) *F*-statistics, to estimate extent of population substructuring, and coefficients over the entire population (F_{IT}), within subpopulations (F_{IS}), and among subpopulations (F_{ST}) for each locus (Weir and Cockerham 1984) in FSTAT (Goudet 2001). We used sequential Bonferroni to adjust nominal significance levels for tests of HW and linkage disequilibrium, and genetic differentiation of pair-wise F_{ST} values (Rice 1989). We also conducted an analysis of molecular variance (AMOVA) (Excoffier et al. 1992), as implemented in Program ARLEQUIN Version 2.0 (Schneider et al. 2000) to test covariance components at a 2-level hierarchy: among subpopulations, and within subpopulations, and at a 3-level hierarchy: among groups (AP and HCWMA vs. BPWMA and WPU), among subpopulations within groups (AP vs. HCWMA, BPWMA vs. WPU), and within subpopulations. We based grouping on geographical distance between subpopulations with those $<$ 60 km apart assumed to be more similar than those $>$115 km apart (Table 2). We used Program BOTTLENECK Version 1.2.02 (Cornuet and Luikart 1996, Luikart et al. 1998) to examine the potential for a reduction in genetic diversity using the 2-phase mutation (TPM) model with 20% variance with the Wilcoxon sign rank test (1,000 iterations). The TPM is a model that is intermediate between the SMM (step-wise mutation model) and the IAM (infinite alleles model), which fits more appropriately to microsatellite data and assumes mutation-drift equilibrium when calculating gene diversity and expected mean heterozygosity (DiRienzo et al. 1994). We expected to observe no significant difference, if the population had not undergone a recent bottleneck, between the calculated expected average heterozygosity (H_E) and the Hardy-Weinberg equilibrium heterozygosity (H_{EQ}).

RESULTS

We found no evidence of linkage disequilibrium among loci, indicating that loci were independent of one another, assuming they were not on the same macrochromosome. However, loci NBGP8 (13.9% failed assays) and NBGP12N (17.94% failed assays) were removed from further analysis due to significant deviations from HW equilibrium ($P < 0.001$) that were caused by significant heterozygote deficiencies ($P < 0.001$). Loci NBGP15 (48.88% failed assays) and LEI 31 (59.64% failed assays) were also removed due to a high proportion of missing alleles. An additional 45 individuals in the remaining subpopulations were removed due to a high frequency of missing alleles (at $>$3 loci). The resulting analysis was conducted using 26, 31, 54, and 57 individuals from HC, WPU, BPWMA, and AP, respectively.

Observed heterozygosity across all loci was on average 0.572 (range = 0.253-0.694), and the numbers of alleles at each locus ranged from 9 to 29 (Table 2). The mean observed number of heterozygotes was lower than the expected number of heterozygotes in each population (Table 3), which coincides with positive estimates of F_{IS} coefficients within each subpopulation (Table 4). However, overall F_{IT} was minimal (0.277 $\pm$ 0.047 [SE]), which suggests only minor loss of heterozygosity over the entire population. Gene diversity per locus over all populations (H_S) was fairly large (mean = 0.769, range = 0.563-0.880), as was overall gene diversity (H_T) (mean = 0.782, range 0.567-0.882) (Table 2). The mean per population gene diversity estimates were similar for the 4 subpopulations (BPWMA = 0.752, HCWMA = 0.772, WPU = 0.754, AP = 0.797). There was a significant difference between mean allelic richness of the 4 bobwhite populations ($P < 0.001$). The mean allelic richness over all populations and loci was 7.888 (Table 5).

The analyses were suggestive that genetic variability was high within the study subpopulations. Therefore, we conducted further analyses to examine for signs of bottleneck effects not apparent in analyses of heterozygosity. No population showed significant heterozygosity excess ($H_E > H_{EQ}$) when assuming the TPM (2-phased model) with the Wilcoxon test (Table 6). Our results suggest bobwhite populations at HCWMA, BPWMA, WP, and AP have not undergone a genetic bottleneck. These results, although not comprehensive (i.e., due to a reduced loci set), suggest subpopulations exhibited no

Table 3. Expected (H_E), and observed (H_O) number of heterozygotes per locus per population estimated using GENEPOP for northern bobwhite in Mississippi and Tennessee, 2002.

WPU			AP		
Locus	H_E	H_O	Locus	H_E	H_O
17	10.766	8.000	17	31.588	26.000
12	14.767	15.000	12	31.273	26.000
114	12.586	11.000	114	34.025	25.000
105	20.373	15.000	105	38.270	30.000
96	15.865	15.000	96	40.614	34.000
9	24.860	19.000	9	43.424	26.000
10	26.339	18.000	10	47.276	36.000
11	17.863	4.000	11	34.371	19.000
Mean	17.927	13.125	Mean	37.605	27.750
BPWMA			HCWMA		
Locus	H_E	H_O	Locus	H_E	H_O
17	18.887	17.000	17	16.294	16.000
12	28.107	19.000	12	15.400	13.000
114	35.259	30.000	114	21.755	18.000
105	38.421	36.000	105	18.444	13.000
96	34.103	24.000	96	16.512	15.000
9	39.747	25.000	9	21.510	17.000
10	40.582	25.000	10	20.404	13.000
11	32.200	13.000	11	10.814	5.000
Mean	33.413	23.625	Mean	17.642	13.750

significant heterozygosity excess and were not subject to bottleneck effects on genetic variability, assuming potential population isolation events occurred recently. Similarly, when allele frequency distributions were plotted for each locus, all populations had a solid L-shaped distribution indicating the lack of bottleneck effects on allelic diversity (Fig. 2).

We also applied 2 different methods (F_{ST}, AMOVA) to examine potential substructuring and differentiation within and among subpopulations. Pair-wise F_{ST} values ranged from 0.012 to 0.043 (mean = 0.020 ± 0.008 [SE]) with BPWMA and HCWMA indicating genetic differentiation following Bonferonni correction ($P < 0.008$; Table 2). Results from AMOVA indicated there was no genetic differentiation among subpopulations. Covariance components at 2- and 3-level hierarchies indicated 97.5% and 97.1% of variance resulted from variation within

Table 4. Within population F_{IS} coefficients per population of northern bobwhite in Mississippi and Tennessee, 2002.

Locus	WPU	AP	BPWMA	HCWMA
LEI 70	0.261	0.178	0.101	0.018
LLSD3	−0.016	0.170	0.327	0.160
LEI 197	0.130	0.268	0.151	0.176
LEI 160	0.268	0.218	0.064	0.300
LEI 142	0.056	0.164	0.298	0.094
NBGP9	0.239	0.404	0.374	0.213
NBGP10	0.320	0.240	0.387	0.368
NBGP11	0.779	0.450	0.599	0.543
Overall	0.250	0.260	0.291	0.225

Table 5. Allelic richness per locus per subpopulation of northern bobwhite in Mississippi and Tennessee, 2002.

Locus	WPU	AP	BPWMA	HCWMA	Overall
17	4.234	5.786	2.966	4.523	4.738
12	6.461	9.687	9.212	8.756	9.210
114	7.000	8.252	6.073	9.500	8.544
105	6.427	7.427	7.451	6.634	7.312
96	8.628	7.110	6.055	7.776	7.410
9	9.448	8.927	9.744	10.098	9.840
10	10.986	11.940	10.915	8.879	11.441
11	5.329	4.377	4.059	4.862	4.606
Mean	7.314	7.938	7.059	7.629	7.888

subpopulations. An isolation by distance effect was also not detected among the 4 bobwhite subpopulations ($r = 0.197$, $P = 0.346$; Mantel test).

DISCUSSION

Previous studies suggest avian populations exhibit low genetic differentiation due to high dispersal rates and enhanced ability to disperse long distances (Smith and Zimmerman 1976, Corbin 1977, Crochet 2000, Rocha and Del Lama 2004). However, sedentary avian populations may exhibit reduced gene flow compared to migratory populations (Arguedas and Parker 2000). Limited dispersal ability decreased gene flow among populations of several galliform species in fragmented habitats (Piertney et al. 1998, Oyler-McCance et al. 1999, Caizergues and Ellison 2002, Caizergues et al. 2003, Oyler-McCance et al. 2005), including evidence of genetic drift among isolated greater prairie-chicken (*Tympanuchus cupido*) populations in North America (Johnsgard 2002, Bellinger et al. 2003, Johnson et al. 2003).

Evidence from related taxa suggests genetic differentiation of disjunct bobwhite subpopulations may also occur; however, our findings indicate presumed isolation of bobwhite subpopulations in Mississippi and Tennessee has, to this point, exhibited negligible effects on gene flow. Extant gene flow among the 4 subpopulations examined here appears to have been sufficient to ameliorate effects of genetic differentiation. The few previous studies evaluating genetic structure of bobwhite subpopulations have revealed conflicting results, which are likely related to issue of scale or limitations in sample size and loci. Ellsworth et al. (1989) revealed adequate gene flow in continuous populations of bobwhite in

Table 6. *P*-values for 1-tailed Wilcoxon sign rank test for heterozygote excess ($H_E > H_{EQ}$) estimated under TPM (two-phased model) for northern bobwhite in Mississippi and Tennessee, 2002.

Population	TPM (Heterozygote excess)
WPU	0.87500
AP	0.67969
BPWMA	0.57813
HCWMA	0.62891

* Significant at $P < 0.05$.

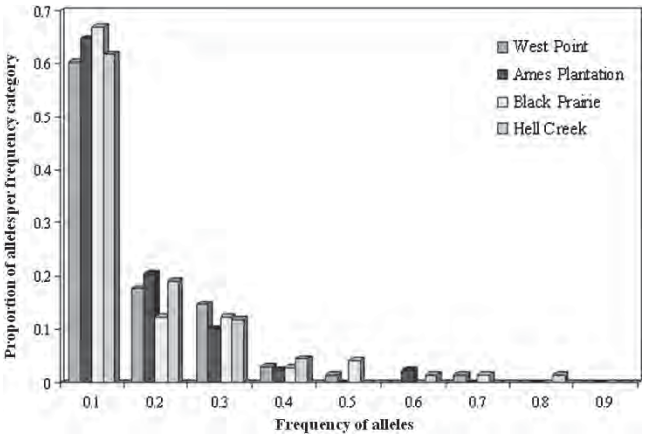

Fig. 2. Distribution of allele frequencies across all loci for populations of northern bobwhite in Mississippi and Tennessee, 2002. An L-shaped distribution of allele frequencies indicates the absence of a genetic bottleneck (Luikart et al. 1998).

Illinois, and Wehland (2006) suggested gene flow was substantial among South Texas subpopulations. However, Eo et al. (2010) suggested the possibility of restricted gene flow over large spatial scales (i.e., the eastern U.S.).

We presumed intensified agricultural practices and associated habitat losses in Mississippi and Tennessee impeded bobwhite dispersal and gene flow. However, open habitats (e.g., row-crop fields, pastures, fallow areas) remain abundant in the landscape and may support bobwhite dispersal. Conversion of the landscape into managed agricultural units may have resulted in a real loss of habitat, but failed to impact gene flow among the observed subpopulations because agricultural landscapes are relatively permeable to dispersing individuals. This is exemplified by a complementary habitat suitability model [HSM] constructed for the Southeastern Coastal Plain Bird Conservation Region in which our study sites occur (R. G. Hamrick, personal communication; Fig. 3). An overlay of suitable bobwhite habitat over our study sites

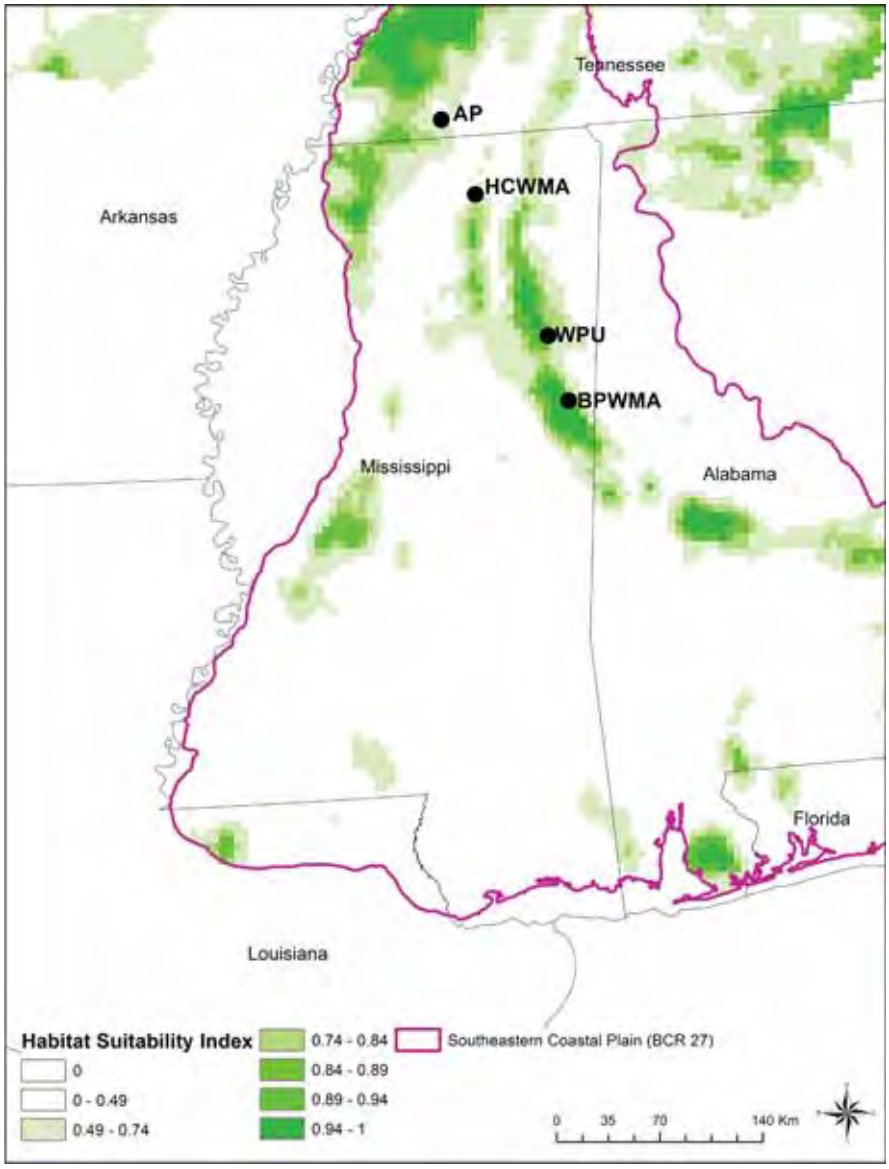

Fig. 3. Predicted bobwhite habitat suitability surface for the Southeastern Coastal Plain Bird Conservation Region (BCR 27) with superimposed geographic locations of sampled bobwhite subpopulations in Mississippi and Tennessee, 2002.

Table 7. Pair-wise F_{ST} values (upper diagonal) for each population of northern bobwhite, and approximated geographical distance (km) between study sites (lower diagonal) in Mississippi and Tennessee, 2002.

	WPU	AP	BPWMA	HCWMA
WPU		0.015	0.012	0.041
AP	173		0.014	0.012[a]
BPWMA	32	203		0.043[b]
HCWMA	118	57	147	

[a] $0.008 < P < 0.05$.
[b] $P < 0.008$.

reveals a distinct crescent of usable space in the Blackland Prairie region of northeast Mississippi whereby gene flow may be promoted (Fig. 3).

Large-scale habitat loss and fragmentation, coupled with low population densities may impede dispersal and gene flow to a significant extent on larger regional scales and across time (e.g., Eo et al. 2010). However, we must approach inference from this and existing bobwhite genetic studies with caution. Most studies are limited in spatial context and sample size and may present limited inference. Relatively small sample sizes and numbers of loci in this study may blur interpretation of results as differentiating effects of sample size and number of loci versus population processes may be difficult. Applicability of short-term genetic data to greater population processes across time is also uncertain, as these processes are expected to encompass decades and even centuries (Eo et al. 2010).

MANAGEMENT IMPLICATIONS

Bobwhite conservation geneticists now have a suite of 23 advanced genetic markers from which they can optimize regional or range-wide analyses and increase precision of parameter estimates beyond the scope presented here (Schable et al. 2004, Faircloth et al. 2009). There is a need for comprehensive studies of genetic structure of bobwhite populations at a larger regional scale in the southeastern United States and, preferably, over the species' range. There is also a need to understand effects of landscape permeability and dispersal efficacy on genetic structure. Knowledge of the extent of genetic differentiation across the species' range will allow researchers to make informed decisions regarding the relative importance of gene flow in bobwhite management. This information will be valuable in formulating a comprehensive management strategy for this species that incorporates information from population dynamics and demography as well as genetics.

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