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COMPARATIVE MORPHOLOGY AND PHYLOGENETIC RELATEDNESS AMONG BOBWHITES IN THE SOUTHERN U.S. AND MEXICO

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ABSTRACT

We analyzed the morphology and phylogenetic relatedness of masked bobwhites (*Colinus virginianus ridgwayi*) and Texas bobwhites (*C. v. texanus*) to determine if the numerically stable Texas bobwhite might serve as a reasonable research and management model for the endangered masked bobwhite. We compared 26 external and 24 internal morphological features. Texas and masked bobwhites had similar body mass; however, masked bobwhites had smaller head and body dimensions and longer wing and thigh bones ($P < 0.01$) than Texas bobwhites. Genomic DNA was extracted from heart or muscle tissue of captive masked bobwhites ($n = 12$) and from northern bobwhites obtained in Florida ($n = 3$), Tennessee ($n = 5$), Texas ($n = 12$), and Oklahoma ($n = 3$). Bobwhites from South Texas and masked bobwhites appear to form a relatively closely related assemblage, possibly representing a separate lineage from other bobwhite populations. Based on gross similarities between Texas and masked bobwhites in morphology and phylogenetic relatedness, as well as in habitat conditions on the semiarid rangelands they occupy, biological and management information from Texas bobwhites seems applicable to masked bobwhites.

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INTRODUCTION

Masked bobwhites formerly inhabited desert grasslands extending from south-central Arizona through much of Sonora, Mexico (U.S. Fish and Wildlife Service 1995). By the early 1900's, much of these subtropical grassland communities had been destroyed by severe drought and grazing by cattle. The masked bobwhite disappeared from Arizona within 50 years of its discovery and it was thought to be extirpated from Mexico. In the 1960's, remnant populations of masked bobwhites were rediscovered in Mexico and the subspecies was listed as endangered under the Endangered Species Conservation Act of 1969. Recovery efforts have been under way for >20 years.

The Texas bobwhite has been used as part of the recovery program for masked bobwhites at Buenos Aires National Wildlife Refuge in Arizona (U.S. Fish and Wildlife Service 1995). Wild males from South Texas are surgically sterilized and used as foster parents for captive-reared masked bobwhite chicks.

Otherwise, experimental research and management techniques for masked bobwhites are limited because of their endangered status. It would be useful to determine the degree to which knowledge available for the well-studied Texas bobwhite is applicable to the masked bobwhite. The 2 races occur in semiarid environments with physiognomically similar habitat structures; common plant species are similar at the generic level (Fitzpatrick and Guthery 1993). However, before information on Texas bobwhites can be used in the management of masked bobwhites, it seems important to determine the degree to which Texas and masked bobwhites are morphological and genetic equivalents. Accordingly, our objective was to examine the comparative morphology and phylogenetic relatedness of Texas and masked bobwhites. We also examined phylogenetic relatedness between these races and other races of bobwhites in the continental United States.

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METHODS

Morphology

Data were collected from 50 (24 F, 26 M) captive-bred masked bobwhites and were compared to like samples of mixed-strain domestic and wild Texas bobwhites. We measured 26 external, 11 skeletal, and 13 organ measurements from each specimen. First-year, non-breeding masked bobwhites aged >150 days were obtained from the Patuxent Environmental Science Center (PESC) and were euthanized with chloroform. Mixed-strain (Texas and northern stock) domestic bobwhites were obtained from Schuenemann Enterprises, a commercial breeder in Nueces County, Texas. Wild Texas bobwhites were obtained from hunters at the Chaparral Wildlife Management Area in Dimmit and LaSalle counties, Texas.

External measurements were taken according to Baldwin et al. (1931) to the nearest 1 mm using a flat ruler or to the nearest 0.1 mm using vernier calipers prior to skinning and dissection. Measurements included body mass (g), total length, wing chord length, culmen length, beak height, beak width, head length, head width, head height, body length, body width, body diameter, tail length, tail width, wing breadth, tarsus length, tarsus diameter, and length and width of each toe.

To obtain skeletal measurements, each specimen was partially skinned to expose the breast, back, and legs. All measurements were taken to the nearest 0.1 mm using vernier calipers with tissue intact according to Robbins and Schnell (1971) and McLelland (1991). Measurements included length of the humerus, radius, ulna, sternum, keel, synsacrum, femur, and tibiotarsus; width and minimum width of the synsacrum; and depth of the keel.

Each specimen was dissected by cutting through the abdominal membrane at the vent and along the contour of the breast muscle through the rib cage until the sternum was removed to expose the body cavity. Mass of internal organs was measured with an analytical balance accurate to 0.0001 g. Linear measurements were taken to the nearest 1 mm using a flat ruler or to the nearest 0.1 mm using vernier calipers. Data were obtained on mass of liver, heart, kidney, adrenal glands, ovaries or testes, proventriculus, gizzard, and spleen. Linear measurements included heart width and height and length of small intestine, large intestine, and caeca.

Molecular Genetics

Of the 50 masked bobwhites obtained from the PESC, 12 (6 M, 6 F) which were not brood mates were chosen for genetic analysis. Wild bobwhites were obtained from Leon County, Florida (n = 3); Fayette County, Tennessee (n = 5); Houston County, Texas (n = 6); Ellis County, Oklahoma (n = 3); Stonewall County, Texas (n = 3); and Brooks County, Texas (n = 3). One pen-reared bobwhite was obtained from a private breeder. Two northern bobwhites from each sample location were chosen for genetics analysis plus the 1 domestic bird, resulting in a total of 25 samples.

Genomic DNA was extracted from heart or muscle tissue using standard proteinase K digestion of the tissue followed by organic extraction of protein using phenol and methylene chloride and isopropanol precipitation of DNA (Maniatis et al. 1982). A segment of the mitochondrial D-loop was amplified using the polymerase chain reaction (PCR; Saikai et al. 1988). PCR amplification solutions and conditions were those described by Bickham et al. (1996) using primers LGL 951 and LGL 1115.

PCR fragments were directly sequenced using the ABI Taq Dye Deoxy^{tm} Terminator Cycle Sequence Kit. Twenty-five samples were sequenced using the 1115 primer. Six samples were also sequenced using the 951 primer to give confirmed double-stranded sequence. Phylogenetic interpretation of data was obtained using the exhaustive procedures of PAUP (Swofford 1993).

Statistical Analyses

Analysis of variance is a robust test so assumptions of normality and homogeneity of variance can be violated if each sample has >20 observations and approximately the same number of observations (Kleinbaum and Kupper 1978:248). Because our data met these conditions, each morphological variable was compared in a 3 × 2 factorial analysis to determine if differences existed between 3 strains and 2 sexes. General linear models (PROC GLM, SAS Institute 1988) were used for the analysis because cell sizes were unequal but cell frequency patterns were proportional. Each analysis tested the null hypotheses that the means were not different for the 3 strains and for the 2 sexes and that no interaction effects were present ($P < 0.01$). We used Tukey's HSD post hoc test to compare means at $P < 0.01$ to increase the power of tests and to control for Type I errors.

RESULTS

Morphology

Forty-five factorial analyses yielded significant results ($P < 0.01$) for all variables except tail width, indicating strain, sex, or interaction effects were present. Interaction effects were present for mid-toe length ($P = 0.0063$) and gizzard mass ($P = 0.0035$). Domestic males (21.1 ± 0.98 mm) (x ± SE) had a longer mid-toe length than domestic females (20.5 ± 1.29 mm); Texas males (19.7 ± 0.72) and females (19.7 ± 0.72) had similar mid-toe length; and masked bobwhite males (18.2 ± 1.11 mm) had a shorter mid-toe length than masked bobwhite females (18.8 ± 0.68 mm). Domestic females (4.1 ± 0.12 g) had the largest gizzard mass. Domestic males (3.4 ± 0.54 g), Texas males (3.3 ± 0.42 g), and Texas females (3.3 ± 0.45 g) had similar gizzard masses, as did masked bobwhite males (2.5 ± 0.25 g) and females (2.4 ± 0.21 g). Because the 2 sexes exhibited different patterns within or between groups, main effects for mid-toe length and gizzard mass could not be determined.

Males had longer mean head length ($P = 0.0003$), tail length ($P = 0.0022$), wing chord length ($P = 0.0079$), and tibiotarsus length ($P = 0.0083$) than females. No other effects due to sex were discovered.

Differences for strains were evident ($P \leq 0.001$) for 42 body components. The most common pattern observed was no statistical difference for masked bobwhites and Texas bobwhites with measurements from these races being smaller than those for the domestic strain (masked = Texas < domestic). This pattern held in 18 of 42 tests (42.9%). Variables included body mass, total length, sternum length, and keel depth. Organ measurements that fit this pattern included heart height and mass of kidneys, adrenal glands, proventriculus, and spleen.

The second most common pattern (13 of 42 tests, 31%) was a gradation in dimensions (masked < Texas < domestic). This pattern held for head width and height, body width and diameter, tarsus length, and length and width of certain toes. Synsacrum length and width and length of the small and large intestines fit this pattern.

A second gradation (Texas < masked < domestic) occurred in 6 of 42 tests (14.3%). Length of the long bones (radius, femur) fit this gradation.

Molecular Genetics

Amplification of the 25 samples representing 7 localities and 1 pen-raised bird gave a fragment of about 650 base pairs. Sequences of these birds using the 1115 primer gave a minimum of 500 base pairs of sequence information. The 6 samples sequenced with the 951 primer confirmed a double-stranded sequence for a minimum of 466 of the 500 or more bases that were called using the 1115 primer. An additional 43–64 bases were determined as single-stranded sequences for the 951 primer for those 6 samples.

Using the region confirmed by double-strand sequence (466 bases) and excluding nucleotide 258, which gave ambiguous sequence results, the exhaustive search algorithm gave 8 trees which collapsed to a phylogenetic network (Figure 1). All branches shown are found in all 8 trees. There are no alternate branching patterns supported using the 50% majority rule consensus trees. Each tick mark on the tree in Figure 1 represents a nucleotide substitution. Although the network cannot be rooted, as no sister taxon was analyzed, the obvious phenetic break is between the masked and south Texas bobwhites, inclusive, and the remainder of the population examined.

The molecular data showed high levels of subdivision among the bobwhite populations analyzed. Only the 2 birds from Ellis County, Oklahoma, 1 bird from northern Texas, and the pen-raised bird shared complete identity for the region sequenced. Birds from other localities had locally unique variants.

High levels of heterogeneity existed within localities. Three variants at reasonably high frequencies were found among the masked bobwhite samples. Three of the 7 localities showed 2 haplotypes, even though only 2 birds were sampled from each locality.

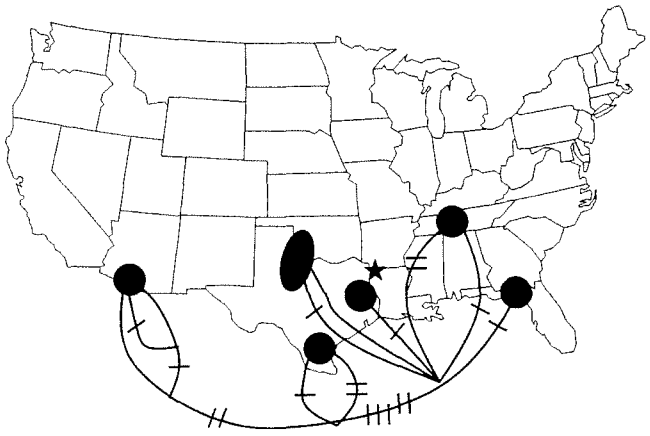

Fig. 1. Unrooted phylogeographic network detailing relatedness among populations of northern bobwhites. Tack marks represent nucleotide substitutions observed among 466 nucleotides of ND 6, glu-tRNA, and D-loop. The starred locality represents the BOG 1 variant which is defined by a nucleotide change observed only using primer 951 (see text).

These data are concordant with probabilities suggesting that most localities possess ≥2 haplotypes.

Sequencing for masked bobwhites revealed ≥3 haplotypes present at reasonably equal frequencies. Therefore, the data suggest that diversity of mtDNA lineages within the captive population of masked bobwhites is not substantially different from the diversity in wild populations of northern bobwhites.

DISCUSSION

Our purpose was to assay the comparative morphology and phylogenetic relatedness of masked bobwhites and Texas bobwhites. Our analyses of comparative morphology is problematic, because of certain confounding effects. For example, masked bobwhites and domestic bobwhites were propagated under different regimes. Also, the masked bobwhites examined in this study arose from a founder population of 57 birds wild-trapped in Mexico in 1968–70 (U.S. Fish and Wildlife Service 1995). The descendants of these founders may not typify historical or extant populations of masked bobwhites in the wild.

In the context given above, we generally found morphological differences that would be expected based on strain (domestic bobwhites larger than Texas or masked bobwhites) or pen-rearing (larger organ masses in pen-reared than in wild birds). The masked bobwhites we examined were structurally smaller and more elongate than wild Texas bobwhites. Whereas these were statistically significant effects, absolute differences were small. White (1995) provides the full set of morphological data collected for this study.

The phylogenetic affinities of bobwhites from south Texas appear to lie with the masked bobwhite population. Only 2 common changes separated the masked bobwhite population from the south Texas population. Five changes separated the masked bobwhite and south Texas bobwhite from all other populations. As the phylogenetic network (Figure 1) is un-

rooted because there was no sister taxon included in our analysis, we cannot at this time suggest that south Texas birds and masked bobwhites represent a separate lineage from other bobwhite populations. Phenetically, however, this is the proper interpretation.

MANAGEMENT IMPLICATIONS

Based on general morphological similarity and phylogenetic relatedness between Texas and masked bobwhites, we found no reason to suspect that the Texas bobwhite would not be a good research and management model for the masked bobwhite. In other words, biological and management knowledge available for Texas bobwhites would seem applicable to masked bobwhites. The implications of our results should be applied cautiously until comparative studies on the habitat ecology of these races have been conducted.

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