TEXAS TECH UNIVERSITY
Natural Science Research Laboratory

OCCASIONAL PAPERS

Museum of Texas Tech University Number 257 27 July 2006

EGGSHELL REMAINS AS A NON-INVASIVE SOURCE OF GENETIC MATERIAL IN WILD TURKEYS (*MELEAGRIS GALLOPAVO*)

ADAM D. BROWN, JOHN H. BRUNJES, IV, RICHARD S. PHILLIPS, WARREN B. BALLARD, MARK C. WALLACE, AND ROBERT J. BAKER

ABSTRACT

Molecular techniques have received increased interest in many ecological studies. The ability of such techniques to determine population structure, gender, and even individuals has provided valuable insight to effective management of wildlife populations. A major drawback of such applications in wildlife studies is the invasiveness of traditional techniques of DNA collection, many of which require capture of individuals. In oviparous chordates, the use of eggshells as sources of genetic material would mitigate the impact of DNA collection on the species of interest. Here we present procedures and protocols used to extract genomic DNA from Rio Grande wild turkey (*Meleagris gallopavo intermedia*) eggs collected at two study sites. While DNA concentrations were highly variable (37.27 ng/µl ± 5.12 SE), the below procedures resulted in reliable genetic data for both gender identification as well as microsatellite analysis using previously developed primers. Based on our findings, eggshell remains appear to have enormous potential as a noninvasive source of DNA.

Key words: DNA, eggshell, *Meleagris gallopavo*, noninvasive, PCR, sex ratio, wild turkey

INTRODUCTION

Genetic analyses of wildlife populations have contributed greatly to our understanding of population structure and dispersal, conservation biology, and wildlife forensics (Haig 1998; Parker et al. 1998). However, the most commonly used sources of wildlife genetic material, blood or tissue samples, are invasive, requiring the capture and restraint of individual animals (Piggot and Taylor 2003). Invasive techniques may negatively affect the species of interest either physically or behaviorally and are often logistically problematic (Spraker et al. 1987; Nicholson et al. 2000). Such concerns, coupled with increasing technologies allow-

ing DNA extraction from dilute or small samples, have lead to exploration of noninvasive alternatives for DNA collection (Taberlet et al. 1999).

Noninvasive genetic sampling techniques have played larger roles in wildlife ecology in recent years (Taberlet et al. 1999; Pearse et al. 2001). DNA extracted from hairs has been used to estimate population size and gene diversity in black bears, *Ursus americanus* (Boersen et al. 2003; Triant et al. 2004). DNA extracted from feces has been used to determine genera in canids (Reed et al. 2005) and felids (Farrell et al. 2000) and for gender determination in several species (e.g., Reed et al. 1997; Ortega et al. 2004). While both hair and feces have been shown effective as noninvasive techniques for mammalian species, noninvasive techniques in the majority of oviparous species are less prevalent.

In avian species, feathers have traditionally been used for DNA extraction (Taberlet and Bouvet 1991), with recent studies acquiring resolutions to address population size, structure, and parentage (McCracken et al. 2001; Segelbacher and Storch 2002; Rudnick et al. 2005). Studies also suggest eggshell remains may be a valuable source of genetic material with distinct advantages for wildlife managers (Pearce et al. 1997; Strausberger and Ashley 2001). Using established primers (D'Costa and Petitte 1998; Latch et al. 2002) and sampling protocols (Longmire et al. 1997) based on more traditional collection methods (e.g. tissue, blood), we attempted to determine the efficiency of DNA extraction from field collected Rio Grande wild turkey (*Meleagris gallopavo intermedia*) eggs for gender determination, as well as its logistic feasibility for wildlife studies.

MATERIALS AND METHODS

Field Collection of Eggshell and Blood Samples.–Rio Grande wild turkey eggs were collected at two study sites in Collingsworth, Cottle, and Donley counties, Texas, (Texas Panhandle) in conjunction with ongoing Rio Grande wild turkey research at Texas Tech University. Only egg remains that could be classified as unique (i.e., collect only blunt or pointed ends at a given nest site) were collected. Each clutch was assigned a unique number, and each egg from that clutch was numbered and placed in separate plastic storage bags. All samples were stored at 4°C within 12 hours of collection and transported from field sites to the laboratory in coolers. For verification purposes, blood samples (2-3 drops) were collected from the brachial vein of adult and yearling wild turkey during capture and placed directly in lysis buffer (Longmire et al. 1997).

Storage of Eggshell Material.–Chorioallantoic membrane (~1.5 cm^2) from each egg sample was excised and placed in a sterile petri dish with 1 ml of lysis buffer (Longmire et al. 1997). To facilitate the appropriate lysing and subsequent digestion steps, the membrane was ground into smaller pieces using a sterile single-edge razor blade. The ground membrane/lysis mixture was placed into a 5 ml polypropylene tube and brought to a total volume of 2 ml with fresh lysis buffer. The work area and all instruments were first washed with 70% EtOH and then wiped clean with sodium hypochlorite (6% bleach) to reduce the risk of cross contamination.

DNA Isolation.–DNA extraction was carried out using the method described by Longmire et al. (1997) with modifications. Extractions began by transferring approximately half of the original membrane/lysis solution from the 5 ml tube into a fresh 15 ml Falcon tube. The volumes in both tubes were brought back up to 1.5 ml with fresh lysis buffer. The remaining original sample was archived in the Natural Science Research Laboratory (NSRL) (http://www.nsrl.ttu.edu/) at the Museum of Texas Tech University. To the Falcon tube, 30 µl of Proteinase K, at a concentration of 10 mg/ml, were added to the 2 ml membrane/lysis solution. Digestion was carried out overnight (~15 hrs) in a 37°C incubator with slow rotation. After overnight digestion, the liquid phase was decanted into a new 15 ml tube, and the remaining solid material was discarded. An equal volume (~1.5 ml) of TE buffer saturated phenol was added to the digested membrane solution. The phenol wash was carried out in a 37°C incubator with slow rotation for approximately 30 minutes. The aqueous and organic phases were separated by centrifugation at

2000 rpm for 10 minutes. The aqueous phase (top) was removed and placed into a clean 15 ml tube, and a second phenol wash was conducted. After the second phenol wash, a final extraction wash using Phenol: Chloroform:Isoamyl alcohol at a mixture of 25:24:1 as outlined above was used.

The remaining aqueous phase was put into dialysis tubing (MWCO 12.14,000) strips approximately 7 cm in length. Dialysis was carried out in a 1X TE buffer solution at 4°C for 72 hours, changing the buffer every 3-4 hours. Total genomic DNA (gDNA) was stored at 4°C for immediate use or frozen (-20°C) for archival purposes. The quantity of isolated DNA was measured using UV spectrophotometric analysis of A260/A280 ratio on an ND-1000 spectrophotometer (NanoDrop Technologies). The isolated DNA was visualized using agarose gel electrophoresis (0.8%) and stained with ethidium bromide to assess quality (Fig. 1).

Sex Identification using PCR.–The PCR reaction required a substantial amount of template due to the small quantities of DNA isolated from the eggshells. The PCR protocol and thermal profile was modified from D'Costa and Petitte (1998) which was developed for sex identification of turkey embryos. Final concentrations of the optimized multiplex-PCR (M-PCR) conditions were: 0.2 mM of each dNTPs, 0.5 μM of each primer (ATP synthase gene and PstI primer sets), 0.8 units of *Taq* DNA polymerase (Promega), 2 mM $MgCl_2$ (Promega) in a 1X Mg free buffer (Promega) with 0.25 mg/ml of bovine serum albumen (bsa) and 150 ng of isolated DNA. All reactions were performed at a final volume of 10 μl in 0.2 ml thin wall PCR tubes. The amplifications were performed using a PTC-200 Gradient Cycler (MJ Research) with the following thermal profile: initial denature at 94°C for 2 minutes; 40 cycles of 94°C for 30 seconds (denature), 51°C for 30 seconds (annealing) and 72°C for 40 seconds (extension); final extension of 72°C for 10 minutes. The isolated gene products for both the ATP synthase (250 bp) and PstI repeat (177 bp) were visualized using agarose gel electrophoresis (2%) and stained with ethidium bromide to visualize the amplicons by band size discrepancy under a UV light source.

Microsatellite Amplification.–We amplified and sequenced the WT75 and WT38-2 loci from 4 individuals (Latch et al. 2002). We used 2 samples (one male and one female) of DNA isolated from whole turkey blood collected during winter capture events (2005) and 2 samples (one male and one female) of DNA isolated from turkey egg shell remains. PCR conditions followed those from Latch et al. (2002), and the final concentrations for PCR conditions were: 0.2 mM of each dNTPs, 0.5 μM of each primer, 0.65 units of *Taq* DNA polymerase (Promega), 1.5 mM $MgCl_2$ (Promega) in a 1X Mg free buffer (Promega) with 10 ng of isolated DNA. All reactions were performed at a final volume of 50 μl in 0.2 ml thin wall PCR tubes. The amplifications were performed using a PTC-200 Gradient Cycler (MJ Research) with the following thermal profile: initial denature at 95°C for 2 minutes; 30 cycles of 95°C for 30 seconds (denature), 55°C (for WT75) or 59°C (for WT38-2) for 30 seconds (annealing), and 72°C for 40 seconds (extension); final

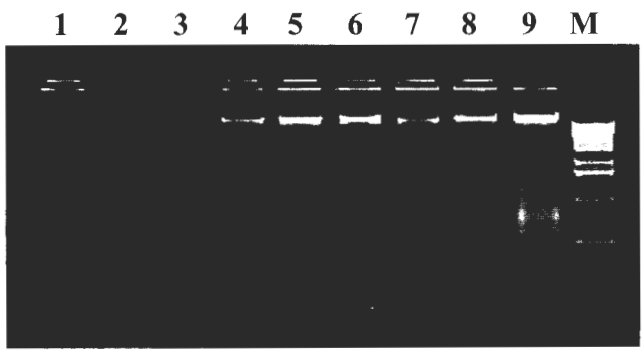

Figure 1. Total genomic DNA isolated from the chorioallantoic membrane of *Meleagris gallopavo intermedia*. Lanes 1-9 represent nine individual samples from one clutch. Lane M is a 1 kilobase (kb) ladder.

extension of 72°C for 10 minutes. The isolated gene products for both the WT75 locus (278-298 bp) and WT38-2 locus (103-119 bp) were visualized using agarose gel electrophoresis (2%) and stained with ethidium bromide to visualize the units by band size discrepancy under a UV light source.

All PCR products were purified before sequencing reactions were performed using the QIAquick® Spin Kit (QIAGEN) according to manufacturer's protocol. Both strands of the individual amplicons were sequenced (Sanger et al. 1977) on an ABI 3100 Genetic Analyzer (ABI) according to ABI protocol. Sequences were proofed, assembled and aligned using ContigExpress and AlignX (respectively) in Vector NTI Suite 7 (InforMax, Invitrogen life science software). Alignments were made based on sequence data deposited in GenBank (NCBI) for the WT75 locus (accession number AF434907) and for the WT38-2 locus (accession number U79365) (Latch et al. 2002).

RESULTS

Sex Identification using PCR.–From eggs collected no more than 9 days post-hatch, we assigned sex identifications for 63 individuals from a total of 7 unique clutches over two separate study sites (Appendix I). Of these 63 individuals, 35 were identified as males, showing a single band, and 28 were females, showing two bands (Fig. 2). The Matador site ($n = 20$) consisted of 70.0% male individuals, and the Salt Fork site ($n = 43$) 48.9% males. To assess accuracy of the technique, we correctly identified 6 individuals ($n = 3$ males and 3 females) using DNA isolated from blood of known male and female turkeys (Fig. 3).

Microsatellite Amplification.–We were able to successfully amplify both the WT75 (Fig. 4) and the WT38-2 loci from both eggshell and blood DNA. The repetitive elements for the Salt Fork site individuals were $[TG]_4$ and $[TCG]_7$ for TK136464 and $[TG]_{11}$ for TK136466. The repetitive elements for the Matador site individuals were $[GT]_5$ and $[CT]_{10}$ for M307-2 and $[GT]_{11}$ and $[CT]_7$ for M307-4. For the WT38-2, the repetitive element for both study sites was $[CA]_{15}$.

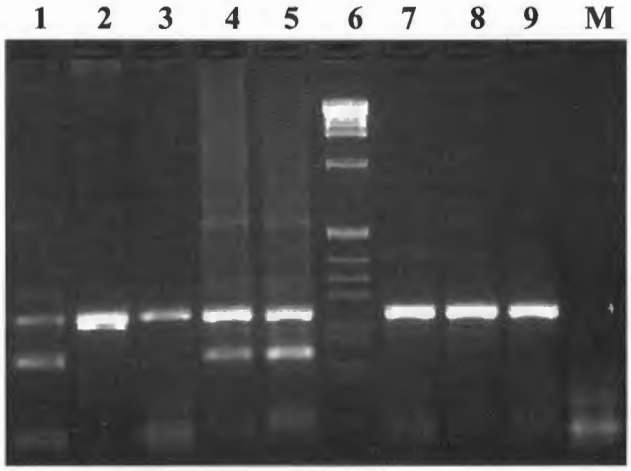

Figure 2. Sex identification of turkey egg shell remains by multiplex PCR (M-PCR) using total genomic DNA. Lane M is a 1 kb ladder. All individuals amplify the 250 bp turkey specific ATP synthase gene, and females are determined by the 177 bp PstI repeat. Male samples are shown with one band in lanes 2-3 and 6-8. Female samples are shown with 2 bands in lanes 1 and 4-5. Lane 9 is a no template control reaction.

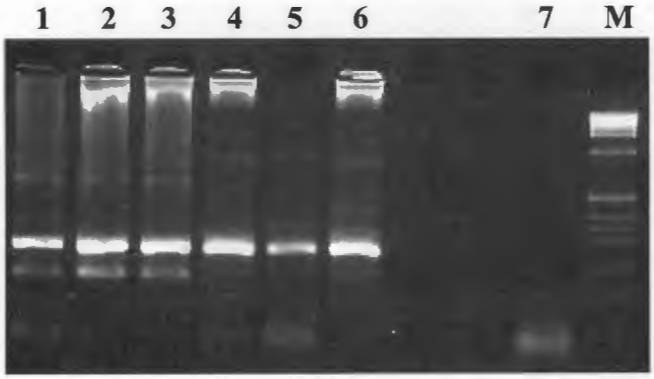

Figure 3. Sex identification by multiplex PCR (M-PCR) using total genomic DNA isolated from blood of adult birds of known sex. Lane M is a 1 kb ladder. Female samples are shown with two bands in lanes 1-3. Male samples are shown with one band in lanes 4-6. Lane 7 is a no template control reaction.

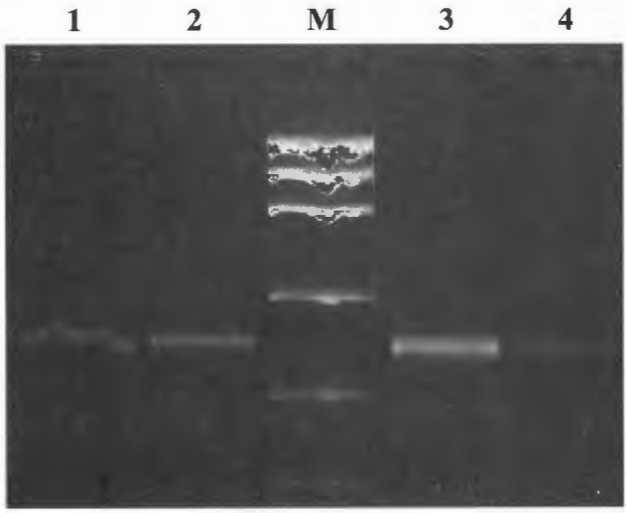

Figure 4. Amplification and PCR purification of the WT75 locus using total genomic DNA isolated from blood and egg shell remains. Lane M is a low mass ladder. DNA from blood is in lanes 1-2. DNA from egg shell remains is in lanes 3-4.

DISCUSSION

Eggshell remains are a logistically feasible, non-invasive source of genetic data. While our study did not empirically address DNA degradation over time, viable DNA was obtainable over a rather large temporal window (9 days) between nest hatch and collection of eggs. Environmental conditions such as heat, sunlight, rain, or insects may have initially affected DNA quantity or quality. Post collection, time to suspension appeared to influence DNA quantity as evidenced by our trend line (Fig. 5). In radio telemetry studies, eggs could be collected with little additional effort and provide a wealth of genetic information on a range of questions, possibly even providing genetic data from the mother (Strausberger and Ashley 2001). DNA from eggshells may prove to be an easily collected and stored warehouse of genetic material.

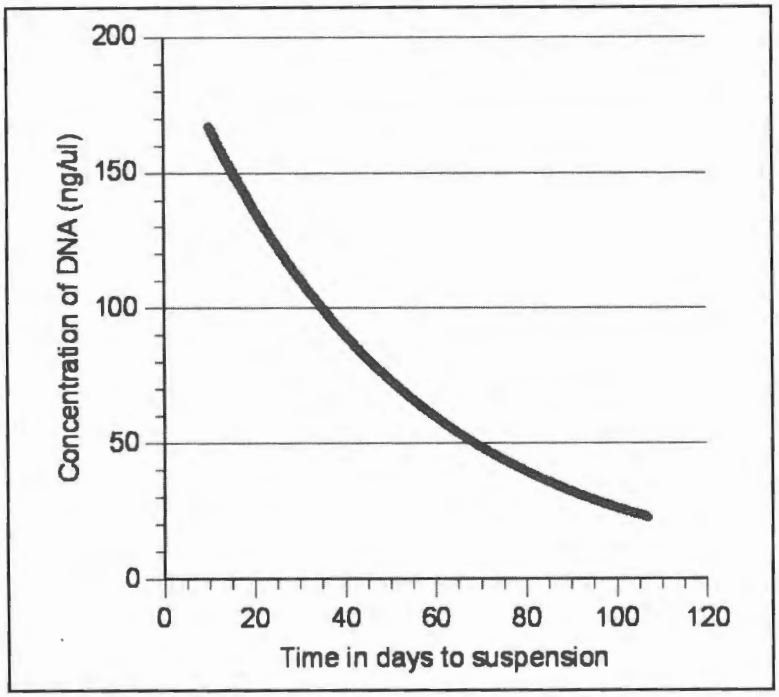

Figure 5. A trend line illustrating degradation of final product DNA concentrations between collection time and suspension in a lysis buffer solution (R^2=0.48, n=63).

For wildlife managers, sex ratios at hatch are a mystery in many game bird species. Several studies suggest equal sex ratios may be inaccurate in some avian species (Hamilton 1967; Rosenfield et al. 1996; Sheldon 1998), yet wild turkey population models typically assume a 50/50 sex ratio (Healy and Powell 1999). While Healy and Powell (1999) suggest skewed sex ratios may influence models, the data presented here are the only datasets known to the authors regarding wild turkey sex ratios at birth. In fact, the paucity of sex ratio data on many game species is indicative of the difficulty associated with capturing cryptically-colored, sexually monomorphic, precocial young. Further, direct capture of young may invite sexually-biased capture probabilities and influence other population estimates associated with study goals (Spraker et al. 1987; Colwell et al. 1988). However, eggshells may be easily collected, inhibit repetitive sampling, and maintain unbiased population estimates. While the authors foresee many potential uses for eggshell DNA (e.g., dump-nesting documentation, parentage), none may be as beneficial as the ability of eggshells to produce estimates of secondary sex ratios.

We believe that we have a straightforward and accurate method for wildlife and molecular biologists to use wild turkey egg shell remains as a source of genetic material. Our primary goal was to test whether or not sex identifications could be made using DNA isolated from egg shell remains (i.e., chorioallantoic membrane). The use of a turkey specific ATP synthase gene may allow for accurate gender determination even in possibly contaminated samples (e.g., avian predation). Amplifying the ATP synthase gene in a multiplex reaction with the female specific PstI element greatly reduces the cost of this method. An important note when using this protocol is the amount of DNA necessary to run the sexing PCR. The range of DNA concentrations was 0.6 to 214.3 ng/µl ($\bar{x}$ = 37.27 ± 5.12) for samples used in this study. This indicates that some reactions, limited by the reaction volume, do not actually have 150 ng of DNA template. While we had a number of reactions work using approximately 100 ng of starting material, 150 ng of starting material is optimal for this protocol and should be the target concentration.

We wanted to further assess the quality and capabilities of DNA from egg shell remains for other molecular techniques. A number of studies on the genetic variation in wild and domestic turkeys using short tandem repeats (also referred to as microsatellites) can be found in the literature (Huang et al. 1999; Latch et al. 2002). Therefore we amplified and sequenced two turkey specific loci based on a study by Latch et al. (2002). While additional samples would be warranted to determine their utility, preliminary results suggest the WT75 locus varies within and between populations, and that the WT38-2 found little to no variation within and between populations. Small sample sizes confound interpretation of these results, yet may be of importance in future studies of Rio Grande wild turkeys in the High Plains (Table 1).

Table 1. A comparison of the WT75 and WT38-2 loci microsatellites used in this study and Latch et al. (2002).

Source Material	Sample Identification	WT75 locus Motif	WT38-2 locus Motif
Blood	TK136464	$[TG]_4 + [TCG]_7$	$[CA]_{15}$
	TK136466	$[TG]_{11}$	$[CA]_{15}$
Egg Shell	M307-2	$[GT]_5 + [CT]_{10}$	$[CA]_{15}$
	M307-4	$[GT]_{11} + [CT]_7$	$[CA]_{15}$
Eastern wild turkey blood	Latch et al. 2002	$[TG]_{16}$	$[CA]_{16}$

Eggshell remains appear to be pragmatic sources of quality genetic material from wild turkeys. Despite low sample sizes, our data suggest no practical differences between the quality of DNA isolated from blood and the quality of DNA isolated from egg shell remains. Further, the quality of DNA isolated from the chorioallantoic membrane of egg shell remains can be used in sex identification and other molecular techniques such as microsatellite amplification.

ACKNOWLEDGMENTS

The field work portion of this study was funded by Texas Parks and Wildlife, the National Wild Turkey Federation, and the Texas Chapter Superfund of the National Wild Turkey Federation. This is manuscript #T-9-1093 of the College of Agricultural Sciences and Natural Resources, Texas Tech University. The field work portion of this project was performed under Texas Tech University Animal Care and Use Committee Approved Protocols 05020-04 and T05047. We appreciate the hard work of James C. Bullock, Matthew J. Butler, Galon I. Hall, Rachel H. Houchin, Ross T. Huffman, Brian E. Petersen, Ryan M. Swearingin, and Ryan N. Walker for eggshell collection in the field and Michelle C. Knapp for laboratory assistance. Steven R. Hoofer, Peter A. Larsen and two anonymous reviewers provided valuable comments which greatly improved this manuscript.

LITERATURE CITED

Boersen, M. R., J. D. Clark, and T. L. King. 2003. Estimating black bear population density and genetic diversity at Tensas River, Louisiana using microsatellite DNA markers. Wildlife Society Bulletin 31:197-207.

Bush, K. L., M. D. Vinsky, C. L. Aldridge, and C. A. Paszkowski. 2005. A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater sage-grouse (*Centrocercus uropihasianus*). Conservation Genetics 6:867-870.

Colwell, M. A., C. L. Gratto, L. W. Oring, and A. J. Fivizzani. 1988. Effects of blood sampling on shorebirds: injuries, return rates, and clutch desertions. Condor 90:942-945.

D'Costa, S., and J. N. Petitte. 1998. Sex identification of turkey embryos using a multiplex Polymerase Chain Reaction. Poultry Science 77:718-721.

Farrell, L. E., J. Roman, and M. E. Sunquist. 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. Molecular Ecology 9:1583-1590.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science 156:477-488.

Healy W.M., and Powell S.M. 1999. Wild turkey harvest management: biology, strategies and techniques. BTP-R5001-1999, -96. Shepherdstown, WV, USFWS. Biological Technical Publication.

Haig, S. M. 1998. Molecular contributions to conservation. Ecology 79:413-425.

Huang, H. B., Y. Q. Song, M. Hsei, R. Zahorchak, J. Chlu, C. Teuscher, and E. J. Smith. 1999. Development and characterization of genetic mapping resources for the turkey (*Meleagris gallopavo*). The Journal of Heredity 90:240-242.

Latch, E. K., E. J. Smith, and O. E. Rhodes Jr. 2002. Isolation and characterization of microsatellite loci in wild and domestic turkeys (*Meleagris gallopavo*). Moleculary Ecology Notes 2:176-178.

Longmire, J. L., M. Maltbie, and R. J. Baker. 1997. Use of "lysis buffer" in DNA isolation and its implication for museum collections. Occasional Papers, Museum, Texas Tech Univeristy 163:1-4.

McCracken, K. G., W. P. Johnson, and F. H. Sheldon. 2001. Molecular population genetics, phylogeography, and conservation biology of the mottled duck (*Anas fulvigula*). Conservation Genetics 2:87-102.

Nicholson, D. S., R. L. Lochmiller, M. D. Stewart, R. E. Masters, and D. M. Leslie, Jr. 2000. Risk factors associated with capture-related death in Eastern wild turkey hens. Journal of Wildlife Diseases 36:308-315.

Ortega, J., M. D. R. Franco, B. A. Adams, K. Ralls, and J. E. Maldonado. 2004. A reliable, non-invasive method for sex determination in the endangered San Joaquin kit fox (*Vulpes macrotis mutica*) and other canids. Conservation Genetics 5:715-719.

Parker, P. G., A. A. Snow, M. D. Schug, G. C. Booton, and P. A. Fuerst. 1998. What molecules can tell us about populations: choosing and using a molecular marker. Ecology 79:361-382.

Pearce, J. M., R. L. Fields, and K. T. Scribner. 1997. Nest materials as a source of genetic data for avian ecological studies. Journal of Field Ornithology 68:471-481.

Pearse, D. E., C. M. Eckerman, F. J. Janzen, and J. C. Avise. 2001. A genetic analogue of 'mark-recapture' methods for estimating population size: an approach based on molecular parentage assessments. Molecular Ecology 10:2711-2718.

Piggott, M. P., and A. C. Taylor. 2003. Remote collection of animal DNA and its application in conservation management and understanding the population biology of rare and cryptic species. Wildlife Research 30:1-13.

Reed, J. E., R. J. Baker, W. B. Ballard, and B. T. Kelly. 2005. Differentiating Mexican wolf and coyote scats using DNA analysis. Wildlife Society Bulletin 32:685-692.

Reed, J. Z., D. J. Tollit, P. M. Thompson, and W. Amos. 1997. Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. Molecular Ecology 6:225-234.

Rosenfield, R. N., J. Bielefeldt, and S. M. Vos. 1996. Skewed sex ratios in Cooper's Hawk offspring. The Auk 113:957-960.

Rudick, J. A., T. E. Katzner, E. A. Bragin, O E. Rhodes, Jr., and J. A DeWoody. 2005. Using naturally shed feathers for individual identification, genetic parentage analyses, and population monitoring in an endangered Eastern imperial eagle (*Aquila heliace*) population from Kazakhstan. Molecular Ecology 14:2959-3967.

Sanger, F., S. Nicklens, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences of the United States of America 74:5463-5467.

Segelbacher, G. S., and I. Storch. 2002. Capercaillie in the Alps: genetic evidence of metapopulation structure and population decline. Molecular Ecology 11:1669-1677.

Sheldon, B. C. 1998. Recent studies of avian sex ratios. Heredity 80:397-402.

Spraker, T. R., W. J. Adrian, and W. R. Lance. 1987. Capture myopathy in wild turkeys (*Meleagris gallopavo*) following trapping, handling and transportation in Colorado. Journal of Wildlife Diseases 23:447-453.

Strausberger, B. M., and M. V. Ashley. 2001. Eggs yield nuclear DNA from egg-laying female cowbirds, their embryos and offspring. Conservation Genetics 2:385-390.

Taberlet, P., and J. Bouvet. 1991. A single plucked feather as a source of DNA for bird genetic studies. Auk 108:959-960.

Taberlet, P., L. P. Waits, and G. Luikart. 1999. Noninvasive genetic sampling: look before you leap. Trends in Evolution and Ecology 14:323-327.

Triant, D. A., R. M. Pace, and M. Stine. 2004. Abundance, genetic diversity and conservation of Louisiana black bears (*Ursus americanus leteolus*) as detected through noninvasive sampling. Conservation Genetics 5:647-659.

Addresses of authors:

ADAM D. BROWN

Department of Biological Sciences
Texas Tech University
Lubbock, TX 79409-3191

Current address:
Children's Cancer Research Institute
University of Texas Health Science Center at San Antonio
San Antonio, TX 78229-3900
e-mail: Adam.D.Brown@ttu.edu

JOHN H. BRUNJES, IV

Department of Range, Wildlife and Fisheries Management
Texas Tech University
Lubbock, TX 79409-2125
email: John.Brunjes@ttu.edu

RICHARD S. PHILLIPS

Department of Range, Wildlife and Fisheries Management
Texas Tech University
Lubbock, TX 79409-2125
e-mail: Richard.Phillips@ttu.edu

WARREN B. BALLARD

Department of Range, Wildlife and Fisheries Management
Texas Tech University
Lubbock, TX 79409-2125
e-mail: Warren.Ballard@ttu.edu

MARK C. WALLACE

Department of Range, Wildlife and Fisheries Management
Texas Tech University
Lubbock, TX 79409-2125
e-mail: Mark.Wallace@ttu.edu

ROBERT J. BAKER

Department of Biological Sciences
Texas Tech University
Lubbock, TX 79409-3191
e-mail: rjbaker@ttu.edu

APPENDIX I

List of specimens examined. Specimens were collected from natural wild populations and are archived in the Natural Science Research Laboratory (NSRL) at the Museum of Texas Tech University (TTU).

TK#	DNA Concentration	Sex	Days from collection to suspension in lysis buffer
140546	NA	Male	73
140547	NA	Male	73
140548	NA	Male	73
140549	NA	Female	73
140550	NA	Male	73
140551	NA	Male	73
140552	NA	Male	73
140553	NA	Male	73
140554	NA	Female	73
140555	NA	Male	73
140556	NA	Female	73
140557	4.9	Female	107
140558	18.8	Unknown	107
140559	18.7	Male	107
140560	20.4	Male	107
140561	18.3	Female	107
140562	11	Male	107
140563	17.6	Female	107
140564	18.3	Male	107
140565	10.7	Male	107
140566	NA	Male	78
140567	NA	Female	78
140568	NA	Male	78
140569	NA	Female	78
140570	NA	Male	78
140571	NA	Female	78
140572	NA	Male	78
140573	NA	Female	78
140574	NA	Female	78
140575	NA	Female	78
140576	NA	Male	78
140577	66	Female	10
140578	170.1	Female	10
140579	166.6	Male	10
140580	117.7	Male	10
140581	125.4	Female	10
140582	99.6	Female	10
140583	126	Female	10
140584	178.8	Male	10
140585	90.7	Female	23
140586	39.2	Female	23
140587	74.2	Male	23
140588	139.9	Male	23
140589	121.3	Female	23
140590	145.8	Female	23
140591	163.6	Female	23
140592	134	Male	23
140593	169.1	Unknown	23

Appendix I (cont.)

TK#	DNA Concentration	Sex	Days from collection to suspension in lysis buffer
140594	227.2	Male	68
140595	63.9	Male	68
140596	108.5	Male	68
140597	335.6	Female	68
140598	77.6	Female	68
140599	111.2	Male	68
140600	161.2	Male	68
140601	437.2	Male	68
140602	302.7	Female	68
140603	33.3	Male	78
140604	27.5	Female	78
140605	44.7	Female	78
140606	45.9	Male	78
140607	26.8	Male	78
140608	19.4	Female	78
140609	19.8	Female	78
140610	23.9	Male	78

TEXAS TECH UNIVERSITY
Natural Science Research Laboratory

PUBLICATIONS OF THE MUSEUM OF TEXAS TECH UNIVERSITY

Institutional subscriptions are available through the Museum of Texas Tech University, attn: NSRL Publications Secretary, Box 43191, Lubbock, TX 79409-3191. Individuals may also purchase separate numbers of the Occasional Papers directly from the Museum of Texas Tech University.

ISSN 0149-175X

Museum of Texas Tech University, Lubbock, TX 79409-3191