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MITOCHONDRIAL-DNA VARIATION WITHIN AND AMONG WILD TURKEY (*MELEAGRIS GALLOPAVO*) SUBSPECIES

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ABSTRACT

Genetic variation within and among wild Turkey, *Meleagris gallopavo*, subspecies was measured using DNA sequencing and PCR-RFLP analysis of a 655 bp portion of the mtDNA D-loop region. DNA sequencing from 25 individual Eastern, Merriam, Rio Grande and domestic Turkeys revealed 16 polymorphic sites. Parsimony and neighbor-joining analysis did not show support for the four wild Turkey subspecies studied. PCR-RFLP analysis of 118 individuals revealed 13 distinct haplotypes. Haplotype variation was detected in all wild Turkey populations, and Wright's F-statistics revealed pronounced differentiation (F_{ST} = 0.302) among populations. Based on these data, non-native wild Turkey populations do not appear to have suffered a genetic bottleneck since their reintroduction.

† † †

Wild Turkey, *Meleagris gallopavo* L., together with Pheasants, *Phasianus* spp., Quails, *Colinus, Coturnix* spp., Partridges, *Alectoris* spp., and Chicken, *Gallus gallus*, belong to the Galliformes family Phasianidae (Gutierrez et al. 1993). Within *M. gallopavo*, 6 subspecies are currently recognized in the United States, including domestic Turkey, *Meleagris gallopavo gallopavo*; Eastern, *Meleagris g. silvestris*; Merriam, *M. g. merriami*; Rio Grande, *M. g. intermedia*; Gould's, *M. g. mexicana*; and Florida, *M. g. osceola* (Aldrich 1967). These subspecies were designated based upon morphological characteristics and historical geographical ranges (Stangel et al. 1992) and provide the basis for subspecies management. However, morphological differences such as plumage and size measurements are unable to consistently discriminate among subspecies (Stangel et al. 1992). In addition, the current status of many populations is questionable because of extensive reintroductions and translocation efforts following the elimination of wild Turkey from much of its original range by excessive hunting, diseases introduced from domestic stocks, and habitat alteration (Beasom and Wilson 1992).

Genetic analysis of wild Turkey may provide insight into the extent of genetic variation within and the extent of genetic differentiation among subspecies. Previous studies on wild Turkey population structure (Boone and Rhodes 1996, Leberg 1991, Leberg et al. 1994, Rhodes et al. 1995, Stangel 1991) have measured allozyme variation in geographically localized and dispersed populations. These studies detected genetic differentiation within localized flocks and populations as well as geographically dispersed populations. They were unable to differentiate subspecies or distinguish among wild, game-farm, and domestic stock based on allele frequencies.

Mitochondrial-DNA (mtDNA) analysis is generally assumed to be more powerful than allozyme analysis for revealing population structure, and has been used for numerous avian systematic and population genetic studies (Avise 1994, Miranda et al. 1997). Wenink et al. (1993) employed sequences from the mtDNA D-loop region to demonstrate significant regional population structure in the dunlin, *Calidris alpina*, a long-dis-

Table 1. Samples of *Meleagris gallopavo* used in this study.

Subspecies	State	Location	Abbv.
M. g. (unknown)	NE	Boyd Co.	NB
	NE	Cherry Co.	NC
	NE	Keya Paha Co.	NKP
	NE	Knox Co.	NK
	NE	Lincoln Co.	NL
	OR	Benton Co.	OR
M. g. silvestris (Eastern)	TN	Fred Stimpson Game Sanctuary	TE
	AL	Upper State Game Sanctuary	AEF
	AL	Coastal Plains	AEU
	SC	Piedmont	SCEC
	SC	North east	SCES
	SD		SDE
M. g. merriami (Merriam)	CO	Dolores Co.	CM
	NM	Fax Co.	NM
M. g. intermedia (Rio Grande)	TX		TR
M. g. *gallopavo* (domestic Turkey)	WI		DTWI
	MN		DTMN
	KS		DTKS

tance migrant shorebird with Holarctic nesting distributions. Mitochondrial DNA has also revealed significant geographical structure over relatively small spatial scales, as in the song sparrow *Melospiza melodia* (Zink 1991). These studies and others have revealed that avian species exhibit a variety of population genetic structures (Avise and Ball 1991).

Our objective was to determine whether there was support of subspecies status based on mitochondrial-DNA (mtDNA) D-loop variation within and among populations. In addition we attempted to describe the genetic structure of Nebraska wild Turkey populations, thus providing a basis for genetic management based on presence or absence of subspeciation.

METHODS

Blood samples from "source" populations without a recorded history of reintroduction were obtained in cooperation with members of the National Wild Turkey Federation Technical Committee from geographically distinct locations across the original range of each subspecies (Table 1). Liver and tissue samples from populations of unknown subspecific status from Nebraska, Oregon, and New Mexico birds were obtained. Tissue samples of domestic Turkey originating from Minnesota, Wisconsin, and Kansas were also obtained (Table 1).

DNA was obtained from a 5.0 µl blood sample or from a 3 × 3 mm tissue sample using the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). The primers LND6-1 (5'–CCCCATAATACGGCG-AAGGATT-3') (Desmond 1997) and WTDL-R (5'-GTTCAGGAGTTATGCATGGGATGT-3') were used to amplify a 3' region of the mtDNA NADH dehydrogenase subunit VI gene, tRNA leucine, and approximately 500 bp of the D-loop region. Primers were synthesized by the Iowa State University DNA Sequencing Facility, Ames, IA. The WTDL-R primer anneals to a conserved region in the middle of the D-loop region and was designed from Chicken, *Gallus gallus* (GenBank accession number X52392), and Quail, *Coturnix coturnix* (GenBank accession number X57245). The 5' ends of the primers are located at 16621 and 516 on the Chicken mtDNA genome (Desjardins and Morais 1990).

For PCR amplification, 20.0 µl of sample DNA from the chelex DNA extraction or 1.5 µl from the phenol-choloform DNA extraction was added to a reaction mixture containing 5.0 µl of reaction buffer (Promega, Madison, WI), 4.0 ul of dNTP mix (10 mM each dATPm dTTP, dCTP, and dGTP) (Promega), 1.0 µl of each primer (20 mM), 2.0 units of *Taq* polymerase (Promega) and nanopure water to a volume of 50.0 µl. Amplifications were done in a Perkin Elmer Cetus (Norwalk, CT) model 480 thermocycler programmed for 35 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 90 s. Amplification products were stored at -20ϒC.

DNA sequencing was conducted by purifying amplified DNA using Micron 50 microconcentrator (Amicon Inc, Beverly, MA), and resuspended to a volume of 10.0

μl using nanopure water. Purified and concentrated DNA, approx. 10.0 ng/100 bp, was sent to the DNA Sequencing Facility, Iowa State University (Ames, IA) for direct sequencing in both directions. Consensus sequences for each individual were derived using the GCG (Genetics Computer Group, Madison, WI) GAP program. The GenBank accession numbers for the DNA sequence are AF172947 to AF172964.

Sequences were aligned with the GCG PILEUP program (with a gapweight of 5.0 and a gap-length weight of 1.0) using Chicken, *Gallus gallus* (GenBank accession number X52392), and common quail, *Coturnix coturnix* (GenBank X57245) as outgroup taxa. Parsimony analyses on the alignments were conducted with PHYLIP v3.57c (Felsenstein, 1993) on phylogenetically informative characters only, with gaps being excluded. Bootstrapping was performed by generating 1000 data sets with the SEQBOOT program. Most-parsimonious trees were constructed using DNAPARS. A majority rule and combinable competent consensus of these trees were constructed using the CONSENSE program in PHYLIP. The DNADIST program of PHYLIP was used to calculate genetic distances according to the Kimura 2-paramter (Kimura 1980) and maximum likelihood models of sequence evolution. Trees were constructed from these distances with the NEIGHBOR and FITCH programs to create neighbor-joining (Saitou and Nei 1987), and UPGMA (Sokal and Michener 1958) trees.

Restriction enzymes sites were predicted from the sequence data using Webcutter 2.0 (Heiman 1997) for PCR-RFLP analysis. Amplified DNA was digested according to manufacturer's recommendations, using the restriction enzymes *Acc* I, *Mnl* I, *Nla* III (New England Biolabs), and *Tai* I (MBI Fermentas Inc., Amherst, NY) per Szalanski et al. (1997). One μl of loading buffer (10% Ficoll 400, 0.25% Bromophenol Blue, 50 mM EDTA, 10 mM Tris-HCL pH 7.5) was added to the 10 μl digest product. Fragments were separated by vertical polyacrylamide gel electrophoresis (PAGE) per Taylor et al. (1997). Restriction profiles for each enzyme were given letter designations in order of discovery, with the first pattern designated "A", the second "B", and so on. Haplotypes of each Turkey were then identified by the combination of letters representing the restriction profiles for each restriction enzyme used.

Haplotype (nucleon) and nucleotide diversities within samples, and nucleotide diversities and divergences between samples, were computed with the Restriction Enzyme Analysis Package (REAP) (McElroy et al., 1992) following the procedures of Nei & Tajima (1981) and Nei & Miller (1990). Genotypic diversity (G) was computed by $(n/[n-1])(1- fi_1^2)$ where fi is the frequency of the ith mtDNA haplotype in a sample of n individuals. G is the probability that random matings will be between individuals with different mtDNA haplotypes (Nei 1987). Nested analysis of variance of haplotype frequencies within and among populations was calculated using Analysis of Molecular Variance (AMOVA) 1.55 (Excoffier et al. 1992).

RESULTS

Polymerase chain reaction resulted in a 655 bp amplicon for all samples (Fig. 1). Twenty-two wild Turkey and 3 domestic Turkey individuals were subjected to DNA sequencing. Among these DNA sequences, 16 of the 655 positions in the sequence were variable. There were 16 substitutions in the 502 bp of the D-loop region, and no variation was detected in the more slowly evolving NADH VI gene and tRNA glucine regions. Genetic distance within *M. gallopavo* ranged from 0.0 to 1.5%, with a mean of 0.7%.

The aligned DNA data matrix, including outgroup taxa, (available upon request, and at the web site http://ianrwww.unl.edu/ianr/plntpath/nematode/aszalans.htm) resulted in a total of 721 characters, including gaps. Of the 655 *Meleagris* characters, 16 were variable and 11 were parsimony-informative among the DNA sequences. Parsimony analysis of the aligned sequences based on a consensus of 1000 bootstrap replicates showed support (>50% of the replications) for only two clades (Fig. 1), one of which consisted of three of the four Colorado *M. g. merriami* DNA sequences. The cladogram inferred from the neighbor-joining analysis was nearly identical in topology to the one derived from parsimony analysis. The only difference between the neighbor-joining and the parsimony trees was that one South Dakota *M. g. silvestris* and the Texas *M. g. intermedia* DNA sequences clustered with the Colorado Merriam clade.

Analysis of polymerase chain reaction-restriction fragment length polymorphism revealed 13 haplotypes among the 116 Turkeys from 18 populations (Tables 2, 3). Mean divergence between the haplotypes (d) was 0.017 (n = 17, SD = 0.021). The mtDNA sequences and the PCR-RFLP haplotypes were not consistent (Fig. 1), possibly because of informative polymorphic sites that were not detected by RFLP analysis.

Haplotype variation was observed within all populations, except for domestic Turkey (Table 3). Of the 13 RFLP haplotypes, 12 were present in Easterns from the southeastern U.S. (Alabama, South Carolina and Tennessee), whereas only six were present in the western (Oregon, South Dakota, Nebraska, Colorado, New Mexico and Texas) populations. Haplotype AAAA was the most common and occurred in 11 of the 18 populations. Six haplotypes were observed in only a single population, five of which occurred only in South Caro-

Table 2. *Meleagris gallopavo* restriction enzyme recognition sites and fragments from PCR-RFLP of mtDNA D-loop amplicon.

Restr. enzyme	Haplo-type	Recognition sites	Size of resultant fragments (bp)
Acc I	A	216, 306	349, 216, 90
	B	216	444, 216
Nla III	A	356, 427, 557, 638	356, 130, 81, 71, 18
	B	356, 401, 427, 557, 638	356, 130, 81, 45, 26, 18
	C	356, 557, 638	356, 201, 81, 18
	D	356, 427, 638	356, 211, 71, 18
Tai I	A	286, 485	286, 200, 169
	B	286	369, 286
Mnl I	A	447, 517, 593, 599, 617, 626	447, 76, 70, 29, 18, 9, 6
	B	517, 593, 599, 617, 626	517, 76, 29, 18, 9, 6
	C	416, 447, 517, 593, 599, 617, 626	416, 76, 70, 31, 29, 18, 9, 6
	D	343, 447, 517, 593, 599, 617, 626	343, 104, 76, 70, 29, 18, 9, 6
	E	416, 517, 593, 599, 617, 626	416, 101, 76, 29, 18, 9, 6

lina. All of the domestic Turkey samples shared a common haplotype, AAAC, which was the second most common wild Turkey haplotype.

Nested analysis of haplotype frequencies revealed that most of the variation occurred within populations and accounted for 86% of the variance, when the Nebraska populations were contrasted with each other. Likewise, 70% and 67% of the variance was accounted for when populations were grouped by states and subspecies, respectively. Pronounced spatial differentiation, F_{ST} = 0.145, was exhibited among the five Nebraska populations, as well as among populations from different states, F_{ST} = 0.226. This was greater than the F_{ST} value of 0.185 observed among the three subspecies.

DISCUSSION

Phylogenetic analysis of four wild Turkey subspecies does not lend support for their subspecific status. However, other extant wild Turkey subspecies that we did not sample may show phylogenetic support for their designation. The high similarity of domestic Turkey to wild Turkey, based on DNA sequence and RFLP data, makes it doubtful that domesticated birds can be differentiated from wild stocks. This is expected given the relatively short period (<400 yrs) since the domestication of Turkey. Also, given a mitochondrial evolutionary clock of 2% sequence divergence per million years (Helm-Bychonski et al. 1986), we predict only a 0.0008% sequence divergence during this time if wild Turkey and domestic Turkey were prevented from interbreeding.

Genetic variation is being maintained within localized native and introduced wild Turkey populations. Although genetic differentiation is pronounced among these populations, there is no correlation with their existing subspecific classification. Previous allozyme studies have observed partitioning of allelic frequencies among localized flocks of Eastern (Boone et al. 1996), and Rio Grande (Rhodes et al. 1995) birds. Leberg (1991) examined Eastern wild Turkey from four states and found the majority of allozyme variation to be partitioned among states. Analysis of 713 wild Turkeys from 22 eastern United States populations revealed five polymorphic allozyme loci (Leberg et al. 1994). Four of the five loci exhibited substantial variation among populations, with an average F_{ST} value of 0.130. Boone et al. (1996) conducted analysis of 72 Eastern wild Turkeys from Aiken County, SC, and detected 5 polymorphic allozyme loci. The mean F_{ST} value was 0.054 and F_{IT} and F_{IS} values were 0.06 and 0.014, respectively. Rhodes et al. (1995) studied localized allozyme variation among Rio Grande flocks in Kansas and found greater variation within than among wintering flocks.

Our data are consistent with the conclusion of the previous studies that gene flow among populations of Turkeys in close proximity is low. In addition, low dispersal rates may help to maintain overall genetic identity within local geographical regions by allowing a large proportion of genetic variation once to be partitioned among flocks. We observed that flocks introduced to Nebraska are maintaining levels of genetic variation similar to those of non-introduced populations.

Table 3. Number of four-enzyme haplotypes of Turkey, in the order *Acc* I, *Nla* III, *Tai* I, and *Mnl* I from 18 populations.

Haplo-	Location																		
type	AEF	AEU	SCEC	SCES	TE	SDE	NB	NKP	NL	NC	NK	OR	CM	NM	TR	DTMN	DTKS	DT	*n*
AAAA 1	3	7	2	2	4	4	0	2	1	1	0	0	1	1	0	0	0	0	30
BAAA 2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
AABA 3	0	1	4	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	9
ABAA 4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
ABBA 5	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
ABAC 6	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
ACAA 7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
ADAC 8	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	3
AABC 9	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AAAB 10	1	0	1	0	1	0	2	0	0	0	1	0	4	5	0	0	0	0	15
AAAC 11	0	0	1	0	0	2	4	0	4	3	5	3	0	0	0	4	4	2	32
AAAD 12	0	1	0	0	0	0	2	3	2	0	0	0	3	0	6	0	0	0	17
AAAE 13	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	4
n	4	11	11	7	8	6	8	5	7	4	6	5	12	6	6	4	4	2	118

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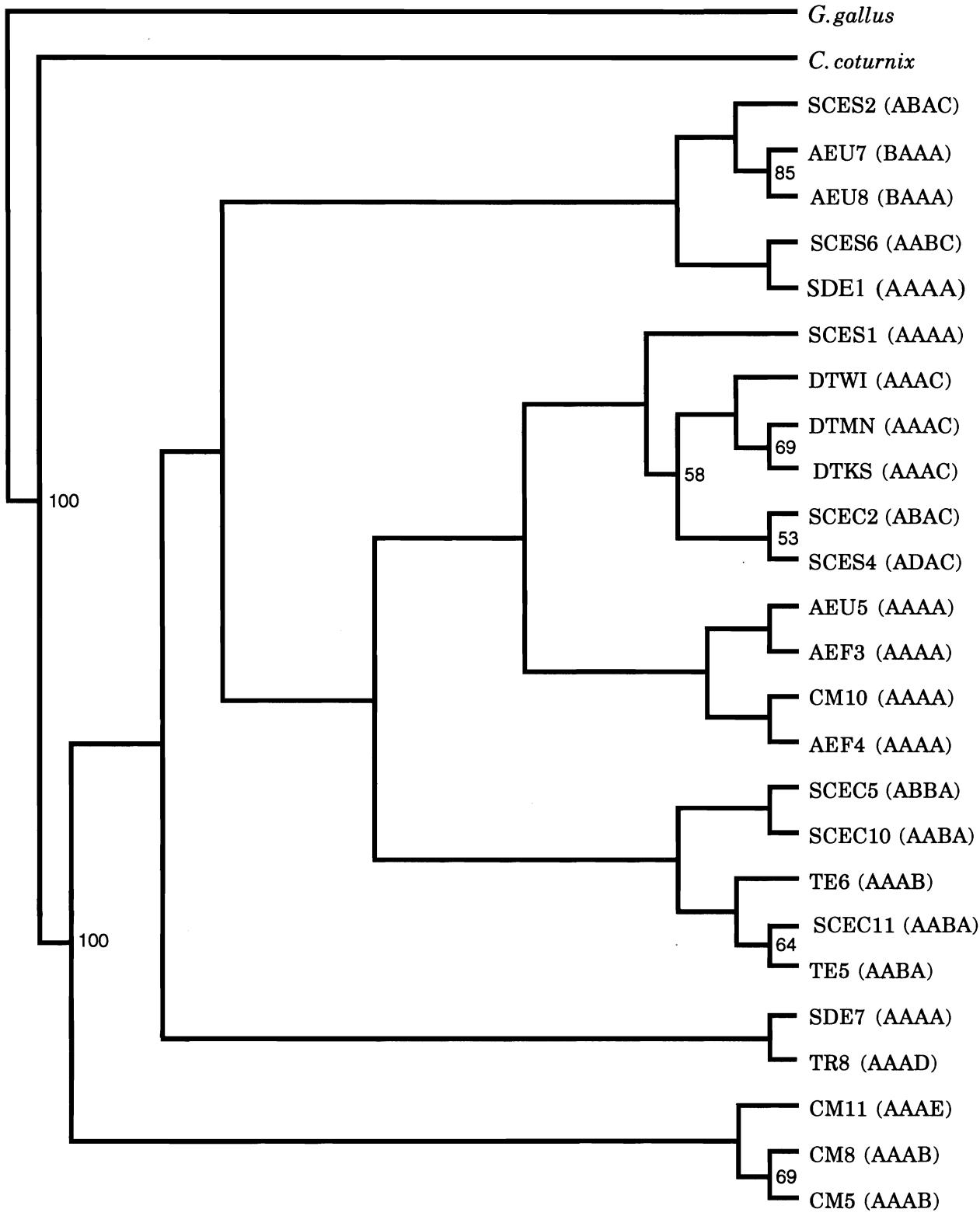

Figure 1. *Meleagris gallopavo* cladogram, derived from parsimony analysis and rooted by the Galliform outgroups, *G. gallus* and *C. coturnix*. Bootstrap values >50% are provided, and PCR-RFLP haplotypes are in parenthesis.

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