Research Article

Assessing Hybridization in Wildlife Populations Using Molecular Markers: A Case Study in Wild Turkeys

EMILY K. LATCH,[1] *Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA*
LOUIS A. HARVESON, *Department of Natural Resource Management, Sul Ross State University, Alpine, TX 79832, USA*
J. SHANE KING,[2] *Department of Natural Resource Management, Sul Ross State University, Alpine, TX 79832, USA*
MICHAEL D. HOBSON, *Texas Parks and Wildlife Department, Alpine, TX 79830, USA*
OLIN E. RHODES, JR., *Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA*

Abstract

*Extensive translocation of wildlife throughout North America has led to concerns regarding taxonomic integrity for a number of species. Often, multiple subspecies or variants were translocated into a common habitat or region, creating the opportunity for hybridization to occur. This issue is of particular concern to managers of wild turkeys (*Meleagris gallopavo*), a species in which considerable mixing of subspecies has occurred. We aim to quantify the subspecific status and degree of hybridization of individuals within an introduced population of Merriam's turkeys (*M. g. merriami*) in the Davis Mountains of Texas, USA, and within nearby Rio Grande turkey populations (*M. g. intermedia*). We used data from the Merriam's source population in New Mexico, USA, as a baseline reference for the genetic characteristics of the Merriam's subspecies. Nineteen years following the introduction event, microsatellite data indicate that the genetic integrity of the introduced population of Merriam's turkeys in the Davis Mountains Preserve has been eroded by both immigration from and hybridization with nearby Rio Grande populations. Data from the mitochondrial control region allow for further characterization of hybrid individuals and indicate that most hybrids were the result of immigrant Rio Grande males mating with resident Merriam's females. Our results attribute to the potential importance of hybridization in wildlife species and suggest that hybridization can be a rapid process capable of drastically altering the evolutionary integrity of animals in a region.* (JOURNAL OF WILDLIFE MANAGEMENT 70(2):485–492; 2006)

Key words

dispersal, gene flow, genetic, hybridization, introduction, Meleagris gallopavo*, microsatellite, mitochondria, subspecies, Texas, translocation, wild turkey.*

For more than a century, translocation of wildlife species for the purpose of reintroduction, introduction, or supplementation has been one of the most commonly used techniques in wildlife management, and for many wildlife species, programs for (re)establishing self-sustaining populations have been overwhelmingly successful (Griffith et al. 1989, Wolf et al. 1996, Fischer and Lindenmayer 2000). Some of the most well-known examples of wildlife species positively influenced by translocation include elk (*Cervus elaphus*; Robbins et al. 1982), white-tailed deer (*Odocoileus virginianus*; Jacobson and Kroll 1994), river otter (*Lontra canadensis*; Serfass et al. 1998), bighorn sheep (*Ovis canadensis*; Fitzsimmons et al. 1997), pronghorn (*Antilocapra americana*; Reat et al. 1999), and wild turkey (*Meleagris gallopavo*; Leberg 1991). Yet the extensive translocation of wildlife throughout North America has led to concerns regarding evolutionary integrity for a number of species. Multiple subspecies or variants often were translocated into a common habitat or region, creating the opportunity for hybridization either among translocated individuals of different subspecies or between subspecies of translocated and native individuals. Biologically, hybridization may result in the loss of unique genetic, morphological, behavioral, or ecological characteristics that have evolved in local populations over time. Furthermore, groups of genes that have evolved to work together (locally adapted gene complexes) may be disrupted, leaving hybrid populations less well-adapted to local environments (Dobzhansky 1970), and potentially leading to extinction of naturally occurring types (Rhymer and Simberloff 1996). The legal implications of hybridization also are critical, particularly when dealing with endangered species and the decision of whether hybrid populations should be protected under the Endangered Species Act (ESA; Allendorf et al. 2001, 2004).

Unfortunately, despite the substantial potential for human-mediated hybridization events and the serious biological and legal implications associated with such mixing, few empirical data exist on human-mediated hybridization for North American wildlife species outside of fish (but see Pilgrim et al. 1998). In fish species, human-mediated hybridization is one of the most significant factors resulting in the loss of native populations (Allendorf and Leary 1988, Ferguson 1990). For example, the decision of how to treat hybridized populations of westslope cutthroat trout under the ESA has been a subject of much debate and remains unresolved (Allendorf et al. 2004).

The potential for hybridization among subspecies is a growing concern for biologists who manage wild turkeys. Although a few natural hybrid zones are thought to exist where ranges of the 5 recognized turkey subspecies overlap, the greatest single factor contributing to these taxonomic concerns pertains to the extensive translocation of wild turkeys throughout North America, making the likelihood of human-mediated hybridization events very high for this species. While thousands of translocation events over the last 50 years have restored the wild turkey to most of its original habitats, these activities also have extended and mixed the historical ranges of many extant turkey subspecies. Thus, literally hundreds of cases exist in which the subspecies of wild turkeys

[1] *E-mail: latche@purdue.edu*
[2] *Present address: Idaho Department of Fish and Game, 1345 Barton Road, Pocatello, ID 83204, USA*

have been artificially combined and now have the opportunity to hybridize. Certainly, there is much debate surrounding subspecies nomenclature, particularly in avian species (Zink and McKitrick 1995). However, the distinctiveness of many recognized wild turkey subspecies is supported by corroboration between genetic data, morphological data, and known historical records (Dickson 1992, Mock et al. 2002). Thus, the specific nomenclature used to describe wild turkeys is inconsequential in this instance, and it does not change the message that wild turkey translocation programs clearly have been responsible for a considerable mixing of distinct forms.

Detection of hybrid individuals traditionally has relied upon morphological methods. However, these methods are subjective, assume that morphological variation has a genetic basis and that hybrid individuals are phenotypically intermediate to parental types, and cannot detect hybrids beyond the first generation. Modern molecular techniques greatly simplify the identification and characterization of hybrid populations and offer opportunities to objectively differentiate types with a high degree of resolution (Mock et al. 2002, Cronin 2003, Scribner et al. 2003). For example, highly variable molecular markers developed for wild turkeys, including nuclear microsatellites (Huang et al. 1999, Latch et al. 2002, Latch 2004) and DNA sequences from the mitochondrial genome (mtDNA; Mock et al. 2002, Latch 2004), can be used to differentiate closely related groups such as subspecies or even distinct populations (Latch et al. 2006). In fact, many recent studies have used molecular markers to objectively evaluate naturally occurring hybridization in wildlife species (e.g., Haig et al. 2004, Schwartz et al. 2004, Tranah et al. 2004).

Biparentally inherited, nuclear markers such as microsatellites are highly polymorphic and provide a level of information content and discriminatory power that previously has not been available for use in wildlife research. The recent development of assignment tests, which are statistically rigorous methods for classifying individuals into randomly mating units by means of likelihood or Bayesian approaches, represent a leap forward in our ability to use markers such as microsatellites to examine issues of population integrity and hybridization. Using statistical approaches such as assignment tests, researchers now can use DNA-based tools to discriminate among potential inter- and intraspecific sources of genetic contributions to populations of conservation or management interest. The DNA-based markers with uniparental modes of inheritance, such as maternally inherited mtDNA, can further elucidate situations in which hybridization is suspected, primarily by revealing the breeding tactics that result in hybrid offspring. For example, by using mitochondrial markers we can potentially determine the taxonomic status of the mother of hybrid offspring. In contrast to the nuclear DNA of a hybrid individual, which will be intermediate between the 2 species or subspecies contributing genes to the gamete, the mitochondrial DNA of a hybrid offspring always will be characteristic of the species or subspecies of the mother (recognizing of course that despite the taxonomic classification of the maternal lineage, the mother also could be a hybrid).

Using both nuclear (microsatellites) and mitochondrial (control region sequences) markers, we investigated the potential for hybridization between 2 wild turkey subspecies that now co-occur in the Davis Mountains of west Texas, USA: the nonnative

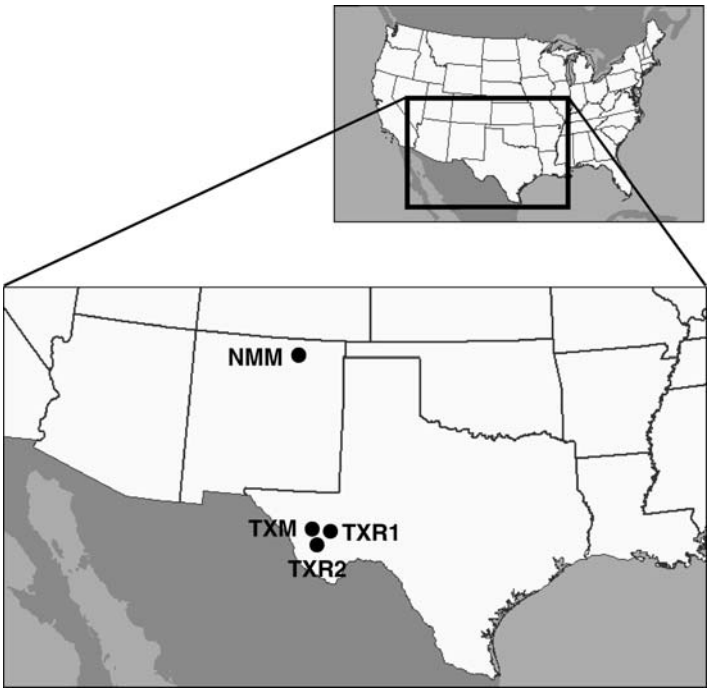

Figure 1. Geographic locations of wild turkey populations sampled from 2001 to 2002. Enlarged region encompasses the south-central United States, where turkeys were sampled from an introduced population in Tex., USA (TXM), its source population in N. M., USA (NMM), and 2 populations near the release site (TXR1 and TXR2).

Merriam's turkey and the endemic Rio Grande turkey. Historically, the Trans-Pecos region of west Texas was exclusively inhabited by Rio Grande turkeys. However, in 1983, Merriam's turkeys from New Mexico (Ute Park, Colfax County) were translocated into the Davis Mountains of Texas (Jeff Davis County, private ranch purchased by The Nature Conservancy in 1997; Fig. 1). Merriam's turkeys were chosen for introduction into this area because 1) it was thought that the habitat in the Davis Mountains would be well suited to Merriam's turkeys, and 2) introduction of Merriam's turkeys would serve to diversify hunting opportunities in Texas. Following the translocation of Merriam's turkeys, the founding population of 43 birds (6 males, 37 females) grew quickly and currently is estimated to be approximately 165 individuals (King 2003).

Currently, 2 small populations of Rio Grande turkeys exist 20–30 km from the introduced Merriam's population (King 2003). One population of around 35 birds is located in Fort Davis, Texas, and the other is located in Alpine, Texas, and consists of around 150 birds. The development of clear management objectives for this complex of wild turkey populations is hindered by a lack of data on the genetic makeup of the individuals within the introduced population in the Davis Mountains as well as the 2 Rio Grande populations nearby.

Our goal was to quantify subspecific status and degree of hybridization of individuals within the introduced Davis Mountains population of Merriam's turkeys and 2 nearby Rio Grande turkey populations in the Trans-Pecos region of Texas. Using nuclear microsatellite data and sequence data from the mitochondrial control region, we determined the subspecies status (Merriam's, Rio Grande, or hybrid) of a sample of individuals

within each of these 3 populations. For comparative purposes, we also collected data on nuclear and mitochondrial variation for a sample of wild turkeys from the Merriam's population in New Mexico used as the source for the introduced Davis Mountains Merriam's population.

Study Area

The Davis Mountains Preserve is an 18,277-acre preserve owned by The Nature Conservancy with conservation easements on 65,830 acres of adjoining property. It is situated in Jeff Davis County, Texas, in the northeastern Chihuahuan Desert. We also collected samples from the source population of Merriam's turkeys used for this introduction in Ute Park, Colfax County, New Mexico (NMM; $n = 25$), and from 2 Rio Grande turkey populations approximately 20 km from the Davis Mountains introduction site (TXR1; $n = 9$ and TXR2; $n = 13$).

Methods

Sample Collection

From November 2001 to March 2002, we collected wild turkey samples from an introduced Merriam's turkey population in the Davis Mountains Preserve (TXM; $n = 27$). Although the number of samples obtained from each population was relatively small, the populations themselves were somewhat small in size. The estimated population sizes of TXM, NMM, TXR1, and TXR2 are 165, 500, 35, and 150 individuals, respectively (King 2003; K. Mower, New Mexico Department of Game and Fish, Santa Fe, N. M., USA, personal communication). Therefore, we have sampled a reasonable percentage (between 5% and 26%) of the total population in each case.

We trapped turkeys using Davis (1994) walk-in style traps and Silvy et al. (1990) improved-modified drop nets at predetermined, baited sites. Upon capture, we took approximately 0.1 cc of blood from the brachial vein of each turkey with a tuberculin syringe equipped with a 25-gauge needle (for males) and a 28-gauge needle (for females). We placed blood in 1.7 mL microcentrifuge tubes filled with 1.5 mL lysis buffer (0.05 M Tris-HCl, 0.1 M EDTA, pH 8.0, 0.01 M NaCl, 0.5% SDS), took samples back to the laboratory, and stored them at −80°C until processed.

DNA Extraction

Approximately 300 μL of each sample was digested by adding 25 units streptokinase, 400 μg proteinase K, and 200 μL fresh lysis buffer, and incubating overnight at 55°C while rotating. We extracted DNA from the digested samples using an AutoGen NA-2000 DNA extraction machine (AutoGen, Inc., Holliston, Massachusetts), using the manufacturer's Tissue no. 1 protocol with 3 modifications: 1) the amount of reagent 3 (phenol/potassium acetate) added to each sample was increased from 0.5 mL to 0.75 mL; 2) the amount of reagent 4 (butanol/ethanol) added to each sample was increased from 0.25 mL to 0.4 mL; and 3) the number of DNA pellet washes was increased from 2 to 3. We resuspended DNA pellets in 100 μL TLE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). We assessed DNA quantity and quality by electrophoresis through a 2% agarose gel stained with ethidium bromide. We diluted all DNA samples to approximately 10 ng/μL in TLE.

Microsatellite Amplification and Electrophoresis

We amplified 9 microsatellite loci from each sample, using the primers and reaction conditions described in Table 1. We chose the 9 loci for this study from a suite of loci developed and screened previously (Huang et al. 1999, Latch et al. 2002, Latch 2004), enabling us to select a panel of loci with no evidence for null alleles. Ten microliter amplification reactions consisted of 5–10 ng genomic DNA (Table 1), 2–4 pmol each primer (Table 1), 1.5–2.0 m*M* $MgCl_2$ (Table 1), 0.2 mM each dNTP, and 0.75 units Taq

Table 1. Locus-specific primers and reaction conditions for nuclear microsatellite and mitochondrial control region loci used for wild turkeys collected from Texas and New Mexico, USA, in 2001 and 2002. Quantities are specific for a 10 μL reaction volume except control region-A, which is specific for a 25 μL volume. Superscripts preceding forward microsatellite primers indicate the fluorescent label colors: B = blue (6-FAM), Y = yellow (TAMRA), or G = green (JOE).

Locus (GenBank accession no.)	Primers (5′ → 3′)	DNA (ng)	Primer (pmol)	$MgCl_2$ (mM)	Annealing temp (°C)
TUM6[a]	[B]F:AAATCAGTGTCATTGTGCAA	5	3	2.0	59
(U79372)	R:TTCTGCTACCTGACCATGTA				
TUM23[a]	[B]F:CGGCATCTCCAGCTCCAT	5	4	1.5	60
(U79332)	R:CCACGGAGAGTCCTGGAT				
TUM50[a]	[B]F:CTGATGTCTTAAAGGCT	5	2	2.0	46
(U79306)	R:ACAAAAACGAACTGATCA				
WT10[b]	[Y]F:TTGGAACAGGAGAAATTTCAGT	10	4	1.5	55
(AF111453)	R:TATTTGTTGCAAGGCAGCAG				
WT54[b]	[Y]F:AAAGAGCAGCGTGTTCCAGT	5	2	1.5	60
(U79330)	R:TTCAAAACAGTGTCACGATTCC				
WT75[b]	[G]F:CCAACTGCAAGATGCTTCTG	10	3	1.5	57
(AF434907)	R:CTGCATTACTGTGCATCATGG				
WT30–2[a]	[G]F:GAAGGAGGAACCAAAAACTACG	5	2	1.5	58
(U79391)	R:CAACCATGGTGTGAGGAGG				
WT38–2[b]	[B]F:GGTTTGAGCAGAGTGAATCTCA	5	3	1.5	60
(U79365)	R:ATTGGTTGGGGGAGGAAC				
WT90–2[b]	[Y]F:AATCAACCCATTTGTTCCCA	5	2	1.5	58
(AF111645)	R:GTGCTTTGATTTAAAAGCCCC				
Control region-A	F:GAAAAATCACAAAATAAGTCA	30	12.5	1.5	53
(AY037889)	R AGTGAGGAGTTCAGGAGTTA				

[a] Loci originally described in Huang et al. (1999), but amplified here using alternative reaction conditions and/or primer sets.
[b] Loci originally described in Latch et al. (2002), but amplified here using alternative reaction conditions.

DNA polymerase (Eppendorf) in 1X reaction buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $Mg[OAc]_2$). We fluorescently labeled forward primers with JOE (green), TAMRA (yellow), or 6-FAM (blue; Table 1). We then amplified reactions via polymerase chain reaction (PCR) according to the following thermocycler conditions: a 2 min initial denaturation step at 95°C was followed by 30 cycles of 30 seconds at 95°C, 30 seconds at the annealing temperature (Table 1), and 30 seconds at 72°C; reactions were completed with a final extension for 5 min at 72°C and a 60°C soak for 45 min. We combined PCR-amplified microsatellites into 4 gel sets based on locus size and fluorescent label color (Table 1). Combined PCR products (0.5 μL) then were added to 0.2 μL ROX400HD internal lane standard (Applied Biosystems, Foster City, California), and electrophoresed through a 5% polyacrylamide gel (Long Ranger Singel Packs; Cambrex Corp., E. Rutherford, New Jersey) on an ABI 377 DNA sequencer. Allele sizes were determined for each locus using GeneScan 3.1 and Genotyper 2.5 software (Applied Biosystems).

We employed several methods to verify the quality of our microsatellite genotype data. First, we developed a set of known alleles for each locus, representing the full range of allele sizes. We combined these into gel sets as described above, and ran this allelic standard on each gel every 12 lanes to minimize genotyping errors due to electrophoretic variability both within and among gels. Second, we independently scored all individuals at a quality-control locus (locus WT38–2 using an alternate primer set). We assessed genotyping errors by comparing genotypes from the 2 loci. Third, we re-electrophoresed or reamplified any ambiguous genotypes, or genotypes with low signal intensity (<100 as determined by Genotyper 2.5 software) to confirm the genotype. Fourth, we discarded unreliable samples prior to analysis. We deemed samples unreliable if they successfully amplified at fewer than 25% of the loci, despite multiple amplification and DNA extraction attempts.

Mitochondrial DNA Amplification and Sequencing

We amplified a portion of the mitochondrial control region (approx. 500 bases of domain I) using the primers described in Table 1. Twenty-five microliter amplification PCRs included 30 ng genomic DNA, 12.5 pmol each primer, 0.2 mM each dNTP, and 2 units Taq DNA polymerase (Eppendorf) in 1X reaction buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $Mg[OAc]_2$). We amplified reactions by PCR according to the following thermocycler conditions: an initial denaturation step of 2 min at 96°C was followed by 35 cycles of 96°C for 30 seconds, 53°C for 30 seconds, and 72°C for 1 min; reactions were completed with a final extension step at 72°C for 10 min. The PCR products were visualized on a 1% agarose gel stained with ethidium bromide and cleaned using Qiagen's PCR Purification Kit. Ten microliter sequencing reactions contained 30 ng cleaned PCR product (as estimated by agarose gel band intensity), 5 pmol forward primer, and 1 μL ABI Big Dye Terminator version 3.1 cut with 3 μL 5X buffer (Applied Biosystems).

Cycle sequencing was performed using the following thermocycler program: an initial denaturation step at 98°C for 5 min was followed by 26 cycles of 98°C for 30 seconds, 50°C for 15 seconds, and 60°C for 2 min. It proved to be extremely important to denature DNA at 98°C and to include a relatively long initial denaturation step; lower denaturation times and temperatures yielded apparently clean sequences but were plagued with inconsistencies (E. K. Latch and O. E. Rhodes, Jr., Purdue University, West Lafayette, Ind., USA, unpublished data.). Sequences were cleaned by precipitating DNA with a low sodium precipitation solution (0.12 mM NaOAc in 100% EtOH) and centrifuging to form a DNA pellet. The pellet was washed twice with 70% ethanol and rehydrated in water. Sequences were run on an ABI 3700 and compiled and edited in Sequencher 4.1 (GeneCodes Corporation, Ann Arbor, Michigan).

We conducted a pilot study prior to our research to verify the consistency of our control-region sequences for wild turkeys. We amplified a set of approximately 150 samples at the control region (Table 1), and we sequenced each product 5 times in the forward direction and 5 times in the reverse direction. Three representative samples of each haplotype were amplified a second time and sequenced additional 5 times in each direction. The protocols described above yielded extremely consistent sequence data in both directions; thus, a clean sequence in the forward direction for each individual used in this study was considered sufficient. To further confirm sequence quality within this study, we sequenced approximately 10% of PCR products in both the forward and reverse directions, and we compared our sequences to wild turkey control-region sequences available in GenBank (e.g., AY037889). Throughout the study, we sequenced forward sequences containing ambiguous bases also in the reverse direction from the original PCR product to confirm the DNA sequence.

Data Analysis

We used the software CONVERT (version 1.2; Glaubitz 2004) to facilitate input file preparation for all software used for microsatellite data analysis. We evaluated each locus within each population for departures from Hardy-Weinberg equilibrium by executing 3,200 iterations of Fisher's exact test in Genetic Data Analysis software (GDA; version 1.1; Lewis and Zaykin 1999). Due to the large number of comparisons involved in the Hardy-Weinberg equilibrium tests, we performed a sequential Bonferroni correction for multiple tests (Holm 1979, Rice 1989) before assessing significance. We assessed overall levels of microsatellite variability within populations by calculating expected and observed heterozygosities and average numbers of alleles per locus for each population using GDA. As measures of control-region genetic variability across the entire dataset and within each population, we calculated the total number of haplotypes (h), haplotype diversity (Hd; Nei 1987, Depaulis and Veuille 1998), and the average number of pairwise nucleotide differences among populations (k; Tajima 1983) using DNASP (version 4.0; Rozas et al. 2003).

To reveal the level of genetic differentiation among populations, we estimated F_{ST} (the proportion of genetic variability in the total population [T] that is due to differences among subpopulations [S]) across the entire dataset and between all population pairs for each molecular marker type using SPAGEDI software (version 1.1; Hardy and Vekemans 2002). We also used SPAGEDI software to test the significance of F_{ST} estimates by randomly permuting individuals among populations 10,000 times and comparing observed and permuted estimates.

To assess whether each population consisted of individuals of a "pure" subspecies or of a mixture, we assigned individuals to

populations based on their multilocus microsatellite genotypes through an assignment test. Assignment tests were performed using program STRUCTURE (version 2.1; Pritchard et al. 2000), which uses a Bayesian model-based clustering method to assign individuals to populations such that Hardy-Weinberg disequilibrium is minimized; thus, our haploid control-region data were inappropriate for these analyses. It is generally recommended that the number of groups (K) be estimated from the data to accurately estimate population structure (Pritchard et al. 2000). We estimated the likelihood of the data for values of K from 1 (where all individuals belonged to a single, randomly mating group) to 4 (where the entire sample could be separated into 4 genetically distinct subpopulations). We performed 5 iterations for each K, in which each iteration consisted of a 30,000 replicate burn-in and a Markov Chain Monte Carlo run of 100,000 replicates. We used the admixture model, which allows individuals to be from more than one of the K populations, and we allowed the allele frequencies among populations to be correlated. The likelihood of the data was greatest when $K = 2$, so this was the value we used to perform our assignment test.

The assignment of each individual was done probabilistically, and therefore the program generates a probability (Q) that each individual belongs to each of the K groups. Because $K = 2$ in this analysis, there was a value Q that represented the proportion of an individual turkey's genome that was characteristic of Merriam's turkeys, and there was a value $1-Q$ that represented the proportion of that turkey's genome that was characteristic of Rio Grande turkeys. Hybrid individuals would be those characterized by an intermediate value of Q (i.e., $Q = 0.5$ for a first-generation hybrid, 0.25 or 0.75 for a second-generation hybrid).

Results

The microsatellite dataset was very robust; we obtained over 97% of all genotypes attempted, and 100% of the samples were scored consistently at WT38–2 and the quality-control locus. Using a sequential Bonferroni correction (tablewide $\alpha_1 = 0.0014$), we determined that none of the 36 populations by locus comparisons deviated significantly from Hardy-Weinberg equilibrium.

Table 2. Number of alleles per locus and observed (Ho) and expected (He) heterozygosities for each microsatellite locus for wild turkeys collected in Tex. and N. M., USA, from 2001 to 2002. The number of alleles unique to the introduced population (TXM only), as well as those shared by TXM and either Merriam's turkeys (NMM) or Rio Grande turkeys (TXR1/TXR2), are provided.

				Allele types		
Locus	No. alleles	He	Ho	NMM and TXM	TXR1/TXR2 and TXM	TXM only
TUM6	2	0.26	0.25	0	1	0
TUM23	8	0.78	0.78	1	1	0
TUM50	17	0.93	0.89	5	3	1
WT10	2	0.49	0.46	0	0	0
WT54	10	0.87	0.80	0	2	4
WT75	10	0.59	0.36	1	0	1
WT30–2	12	0.66	0.56	2	3	0
WT38–2	9	0.80	0.82	0	0	2
WT90–2	10	0.80	0.61	1	2	1
Overall	8.89	0.69	0.61	10	12	9

Across the entire dataset, there were between 2 and 17 microsatellite alleles per locus with an overall average multilocus observed heterozygosity of 0.61 (Table 2). Average levels of observed heterozygosity were quite variable among loci, ranging from 0.25 to 0.89 (Table 2). On average, each population contained 5.3 alleles per locus (range: 4.8–6.6), and average multilocus observed heterozygosity values ranged from 0.51 to 0.70 (data not shown).

We detected 24 unique microsatellite alleles (out of 80 alleles total) within specific populations, but none occurred at very high frequencies and many could be artifacts of small sample sizes. Of the 80 total microsatellite alleles detected across the 10 loci surveyed, 10 were found in only NMM and TXM, suggesting that these may be alleles present in NMM that were retained in TXM during the translocation (Table 2). We found 12 alleles only in TXR1, TXR2, and TXM, suggesting that these may be alleles that were brought into the TXM population after the translocation event (Table 2). We found 9 alleles in only the TXM population; these either may be alleles that were generated via mutation in the TXM population subsequent to the translocation or unsampled alleles that exist in one of the other populations (Table 2).

The overall F_{ST} value indicated the presence of significant microsatellite differentiation among the 4 populations surveyed ($F_{ST} = 0.128$; 1-sided $P < 0.0001$). The F_{ST} estimates between all pairs of populations were significant, ranging from 0.06 (TXR1 – TXR2) to 0.26 (NMM – TXR1; Table 3).

Across 71 individual wild turkeys, 432 nucleotides within the control region were aligned. Sixteen sites were variable within this region (11 of which were parsimony-informative), resulting in detection of 18 control-region haplotypes. Only 6 of these haplotypes were found in more than 1 population. The remaining 12 haplotypes each were found in low frequencies within individual populations, suggesting that these haplotypes may exist in other populations but were not sampled in our study. The overall estimate of F_{ST} (0.190; 1-sided $P < 0.0001$) was significant, indicating that the distribution of haplotypes differed among the 4 populations. Pairwise F_{ST} estimates between populations ranged from 0.12 (TXR1 – TXR2) to 0.25 (TXR2 – NMM; Table 3).

Assignment tests based on our multilocus microsatellite data proved to be particularly useful in our study. Our initial effort to identify the most likely value of K yielded results consistent with expectations, suggesting that our data strongly supported a genetic separation of the total sample into 2 distinct groups. Therefore, we

Table 3. Matrix of pairwise F_{ST} values indicating genetic differentiation among an introduced population of wild turkeys in Tex., USA (TXM), its source population in N. M., USA (NMM), and 2 populations near the release site (TXR1 and TXR2), collected from 2001 to 2002. F_{ST} estimates were based on 432 bases of control-region sequence (above diagonal) or 9 microsatellite loci (below diagonal).

	TXR1	TXR2	NMM	TXM
TXR1	—	0.121*	0.231*	0.130*
TXR2	0.058*	—	0.247*	0.245*
NMM	0.259*	0.194*	—	0.160*
TXM	0.096*	0.067*	0.107*	—

* Significant F_{ST} estimates ($p < 0.05$, 1-sided test), based on 10,000 permutations of individuals among populations.

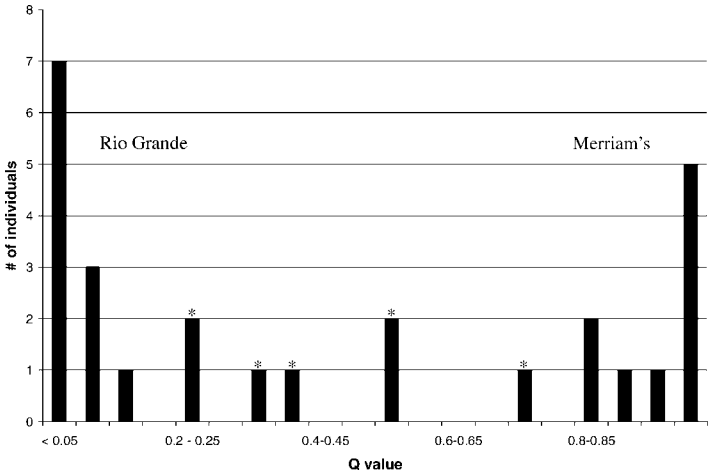

Figure 2. Frequency distribution of Q values for the introduced (TXM) wild turkey population collected in Tex., USA, from 2001 to 2002. Q represents the proportion of each turkey's genome that was assigned to the Merriam's subspecies, and ranges from zero (the turkey's genome was characteristic of the Rio Grande subspecies [*Meleagris gallopavo intermedia*]) to 1 (the turkey's genome was characteristic of the Merriam's subspecies [*M. g. merriami*]). We obtained Q values using program STRUCTURE (version 2.1; Pritchard et al. 2000), and they were based on 10 microsatellite loci. The X axis is divided into 20 intervals, each representing a range of 0.05. Putative hybrids were identified as those with Q values between 0.2 and 0.8 and are marked with an asterisk.

could proceed to assign each individual in the entire sample to the Merriam's group or the Rio Grande group. We assigned all individuals from NMM to the Merriam's group, with extremely high Q values ($\bar{Q}_{NMM} = 0.978$). Similarly, we assigned all turkeys from TXR1 and TXR2 to the Rio Grande group with very little ambiguity ($\bar{Q}_{TXR1} = 0.986$; $\bar{Q}_{TXR2} = 0.959$). In the TXM population, 33% (9 of 27; 4 males, 5 females) of the individuals were assigned with high Q values to the Merriam's group and 41% (11 of 27; 7 males, 4 females) were assigned with high Q values to the Rio Grande group. The remaining 26% of individuals in the TXM population (7 of 27; 2 males, 5 females) could not be reliably assigned to either subspecies and exhibited intermediate Q values, suggesting that these turkeys were putative hybrids. A frequency distribution of Q values for the TXM population illustrated the relative proportion of turkeys that were characteristic of Merriam's or Rio Grande subspecies, as well as potential hybrids (Fig. 2).

Intermediate Q values (0.20–0.80) for the 7 ambiguously assigned turkeys in the TXM population suggested that these turkeys possessed characteristics of both Rio Grande and Merriam's subspecies (Fig. 3). The Q values near 0.5 indicated potential first-generation hybrids, and Q values near 0.25 (or 0.75) indicated potential second-generation hybrids. The control-region haplotypes of the 7 suspected hybrid turkeys provided some additional information about the likely subspecies of their maternal parents. One of the hybrids possessed a haplotype found only in Rio Grande turkeys from TXR1 or TXR2 (at a frequency of 14%), and thus was almost certain to have had a Rio Grande mother. Four hybrids exhibited a haplotype that was only found in Merriam's turkeys from NMM (at a frequency of 4%), making a Merriam's mother most probable. The remaining 2 hybrids shared a haplotype that was present in both subspecies; however, it was present at a frequency of 56% in the Merriam's subspecies and only 5% in the Rio Grande subspecies. Therefore, it was most likely that these hybrids were derived from a Merriam's maternal lineage.

Discussion

The genetic integrity of the introduced population of Merriam's turkeys in the Davis Mountains (TXM) likely has been eroded by both immigration of and hybridization with Rio Grande turkeys. Our data indicate that approximately two-thirds of the turkeys sampled from the TXM population were Rio Grande turkeys or recent Rio Grande × Merriam's hybrids. Historical records of wild turkeys in the Davis Mountains are limited. Turkeys may have existed in the Davis Mountains prior to the introduction of Merriam's turkeys in 1983; however, information is anecdotal at best (Texas Game, Fish, and Oyster Commission 1946). If Rio Grande turkeys historically were present, it could have implications for the origin of hybrid individuals in the TXM population because hybrids could have arisen in the absence of immigration. However, it does not change the overall pattern of hybridization we observed. If Merriam's turkeys were present in the Davis Mountains prior to the translocation of individuals from New Mexico, then perhaps we underestimated the overall degree of hybridization in this population by failing to detect native Merriam's × introduced Merriam's hybrids.

The 2 Rio Grande populations nearest to the Davis Mountains Preserve release site were approximately 20–30 km away, demonstrating the high potential for dispersal in this species, despite the relatively short time (19 years) since the initial translocation event. Dispersal of both male and female wild turkeys has been documented across the species' range (Glazener 1967, Healy 1992). Although we found slightly more immigrant males than females in TXM (7:4), the number of immigrants was not significantly different between sexes as determined by a chi-square goodness-of-fit test ($\alpha = 0.05$; $p = 0.30$).

Radiotelemetry data collected on turkeys in the TXM population verify the lack of subspecies-specific spatial segregation of turkeys and indicate that at least spatially the opportunity for hybridization indeed exists in the Davis Mountains Preserve. King (2003) documented movements of turkeys within the Davis Mountains Preserve. Upon incorporating our genetic assignments, we found no apparent trend to the distribution of turkeys therein, suggesting a thorough mixing of all resident turkeys regardless of their genetic makeup or population of origin. In both breeding and nonbreeding seasons, Merriam's and Rio Grande turkeys were caught in the same traps, were monitored via telemetry in close proximity to one another, and used habitats similarly (J. S. King and L. A. Harveson, Sul Ross State University, Alpine, Tex., USA, unpublished data).

Microsatellite data revealed a relatively high percentage of hybrid individuals in the TXM population (26%), indicating that immigrant Rio Grande turkeys were not merely coexisting with resident Merriam's turkeys. Further characterization of hybrids in the TXM population using control-region data showed that 86% of the identified hybrid individuals likely were the result of immigrant Rio Grande males mating with resident Merriam's females. Although Rio Grande females were immigrating into the population, our data do not indicate that they were contributing a significant number of hybrid offspring to the TXM population.

There are numerous potential reasons for differential rates of introgression between these subspecies, including reduced survival of Rio Grande female × Merriam's male hybrids, preference for Merriam's females by Rio Grande males, and dominance of Rio Grande males over Merriam's males. However, we did not test these hypotheses directly.

Although the data identified a large influx of Rio Grande turkeys into the Davis Mountains Preserve, there was no evidence of immigrant Merriam's turkeys or of hybrid Merriam's × Rio Grande turkeys in TXR1 or TXR2. Such unidirectional migration patterns suggest limited dispersal of introduced Merriam's turkeys from their release site and relatively unrestricted dispersal of nearby Rio Grande turkeys into the Davis Mountains Preserve. Merriam's turkeys have relatively restricted habitat requirements and are rarely found in low elevations (MacDonald and Jansen 1967, Eaton 1992). Thus, the introduced population in the Davis Mountains may represent an island of habitat from which Merriam's turkey dispersal is restricted. However, the limited dispersal of Merriam's turkeys observed in Texas cannot be assumed elsewhere and may be due to site-specific conditions in and around the TXM release site. For example, genetic data from wild turkeys in southwest Kansas also document a large number of Merriam's × Rio Grande hybrids, but show the exact opposite patterns of hybridization, with immigration of Merriam's turkeys into an area repeatedly restocked with Rio Grande turkeys (Latch et al. 2006).

Perhaps a more logical explanation for limited dispersal of Merriam's turkeys from the TXM population is limited dispersal of translocated individuals from their release site, a phenomenon that has been documented in wild turkeys (Leberg et al. 1994, Latch and Rhodes 2006). In white-tailed deer, a series of empirical studies found that although restocked animals had substantial genetic impacts on recipient populations, they had minimal effects on the genetic composition of nearby native populations (Ellsworth et al. 1994, Leberg et al. 1994, Leberg and Ellsworth 1999). Our data corroborate these findings, and further suggest that whereas dispersal from a release site may be limited, immigration of native individuals into reintroduced populations may be relatively unrestricted. Limitations to dispersal from the release site may be further exacerbated in the wild turkey by releasing related individuals. Current methods for trapping turkeys make it difficult to avoid capturing related individuals, and the release of related individuals has been shown to limit dispersal of turkeys from release sites (Sylvester and Lane 1946, Lewis 1959, Schorger 1966).

Management Implications

Management of wildlife species will continue to rely on the translocation of individuals, often into areas where they did not historically occur. Such movements of wildlife often connect populations that traditionally have been geographically separated, creating the opportunity for hybridization to occur. Our results suggest that hybridization may be common, urging for extreme care when selecting source stock for translocations. Management should be directed at selecting source stock from as near the release site as possible so that translocated individuals will be adapted to local environmental conditions and will not threaten the genetic integrity of native populations (Falconer and Mackay 1996, Jacobson and Lukefahr 1998). In areas where hybridization is a concern, molecular markers are a useful tool for managers to identify and characterize hybrid individuals.

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