Conservation Genetics (2005) 6:981–997
DOI 10.1007/s10592-005-9089-2

The effects of gene flow and population isolation on the genetic structure of reintroduced wild turkey populations: Are genetic signatures of source populations retained?

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Received 23 November 2004; accepted 5 May 2005

Key words: wild turkey, translocation, gene flow, assignment, microsatellite

Abstract

To counter losses of genetic diversity in reintroduced populations, species sometimes are reintroduced into networks of populations with the potential to exchange individuals. In reintroduced populations connected by gene flow, patterns of genetic structure initiated by the founding event may become obscured, and populations may eventually follow an isolation-by-distance model of genetic differentiation. Taking advantage of well-documented reintroduction histories of wild turkey populations in Indiana, we assessed the degree to which gene flow among reintroduced populations has obscured genetic signatures left by the founding events. Using a suite of nuclear microsatellite loci and sequence data from the mitochondrial control region, we characterized the level of genetic diversity and degree of genetic structure within and among: (1) reintroduced populations in isolated northern Indiana Fish and Wildlife Areas, (2) reintroduced populations in southern Indiana Fish and Wildlife Areas, where the distribution of populations is more continuous, and (3) source populations used for these reintroductions. We also utilized individual-based assignment tests to determine the relative contribution of source populations to the current distribution of alleles in reintroduced populations. Our results indicate that wild turkey reintroductions in Indiana have left distinct genetic signatures on populations that are detectable even after several decades. Although we found some case-specific evidence for gene flow, particularly in regions where populations are in close proximity, our data indicate on overall paucity of gene flow at a regional scale. Such post-reintroduction genetic monitoring has immediate implications for the design of optimal strategies to reintroduce wildlife for conservation and management.

Introduction

Species reintroductions are used extensively in wildlife management, to meet recreational, aesthetic, and conservation objectives. A primary concern pertaining to most species reintroduction programs is the conservation of genetic diversity in newly founded populations. Human-mediated founder events and post-reintroduction genetic drift often result in the creation of populations wherein only a small portion of the total genetic variability of a source population is retained (Nei et al. 1975). Indeed, a number of empirical studies have demonstrated significant reductions of genetic variability in translocated populations relative to their sources (Fitzsimmons et al. 1997; Williams et al. 2000, 2002; Mock et al. 2004). Losses of genetic variability due to founder effects will be greater in populations established with few individuals (Baker & Moeed 1987; Merila et al. 1996; Mock et al. 2004). Small founding populations also will intensify the effects of genetic drift following a

translocation event, potentially leading to shifts in the allele frequency distribution of the translocated population relative to its source (Fitzsimmons et al. 1997; Luikart et al. 1998b; Rowe et al. 1998; Williams et al. 2002; Mock et al. 2004), relative to other native populations (Leberg 1991; Baker & Moeed 1987; Perez et al. 1998), or relative to theoretical expectations (Scribner & Stuwe 1994; Fitzsimmons et al. 1997).

To counter the loss of genetic diversity in newly founded populations, species sometimes are established in networks of populations that have the potential to exchange individuals. In theory, multiple reintroductions into a region can create the opportunity for gene flow among populations, thereby alleviating the potential negative effects associated with genetic drift and low population sizes following a reintroduction event (Wright 1978; Allendorf 1983). It has been hypothesized that by creating a network of reintroduced populations connected by gene flow, the genetic relationships among the re-established populations might eventually follow an isolation-by-distance model (Sokal & Wartenberg 1983), ultimately obscuring the reintroduction history of any specific population (Baker 1992; Merila et al. 1996). Alternatively, reintroduced populations isolated from conspecifics may lack opportunities for gene flow and might be expected to retain the genetic signatures of their source populations long after the reintroduction event (Leberg et al. 1994; Williams et al. 2000; Mock et al. 2004).

Despite the overwhelming number of species translocation programs that have been undertaken in North America, surprisingly few empirical studies have directly investigated the effects of genetic drift and gene flow in translocated populations at a regional scale, where interactions among populations are possible (Ellsworth et al. 1994; Leberg et al. 1994; Rhodes et al. 1995, 2001; Williams et al. 2002; Mock et al. 2004). A number of confounding factors may be partly responsible for the deficit of such studies, including: (1) significant alterations to source populations after the reintroduction events, (2) reintroductions into the same area from multiple sources, (3) incomplete or erroneous recordkeeping, and (4) reintroduction of individuals into pre-existing but previously uncharacterized gene pools. In addition, rigorous assessments of the genetic effects of gene flow and genetic drift in reintroduced populations often require large-scale studies involving multiple, paired sets of source and reintroduced populations. Such large-scale studies often can be difficult to accomplish both in terms of time and cost.

Regardless of the potential difficulties, North American game species in particular offer a plethora of opportunities to empirically investigate the gene dynamics of reintroduced populations by direct comparison to their sources and by comparisons among reintroduced populations in a region. Game species often are managed extensively via translocations, offering numerous opportunities for study replication both within and among species. For many reintroduction programs involving game species, meticulous records are kept which outline the specific geographic and biological details of each translocation event. Furthermore, comprehensive information regarding past management histories and changes in population size exist for many North American game species. Basic biological and demographic parameters that govern the distribution and maintenance of genetic variation also are typically well-characterized for such intensively managed species.

In this research we investigated the gene dynamics of reintroduced eastern wild turkey (Meleagris gallopavo silvestris) populations in Indiana. The current Indiana wild turkey population represents an excellent model system through which to investigate the effects of gene flow and genetic drift on the genetic structure of reintroduced populations within a region. Although wild turkeys were extirpated in Indiana by the early 1900s, a massive reintroduction effort over the last several decades has restored this species to most of Indiana, and currently over 90,000 turkeys reside throughout the state (S. Backs, Indiana Department of Natural Resources, pers. commun.). For each of the nearly 3,000 birds reintroduced into Indiana, information on the trapping location and date, reintroduction site, sex, age, weight, and condition of the bird was cataloged. Early in the reintroduction effort, turkeys were reintroduced primarily into southern portions of the state, where forest habitats are relatively continuous. After the successful reintroduction of turkeys into southern Indiana, turkey populations also were established in the more fragmented habitats of northern Indiana. These later reintroductions into northern Indiana also

proved to be quite successful, despite the fact that forest habitats in this region were generally more isolated than in the southern portion of the state. Most reintroduced populations became established quickly and were eligible for hunting within a few years (S. Backs, pers. commun.).

Overall, the Indiana wild turkey reintroduction program set out to create one large, robust turkey population. Fairly continuous habitat in southern Indiana allowed managers to implement programs to maximize interactions among populations. In particular, numerous reintroductions often were performed in an area (block stocking) using founders from multiple source populations (Backs and Eisfelder 1990). Northern Indiana generally lacks the habitat continuity necessary for block stocking. Thus, reintroduced populations in northern Indiana are more isolated from one another, reducing the potential for gene flow among populations.

We took advantage of the well-documented, straightforward reintroduction histories of turkey populations in Fish and Wildlife Areas across Indiana to assess the degree to which genetic drift within and gene flow among reintroduced populations have obscured initial patterns of genetic structure created by the founding events. Using both nuclear and mitochondrial DNA markers, we aimed to characterize the degree of genetic structure within and among: (1) reintroduced populations in northern Indiana Fish and Wildlife Areas, where reintroduced populations are relatively isolated, (2) reintroduced populations in southern Indiana Fish and Wildlife Areas, where networks of potentially interconnected populations were established, and (3) source populations used for these reintroductions. We expected wild turkey populations in southern Indiana Fish and Wildlife Areas to show signs of genetic isolation by distance more quickly than in northern Indiana, as a result of greater connectivity among populations and opportunity for gene flow from neighboring populations. Likewise, we predicted that wild turkey populations in northern Indiana Fish and Wildlife Areas would retain genetic characteristics of their source populations for a longer period than populations in southern Indiana, due to population isolation and a subsequent reduction in gene flow potential.

Methods

Study populations

This study involved reintroduced wild turkey populations in 11 Indiana Fish and Wildlife Areas (Table 1). Six of these Fish and Wildlife Areas are located in the northern third of Indiana, where reintroduced populations are fairly isolated (JP, WI, PR, LS, WS, and KI; Table 1). The remaining five Fish and Wildlife Areas are in the southern third of the state, where reintroduced populations are in close proximity to other turkey populations (HL, SR, MI, CS, and GL; Table 1). Compared to the approximately 25 reintroductions performed in northern Indiana, well over 100 reintroductions were performed in southern portions of the state, thereby creating a network of populations and an opportunity for high amounts of gene flow among them. Fish and Wildlife Areas proved to be particularly useful units for study, mainly because they are properties to which access is controlled during turkey hunting season, and thus served as distinct areas from which samples could be taken. Furthermore, the reintroduction histories that led to the establishment of turkey populations in Fish and Wildlife Areas were relatively straightforward with respect to the number of source populations used and the number of reintroduction events. All reintroductions into the Fish and Wildlife Areas used in this study were performed within a decade of one another (1983–1992), had similar founder compositions (i.e., number and sex ratio), and each reintroduced population was founded with turkeys from one of three source populations – Missouri, Iowa, and Crane, Indiana (Table 1). Missouri and Iowa retained viable wild turkey populations throughout the decline of the species, and thus were used extensively as source populations by a number of Midwestern states, including Indiana. The turkey population at the Naval Surface Warfare Center in Crane, Indiana (Crane) was extirpated from the area in the early 1900s. This population was formally re-established using only 5 turkeys (1 male and 4 females) from Arkansas in 1956. Although the Crane population was relatively slow to grow, once population sizes were adequate it was quickly used as a source of founders for subsequent reintroductions into other portions of the state.

Table 1. Details of wild turkey reintroductions into selected Indiana Fish and Wildlife Areas (FWA). For each sampling location, the number of samples collected and the sampled population's two-letter designation is provided. Sampled populations are grouped into regions (North or South) based on their geographic locations within Indiana. The number of reintroduction events into each Fish and Wildlife Area and the source of founding individuals for each reintroduction are provided. For each reintroduction, the number of male and female founders is given, as well as the year in which the reintroduction was performed

Sample population	Sample size	Location	Region	# of reintroductions	Source(s)	# founders		Year of reintroduction(s)
						male	female	
JP	36	Jasper-Pulaski FWA, Medaryville, IN	North	1	Iowa	4	12	1987
WI	36	Winamac FWA, Winamac, IN	North	1	Iowa	6	11	1987
PR	40	Pigeon River FWA, Mongo, IN	North	1	Missouri	4	12	1983
LS	39	LaSalle FWA, Lake Village, IN	North	1	Crane[1]	6	11	1992
WS	36	Willow Slough FWA, Morocco, IN	North	1	Crane	6	11	1989
KI	39	Kingsbury FWA, LaPorte, IN	North	1	Crane	6	11	1992
HL	39	Hovey Lake FWA, Mount Vernon, IN	South	1	Crane	6	13	1989
SR	40	Sugar Ridge FWA, Winslow, IN	South	0[2]	Crane			1969–1991
MI	40	Minnehaha FWA, Sullivan, IN	South	1	Missouri	2	0	1988
					Iowa	3	11	
CS	25	Crosley FWA, North Vernon, IN	South	1	Missouri	1	9	1988
					Crane	2	4	
GL	40	Glendale FWA, Montgomery, IN	South	2	Iowa	2	0	1988
					Crane	3	11	1989

[1] Crane = Crane Naval Surface Warfare Center, located in southern Indiana
[2] no reintroductions were made into SR directly; 4 reintroductions were made into this county, all from sources in or near Crane (1969, 1972, 1990, 1991).

Sample collection

We collected 410 blood or blood clot samples from turkeys harvested on 11 Fish and Wildlife Areas in Indiana from 1999–2003, all of which were founded via reintroductions (Figure 1). We also collected 51 blood clot samples from the Crane source population, 51 blood clot samples from the Missouri source population, and 29 feather samples from the Iowa source population (Figure 1). In Missouri and Iowa, turkeys from several areas throughout the state were used as source stock for reintroductions (Figure 1). We collected samples only from those areas where founder turkeys were trapped for reintroductions into the Indiana Fish and Wildlife Areas included in this study. All samples from Indiana Fish and Wildlife Areas were collected from hunter-harvested birds at local check stations from 1996 through 2003, with the assistance of local wildlife biologists, hunter check station operators, and Purdue University personnel. Upon collection, each sample of blood or blood clot was placed immediately into 1.5 mL of lysis buffer (0.1 M Tris-EDTA, pH8.0, 0.1 M EDTA, 0.01 M NaCl, 0.5% [w/v] SDS), and was transferred to a laboratory where it was stored at −80°C until processing. Feather samples were placed in separate envelopes and stored at room temperature until processing.

DNA extraction

We extracted DNA from blood, blood clots, and feathers, using methods previously described (blood and blood clots, Latch et al. 2006; feathers, Pearce et al. 1997). The quality (fragment size range) and quantity of each DNA sample was assessed by electrophoresis through a 1% agarose gel stained with ethidium bromide. Stock DNA was stored at −80°C to minimize DNA degradation during long-term storage. A portion of each sample was diluted to approximately 10 ng/ μL in TLE (10 mM Tris-HCl, 0.1 mM EDTA) to serve as a working stock, and was stored at −20°C to minimize DNA degradation and maximize accessibility.

Control region data collection and analyses

We amplified approximately the first 500 bp of the mitochondrial DNA control region using primers and reaction conditions described in Table 2. Initially, the control region-A primer set was used to

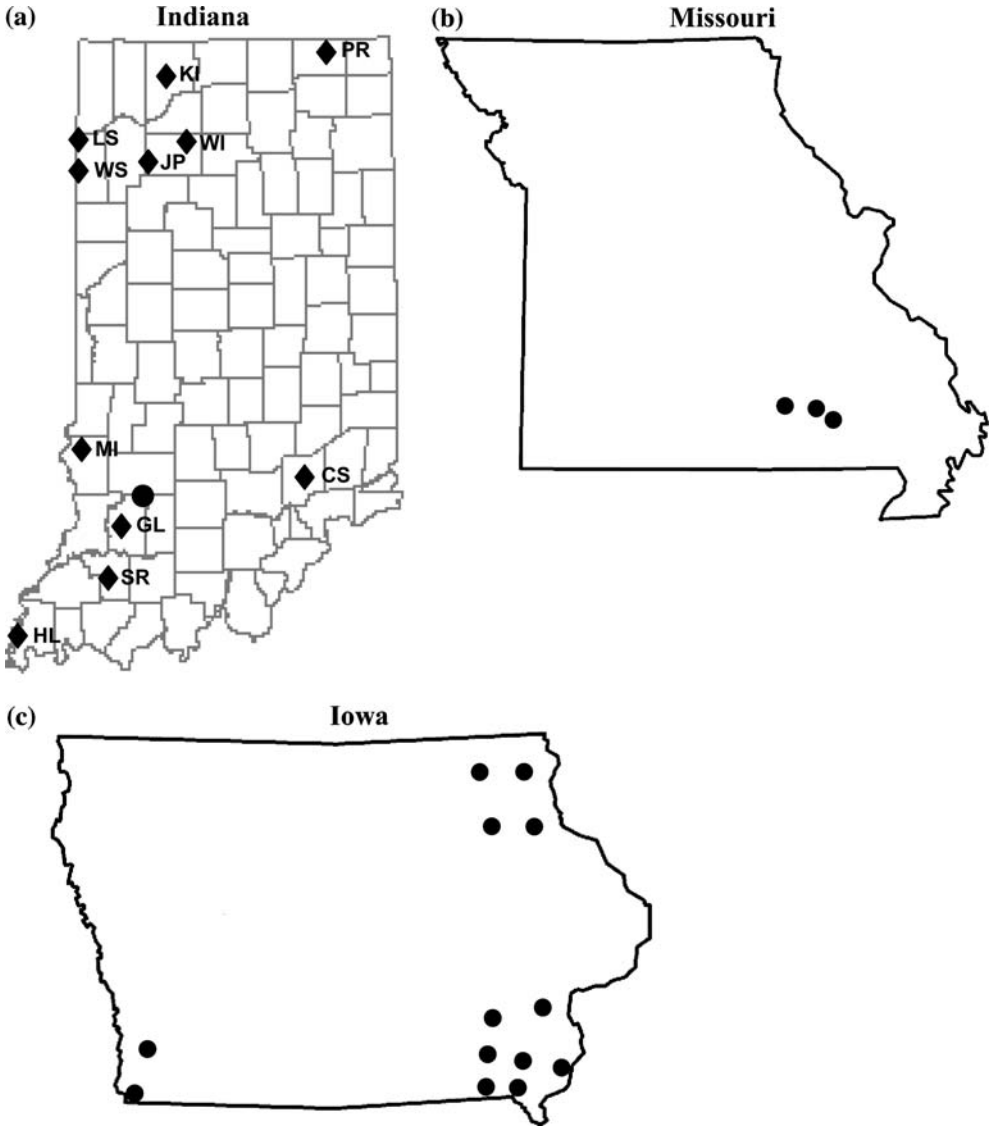

Figure 1. Wild turkey sampling locations for source populations (•) in (a) Indiana, (b) Missouri, and (c) Iowa. Turkeys from these source populations were used to re-establish populations in Fish and Wildlife Areas in Indiana (⧫), each of which is designated by a two-letter code.

amplify and sequence the control region in turkeys for this study. However, these primers became increasingly unstable and required a 25μL reaction volume (requiring large quantities of reagents). These primers eventually were replaced with the control-region-B primer set, which was much more reliable, included the entire mtDNA sequence contained by the control region-A primer set, and could be amplified in a 10μL reaction volume. Approximately 50% of the samples were amplified and sequenced using each primer set. We ensured the consistency of the data by comparing sequences from approximately 10% of the samples using both primer sets. Both primer sets were subjected to the same PCR thermocycler profile: a 2-min denaturation step of 2 min at 96°C, followed by 35 cycles of 96°C for 30 s, 53°C for 30 s, and 72°C for 1 min, and a final extension step at 72°C for 10 min. The quality and relative quantity of PCR products were estimated by electrophoresis through 1% agarose gels stained with ethidium bromide. PCR products were

Table 2 Locus-specific primers and reaction conditions for nuclear microsatellite and mitochondrial control region loci used for wild turkeys in this study. Quantities are specific for a 10μL reaction volume except control region-A, which is specific for a 25μL 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 #)	Gel Set	Primers (5′ → 3′)	DNA (ng)	Primer (pmol)	$MgCl_2$ (mM)	Annealing temperature (°C)
TUM6[1] (U79372)	D	^{B}F:AAATCAGTGTCATTGTGCAA R:TTCTGCTACCTGACCATGTA	5	3	2.0	59
TUM23[1] (U79332)	C	^{B}F:CGGCATCTCCAGCTCCAT R:CCACGGAGAGTCCTGGAT	5	4	1.5	60
TUM50[1] (U79306)	B	^{B}F:CTGATGTCTTAAAGGCT R:ACAAAAACGAACTGATCA	5	2	2.0	46
WT10[2] (AF111453)	D	^{Y}F:TTGGAACAGGAGAAATTTCAGT R:TATTTGTTGCAAGGCAGCAG	10	4	1.5	55
WT32[1] (U79387)	B	^{G}F:TGTTGGAGCTGACTGGAACA R:TGTCTAGATGAGCAGCTGAATG	5	3	1.5	59
WT54[2] (U79330)	A	^{Y}F:AAAGAGCAGCGTGTTCCAGT R:TTCAAAACAGTGTCACGATTCC	5	2	1.5	60
WT75^{B} (AF434907)	C	^{G}F:CCAACTGCAAGATGCTTCTG R:CTGCATTACTGTGCATCATGG	10	3	1.5	57
WT30–2[1] (U79391)	D	^{G}F:GAAGGAGGAACCAAAAACTACG R:CAACCATGGTGTGAGGAGG	5	2	1.5	58
WT38–2[2] (U79365)	A	^{B}F:GGTTTGAGCAGAGTGAATCTCA R:ATTGGTTGGGGGAGGAAC	5	3	1.5	60
WT90–2[2] (AF111645)	C	^{Y}F:AATCAACCCATTTGTTCCCA R:GTGCTTTGATTTAAAAGCCCC	5	2	1.5	58
Control region-A (AY037889)	N/A	F:GAAAAATCACAAAATAAGTCA R AGTGAGGAGTTCAGGAGTTA	30	12.5	1.5	53
Control region-B (AY037889)	N/A	F: AGAAAAATCACGAAATAAGTCA R: GTGAAAAGTGAGGAGTTCAG	10	5	1.5	65

[1] Described in Huang et al. (1999), but amplified here using alternative reaction conditions and/or primer sets.
[2] Described in Latch et al. (2002), but amplified here using alternative reaction conditions.

cleaned using a low sodium precipitation protocol, in which the DNA was precipitated with a sodium acetate solution (0.12 mM NaOAc in 100% EtOH), centrifuged to form a pellet, washed twice with 70% EtOH, and resuspended in water.

Ten microliter sequencing reactions contained approximately 30 ng PCR product, 5 pmol forward primer, and 1μL ABI Big Dye Terminator version 3.1 cut with 3μL 5X buffer (Applied Biosystems). Sequencing reactions were carried out as follows: 98°C for 5 min, followed by 26 cycles of 98°C for 30 s, 50°C for 15 s, 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 (Latch and Rhodes, unpubl. data). Sequenced products were cleaned using the same low sodium precipitation protocol as above, and were sequenced on an ABI 3730 DNA sequencer by the Purdue University Core Genomics Center. Sequences were compiled and edited in Sequencher 4.1 (GeneCodes Corp.).

Several measures were taken to ensure the quality and consistency of our sequence data. Although we used only forward primers for the majority of sequencing in this study, we had conducted an earlier pilot study to confirm the consistency of our control region sequences obtained using forward and reverse primers for a set of 230 turkeys (Latch and Rhodes, unpubl. data). To further confirm sequence quality within this study, we sequenced approximately 10% of PCR products in both the forward and reverse directions. We also compared sequences to wild turkey control region sequences available in GenBank (e.g., AY037889). Throughout the study, forward

sequences containing ambiguous bases also were sequenced in the reverse direction from the original PCR product to confirm the DNA sequence.

We split the overall sample into three subsets for analysis: (1) the three source populations (Missouri, Iowa, and Crane), (2) northern Indiana Fish and Wildlife Areas, and (3) southern Indiana Fish and Wildlife Areas (Table 1). All data analysis was performed separately for each subset of the data. For control region sequence data, we assessed levels of genetic diversity by calculating the following diversity indices in DNASP software (version 4.0; Rozas et al. 2003): the number of haplotypes (h), number of unique haplotypes, haplotype diversity (Hd; Nei 1987; Depaulis & Veuille 1998), and the average number of pairwise nucleotide differences (k; Tajima 1983).

We estimated the amount of genetic differentiation among population pairs by calculating F_{ST} values 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.

Microsatellite data collection and analyses

We amplified 10 microsatellite loci from each sample, using the primers and reaction conditions outlined in Table 2. We chose the 10 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 loci with no evidence of null alleles. Ten microliter amplification reactions were carried out with 5–10 ng genomic DNA (Table 2), 1–4 pmol each forward and reverse primer (Table 2), 0.2 mM each dNTP, and 0.75 Units Taq DNA polymerase (Eppendorf) in 1X reaction buffer [50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $Mg(OAc)_2$]. Microsatellites were amplified via PCR according to the following thermocycler conditions: a 2-minute initial denaturation step at 95°C, 30 cycles of 30 s at 95°C, 30 s at the annealing temperature (Table 2), and 30 s at 72°C, followed by a final extension for 5 min at 72°C, and a 60°C soak for 45 min. Amplified loci were combined into 4 gel sets based on locus size and fluorescent label color, with each gel set containing 2–3 loci. Combined PCR products then were added to 0.2μL 400 HD ROX internal lane standard (Applied Biosystems) and electrophoresed through a 5% polyacrylamide gel on an ABI 377 DNA sequencer. Allele sizes were determined for each locus using GeneScan 3.1 and Genotyper 2.5 software.

We employed a number of different methods to ensure the quality of our microsatellite genotype data. First, we developed a pooled set of 5–6 known alleles for each locus, spanning the full range of allele sizes. We combined these into gel sets as above and ran this allelic standard on each gel every 12 lanes. Genotyping error can occur if allelic categories are not consistently and accurately defined, and our allelic standards allowed us to detect and correct for any microvariability within or among gels in the migration of alleles relative to the internal lane standard. Second, we independently scored a random set of 192 individuals at a quality control locus (locus WT38-2 amplified using a separately designed primer set TUM17 from Huang et al. 1999). Genotyping errors were assessed by comparing genotypes from the two amplicons (representing the same microsatellite locus). Third, we re-electrophoresed or re-amplified 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. Samples were deemed unreliable if they successfully amplified at fewer than 25% of the loci, despite multiple amplification and DNA extraction attempts.

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 across the entire dataset by executing 3200 iterations of Fisher's exact test in GDA software (version 1.1; Lewis & Zaykin 1999). Due to the large number of comparisons involved, we performed a sequential Bonferroni correction for multiple tests (Holm 1979; Rice 1989) before assessing significance.

We split the overall sample into three subsets for analysis, just as we did for control region data: (1) the three source populations (Missouri, Iowa, and Crane), (2) northern Indiana Fish and Wildlife Areas, and (3) southern Indiana Fish and Wildlife Areas (Table 1). All data analysis was performed separately for each subset of the data. We

estimated genetic variability by calculating the average number of alleles per locus (A), the number of unique alleles, and observed (Ho) and expected (He) heterozygosities in GDA software. We adjusted the average number of alleles per locus for variation in sample sizes with rarefaction in FSTAT software (version 2.9.3; Goudet 2001) to alleviate the sensitivity of allelic richness to sample size (Hurlbert 1971, Petit et al. 1998). We tested for significant differences in allelic richness (rarefacted average number of alleles per locus) among subsets of the data in FSTAT software using 1,000 permutations.

Within each subset of the data, we estimated F_{ST} between pairs of populations using SPAGEDI software. We tested for significant differentiation between populations by comparing observed F_{ST} values to those obtained by permuting individuals among populations 10,000 times. We used pairwise microsatellite F_{ST} estimates between all reintroduced populations in the dataset to generate a neighbor-joining tree in GDA software, and imported it into TREEVIEW (version 1.6.6; Page 1996) to facilitate visualization of genetic relationships among populations.

To address the question of whether reintroduced northern (or southern) Indiana populations followed an isolation by distance model, we employed Mantel tests (Mantel 1967) using GENEPOP software (version 3.2; Raymond & Rousset 1995). We performed pairwise comparisons of matrices of the natural log of geographic distances (in miles) to matrices of genetic differentiation, as estimated by $F_{ST}/(1 - F_{ST})$ for microsatellite data from the data subsets of northern and southern Indiana Fish and Wildlife Areas, respectively (Rousset 1997). We tested the null hypothesis of no correlation between genetic and geographic distance matrices by calculating a Spearman Rank correlation coefficient for each of 10,000 permutations of the data and comparing the distribution of observed values to the expected distribution of random outcomes. Positive correlations indicate isolation by distance, where gene flow between populations results in greater similarity between neighboring populations than between distant populations (Slatkin 1993). Additionally, we plotted the natural log of geographic distances against $F_{ST}/(1 - F_{ST})$ for all pairwise comparisons within northern and southern population sets to visualize correlations.

We employed an individual-based assignment test in the program STRUCTURE (version 2.1; Pritchard et al. 2000) to investigate the alternate question of whether reintroduced northern (or southern) populations still retained genetic characteristics of their source populations. The program STRUCTURE uses a model-based clustering approach to infer population structure from multilocus genotypes. The model assumes that within a sample there are K subpopulations, each of which is characterized by a set of allele frequencies at each locus. It is further assumed that loci are at Hardy–Weinberg equilibrium within populations. STRUCTURE then attempts to decompose the entire sample set into K genetically distinct clusters by assigning individuals to one or more of the clusters such that Hardy-Weinberg and linkage disequilibrium (expected in a mixed sample of a subdivided population; Nei and Li 1973) are minimized. The reliance of assignment methods such as STRUCTURE on Hardy–Weinberg and linkage equilibrium makes this analysis appropriate only for our diploid microsatellite data. Assignments are done probabilistically, and therefore STRUCTURE generates a probability (q) that each individual belongs to each of the K subpopulations. In this study, q values represent the proportion of an individual turkey's genome that is characteristic (or uncharacteristic) of its source population. By employing the 'use population information' option in program STRUCTURE, we were able to define Missouri, Iowa, and Crane as known source populations. We assigned individuals from reintroduced northern (or southern) Indiana populations to one of the three known source populations, using 100,000 replicates following a burn-in of 30,000 replicates. Individuals were assigned to the population in which the Q value was highest.

Results

Mitochondrial DNA

Control region analyses were based on 254 bases of aligned sequence for 464 individuals. Eleven characters were variable in this region, resulting in the detection of 9 closely related haplotypes across the entire sample. Five of these haplotypes

were distributed widely across most populations in all subsets of the data. Of the remaining 4 haplotypes, 3 were unique to a single population; one was found only in Iowa, one was found only in Crane, and one was found only in PR, a northern Fish and Wildlife Area (Table 3). The Iowa and Crane haplotypes were found at somewhat low frequencies (4%) in their respective populations, but the PR haplotype was found at a higher frequency of 15% (Table 3). The final haplotype was not unique to a particular population, but also was not widely distributed. It was found at extremely high frequency in the MI Fish and Wildlife Area in southern Indiana (52%), and also was found at a frequency of 4% in WI, a northern Indiana Fish and Wildlife Area (Table 3).

Haplotype diversity averaged 0.595 across all populations (Table 3). Within populations, haplotypes differed by an average of approximately 1.3 nucleotides, although this number varied among populations. For example, haplotypes in the JP (northern Indiana) population were very closely related to one another ($k = 0.464$), whereas haplotypes in the HL (southern Indiana) population differed by over 2 nucleotides on average ($k = 2.003$; Table 3).

Overall, source populations were genetically differentiated from one another based upon mitochondrial control region data ($F_{ST} = 0.071$; $p = 0.0010$, one-sided test). Pairwise control region F_{ST} estimates indicated significant genetic differentiation between Crane and Iowa (0.122) and between Crane and Missouri (0.070); however, no differentiation was detected between Missouri and Iowa (−0.005; note that the Weir and Cockerham (1984) method of F_{ST} estimation used in SPAGEDI software allows estimates to fall below zero; Table 4). In northern Indiana, Fish and Wildlife Areas also were considerably differentiated from one another, although there was a large variance in pairwise F_{ST} values among populations. The overall estimate of F_{ST} was 0.191, with pairwise values ranging from 0.029 between WI and KI to 0.427 between JP and PR (Table 4). Similarly, we found a high level of genetic differentiation among southern Indiana Fish and Wildlife Areas ($F_{ST} = 0.223$) and a large variance in pairwise values, which ranged from 0.005 (SR–GL) to 0.376 (MI–CS; Table 4).

Table 3 Estimates of mitochondrial control region diversity for wild turkey source populations and reintroduced populations in northern and southern Indiana Fish and Wildlife Areas (FWAs). h = number of haplotypes, Uh = number of unique haplotypes, Hd = haplotype diversity, and k = average number of nucleotide differences among haplotypes

Subgroup	Population	Sample size	h	Uh	Hd	k
Sources	Missouri (MO)	51	5	0	0.557	1.053
	Iowa (IA)	26	5	1	0.465	1.086
	Crane[1] (CR)	50	5	1	0.762	1.626
	Overall	127	7	2	0.650	1.330
Northern FWAs	Jasper-Pulaski FWA (JP)	30	3	0	0.343	0.464
	Winamac FWA (WI)	27	5	0	0.632	1.043
	Pigeon River FWA (PR)	39	5	1	0.636	1.072
	LaSalle FWA (LS)	34	5	0	0.665	0.945
	Willow Slough FWA (WS)	36	5	0	0.692	1.862
	Kingsbury FWA (KI)	38	4	0	0.745	1.341
	Overall	196	7	1	0.755	1.425
Southern FWAs	Hovey Lake FWA (HL)	28	4	0	0.672	2.003
	Sugar Ridge FWA (SR)	30	3	0	0.497	1.343
	Minnehaha FWA (MI)	31	4	0	0.632	1.260
	Crosley FWA (CS)	20	3	0	0.343	0.686
	Glendale FWA (GL)	24	5	0	0.685	1.399
	Overall	112	7	0	0.708	1.871

[1] Crane = Crane Naval Surface Warfare Center, located in southern Indiana

Table 4 Pairwise F_{ST} values for microsatellite data (above diagonal) and control region sequences (below diagonal) for wild turkeys in (a) source populations, (b) reintroduced northern Indiana Fish and Wildlife Areas, and (c) reintroduced southern Indiana Fish and Wildlife Areas. F_{ST} estimates significantly ($p < 0.05$, one-sided test) greater than zero are denoted with an asterisk

	MO	IA	CR			
(a) source populations						
MO	–	0.022*	0.019*			
IA	−0.005	–	0.032*			
CR	0.070*	0.122*	–			
(b) reintroduced northern Indiana Fish and Wildlife Areas						
	JP	WI	PR	LS	WS	KI
JP	–	0.028*	0.096*	0.077*	0.076*	0.063*
WI	0.074	–	0.084*	0.069*	0.071*	0.032*
PR	0.427*	0.253*	–	0.045*	0.034*	0.074*
LS	0.254*	0.127*	0.214*	–	0.019*	0.054*
WS	0.254*	0.072*	0.212*	0.151*	–	0.052*
KI	0.200*	0.029	0.247*	0.059*	0.102*	–
(c) reintroduced southern Indiana Fish and Wildlife Areas.						
	HL	SR	MI	CS	GL	
HL	–	0.071*	0.066*	0.056*	0.058*	
SR	0.191*	–	0.090*	0.037*	0.046*	
MI	0.311*	0.297*	–	0.056*	0.034*	
CS	0.351*	0.037	0.376*	–	0.038*	
GL	0.118*	0.005	0.249*	0.058	–	

Microsatellites

Our microsatellite dataset was very robust; our final dataset contained less than 3% missing data, and 190 of the 192 samples used for quality control scored consistently at the WT38-2 locus using both the standard and alternate primer sets. All three subsets of the data (source populations, northern Fish and Wildlife Areas, and southern Fish and Wildlife Areas) exhibited high levels of genetic variability (Table 5). Northern Fish and Wildlife Areas had significantly reduced allelic richness in comparison to the source populations, but this trend was absent when we compared southern Fish and Wildlife Areas with the sources. There also was no significant difference in allelic richness between northern and southern Fish and Wildlife Areas. Several unique alleles were found within all populations except SR; however, the ubiquitous distribution of low frequency (< 8%) unique alleles across loci and populations suggests that at least a portion of these may be sampling artifacts.

The overall estimate of F_{ST} among source populations indicated that they were significantly differentiated from one another ($F_{ST} = 0.023$; $p = 0.0000$, one-sided test) and considerable levels of genetic differentiation were detected among all source population pairs; pairwise F_{ST} values between source populations ranged from 0.019 between Missouri and Crane to 0.032 between Iowa and Crane (Table 4). Microsatellites also demonstrated significant genetic differentiation among northern Fish and Wildlife Areas; the overall estimate of F_{ST} was 0.059 ($p = 0.0000$, one-sided test) and pairwise F_{ST} values between populations ranged from 0.019 to 0.096 (Table 4). Similarly, southern Indiana Fish and Wildlife Areas exhibited considerable genetic differentiation among populations ($F_{ST} = 0.057; p = 0.0000$, one-sided test), with pairwise estimates of F_{ST} ranging from 0.034 to 0.090 (Table 4).

The neighbor-joining tree, generated from pairwise F_{ST} estimates for all Indiana Fish and Wildlife Areas, reflected genetic differentiation among populations with considerable branch lengths between most population pairs (Figure 2). Although relationships among populations did not follow geographical patterns, general associations between reintroduced populations and their sources were evident (Figure 2).

We found no evidence for isolation by distance in either of the reintroduced northern or southern Indiana population sets using Mantel tests. There were no significant positive correlations between

Table 5 Estimates of nuclear microsatellite diversity for wild turkey source populations and reintroduced populations in northern and southern Indiana Fish and Wildlife Areas (FWAs). A = average number of alleles per locus, UA = number of unique alleles per locus, He = expected heterozygosity, and Ho = observed heterozygosity

Subgroup	Population	Sample size	A (w/rarefaction)	UA	He	Ho
Sources	Missouri (MO)	51	9.7 (8.5)	4	0.78	0.78
	Iowa (IA)	29	8.6 (8.0)	2	0.74	0.70
	Crane[1] (CR)	51	9.8 (8.4)	1	0.76	0.72
	Overall	131	11.5 (11.5)	7	0.77	0.74
Northern FWAs	Jasper-Pulaski FWA (JP)	36	7.7 (6.9)	1	0.69	0.66
	Winamac FWA (WI)	36	7.5 (6.9)	1	0.70	0.68
	Pigeon River FWA (PR)	40	7.7 (6.8)	2	0.74	0.69
	LaSalle FWA (LS)	39	8.6 (7.7)	2	0.72	0.68
	Willow Slough FWA (WS)	33	7.8 (7.5)	1	0.75	0.69
	Kingsbury FWA (KI)	39	7.7 (7.0)	0	0.69	0.67
	Overall	223	11.6 (10.9)	7	0.75	0.68
Southern FWAs	Hovey Lake FWA (HL)	39	7.3 (6.8)	1	0.70	0.71
	Sugar Ridge FWA (SR)	40	8.2 (7.4)	0	0.71	0.64
	Minnehaha FWA (MI)	40	8.8 (7.9)	1	0.71	0.69
	Crosley FWA (CS)	25	8.7 (8.4)	1	0.78	0.74
	Glendale FWA (GL)	40	9.1 (8.3)	0	0.74	0.68
	Overall	184	11.5 (11.3)	3	0.76	0.69

[1] Crane = Crane Naval Surface Warfare Center, located in southern Indiana.

genetic differentiation and geographic distances among northern Indiana ($r^2 = 0.178; p = 0.14$; one-tailed test) or southern Indiana ($r^2 = 0.001$; $p = 0.49$; one-tailed test) populations. Plots of pairwise geographic distance versus genetic differentiation illustrate this lack of correlation between genetic and geographic distance (Figure 3).

Most individuals in reintroduced northern Indiana populations (JP, WI, PR, LS, WS, and KI) could be assigned back to their source populations (Table 6). Individual turkeys from Iowa-sourced northern populations (JP and WI) were assigned to their source with very high Q values (Table 6). Only a total of 4% of individuals from JP and WI were assigned to a source population other than Iowa. Ninety percent of PR individuals, reintroduced from Missouri, also were assigned to their source with high Q values (Table 6). The 10% of individuals in the PR population that were assigned to other sources had relatively low Q values, and mostly were those individuals with incomplete multilocus genotypes. Assignments of individuals from LS and WS, populations founded with turkeys from Crane, were somewhat ambiguous; however, most individuals could be assigned to their source population (LS = 69%, WS = 64%; Table 6). Individuals in the KI population were an exception to the overall pattern of correct assignments in northern Indiana. Only 28% of the individuals in this population were assigned to their Crane source, while over 64% were assigned to Iowa. The proportion of each KI individual's genome assigned to both sources was fairly high, as indicated by average Q values near 79% (Table 6).

In reintroduced southern Indiana populations, there was more ambiguity and less confidence in assignments than in northern Indiana. Overall, each turkey's genome was not overwhelmingly characteristic of any of the source populations, with average Q values ranging from 43% to 72% among populations (Table 6). Moreover, we found a higher proportion of misassignments than in northern Indiana. Of the five reintroduced southern Indiana Fish and Wildlife Areas, only HL was founded from a single reintroduction from Crane. Approximately 44% of individuals in this population were assigned back to Crane; however, an almost equal percentage of turkeys in HL were assigned to Missouri (41%; Table 6).

In each of the remaining four reintroduced populations in southern Indiana Fish and Wildlife Areas, the source populations could still be reliably identified. Although there were no reintroductions directly into the SR Fish and Wildlife Area, there were four reintroductions into Pike County, where

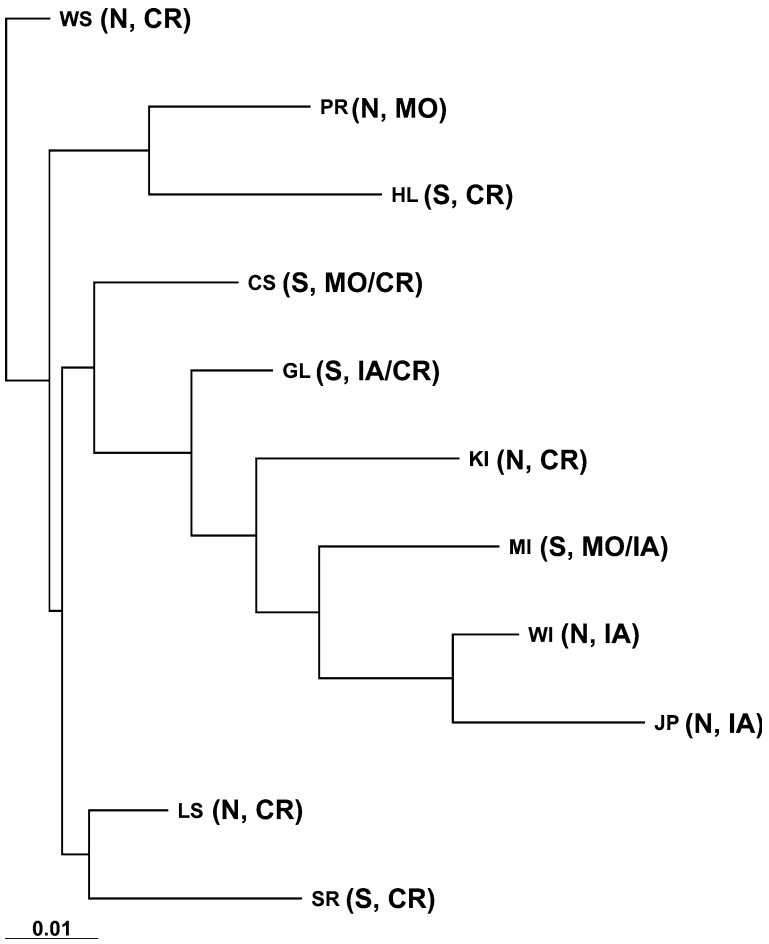

Figure 2. Neighbor-joining tree based on F_{ST} estimates from 10 microsatellite loci, illustrating relationships among wild turkey populations in Indiana Fish and Wildlife Areas. Letters in parentheses following population designations indicate geographic location (N = North or S = South) and source population (CR = Crane, MO = Missouri, and IA = Iowa).

SR is located. All 4 reintroductions were from in or near Crane, and thus we expected SR individuals to assign back to Crane. Our results agreed with our expectations; approximately 75% of individuals from SR were assigned to Crane as the source population (Table 6). Assigning individuals from MI, CS, and GL was slightly more complicated; both MI and CS were founded from a single reintroduction event using multiple sources, and GL was founded in two reintroduction events from two different sources in two consecutive years. In the MI reintroduction, approximately 13% of the founder individuals were from Missouri and 87% were from Iowa. We were able to assign 18% of individuals back to Missouri and 74% to Iowa, nearly matching the original founder proportions (Table 6). The CS population was founded from approximately 62% Missouri and 38% Crane individuals. We also were able to assign individuals to these sources in proportions similar to those of the founding event (44% and 32%, respectively; Table 6). The GL reintroduction was performed using an initial stocking of only two adult males from Iowa and a later supplementation of 15 turkeys of mixed sex and age from Crane (Table 1). We assigned 43% and 48% of individuals to Iowa and Crane, respectively.

Discussion

Reintroductions of wild turkeys into Indiana have left distinct genetic signatures on established

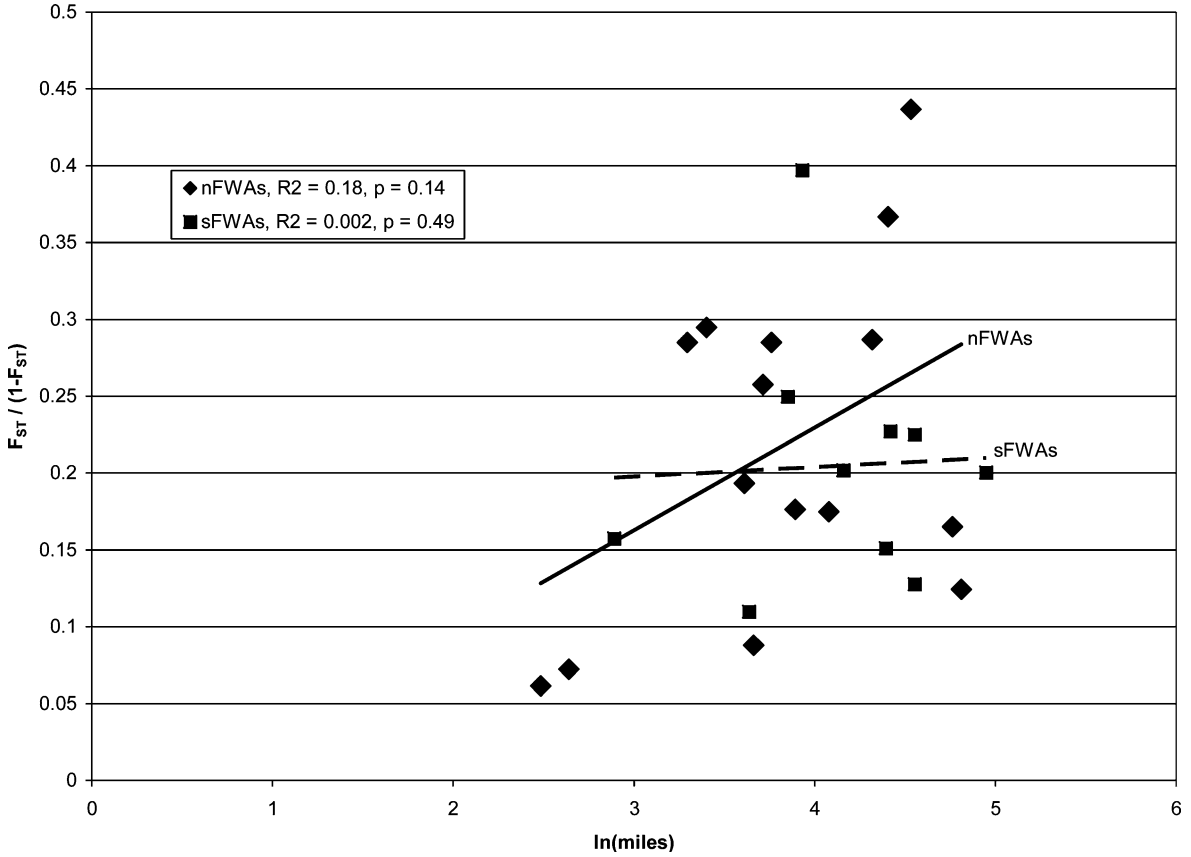

Figure 3. Mantel test results for Indiana wild turkey populations in northern Fish and Wildlife Areas (nFWAs) and southern Fish and Wildlife Areas (sFWAs). The plot represents the correlation between matrices of pairwise geographic distances [ln (distance in miles)] and genetic differentiation ($F_{ST}/1 - F_{ST}$) at ten microsatellite loci.

populations, even several decades after the reintroduction events. The strength of genetic relationships between reintroduced populations and their sources was greater for northern Indiana populations than for southern Indiana populations, reinforcing the premise that population isolation accentuates the genetic legacy of reintroduction events. Similar results have been demonstrated for isolated populations of Merriam's turkey (*M. g. merriami*; Mock et al. 2004) and pronghorn (*Antilocapra americana*; Rhodes et al. 2001). However, data for other species, such as elk (*Cervus elaphus*), have demonstrated that the genetic legacy of source populations may be almost totally obscured in isolated populations, particularly if subjected to extreme bottlenecks over prolonged time periods (Williams et al. 2002). Although the strength of genetic relationships between reintroduced turkey populations in southern Indiana and their sources was less clear than that for the more isolated northern populations, the genetic legacy of reintroduced turkey populations in southern Indiana was not completely obscured by gene flow from neighboring populations despite their proximity. These results corroborate the allozyme-based studies of other researchers for white tailed deer (*Odocoileus virginianus*; Leberg et al. 1994; Leberg & Ellsworth 1999) and fisher (*Martes pennanti*; Williams et al. 2000), where the genetic legacy of reintroduced populations in a region remained detectable for many decades.

We found substantial levels of genetic variability in most reintroduced populations, as indicated by both nuclear microsatellite and mitochondrial control region data. For microsatellites, all sampled populations exhibited high levels of heterozygosity and allelic richness. In northern Indiana, we saw a significant reduction in the allelic diversity of reintroduced populations relative to their sources, as might be expected

Table 6 Assignments of wild turkeys from reintroduced Fish and Wildlife Areas in northern or southern Indiana to source populations based on multilocus microsatellite genotypes (10 loci). For each Fish and Wildlife Area, the number of individuals (N) assigned to each source population (Missouri, Iowa, or Crane), the percent of total individuals assigned to each source, and the average *Q* values for those assignments are provided. The percent of total individuals assigned to the documented source population is in bold for each population

Region	Population	Source(s)	Missouri			Iowa			Crane		
			N	%	*Q*	*N*	%	*Q*	*N*	%	*Q*
North	JP	Iowa	0	0	0	35	**97**	89.8	1	3	68.2
North	WI	Iowa	2	6	74.7	34	**94**	88.2	0	0	0
North	PR	Missouri	36	**95**	83.1	0	0	0	4	10	49.3
North	LS	Crane[1]	8	21	76.0	4	10	61.1	27	**69**	78.0
North	WS	Crane	10	30	66.9	2	6	70.6	21	**64**	68.7
North	KI	Crane	3	8	56.7	25	64	78.7	11	**28**	79.3
South	HL	Crane	16	41	58.6	6	15	60.0	17	**44**	59.9
South	SR[2]	Crane	3	8	51.2	7	18	51.6	30	**75**	67.8
South	MI	Missouri, Iowa	7	**18**	58.7	29.5[3]	**74**	71.6	3.5	9	43.3
South	CS	Missouri, Crane	11	**44**	65.4	6	24	66.3	8	**32**	56.3
South	GL	Iowa, Crane	4	10	55.6	17	**43**	60.4	19	**48**	68.3

[1] Crane = Crane Naval Surface Warfare Center, located in southern Indiana
[2] no reintroductions were made into SR directly; 4 reintroductions were made into this county, all from sources in or near Crane (1969, 1972, 1990, 1991).
[3] Assigned with equal probability to both Iowa and Crane source populations.

during a founder event. However, we did not observe a similar reduction in allelic diversity in southern Indiana. The common practice of block stocking used in this region may have increased connectivity among recently established populations. Such connectivity is likely to encourage population growth and decrease the severity of genetic drift, thereby alleviating the impact of the founder event. Most populations also exhibited high levels of variability within the mitochondrial control region, evident from high numbers of haplotypes, haplotype diversity values, and average numbers of nucleotide differences among haplotypes. There was not an obvious reduction in haplotype diversity in reintroduced populations relative to their sources. Fewer mtDNA haplotypes than microsatellite alleles in source populations means that a higher proportion of mtDNA haplotypes likely were sampled when selecting founders for reintroductions. The polygynous mating system of the wild turkey may have further encouraged the establishment of those maternal lineages, lessening the impact of genetic drift on mtDNA diversity.

The rare haplotype found in high frequency in the MI Fish and Wildlife Area is particularly interesting, as it suggests a considerable skew in the distribution of offspring among maternal lineages early in the development of this population. The detection of this haplotype in the WI (4%) and MI (54%) populations, which share Iowa as a source of founders, suggests that it may be a haplotype that exists at low frequency in the Iowa population but was not sampled in this study. The combination of a small founding population and several generations of genetic drift can result in extreme shifts in haplotype frequencies between source and translocated populations (Maruyama & Fuerst 1985; Fuerst & Mauyama 1986; Luikart et al. 1998a, b). In a reintroduced elk herd in Pennsylvania, multiple dramatic shifts in microsatellite allele frequencies away from those of its source herd were observed and, in one instance, a low-frequency allele (7.5%) in the source population became fixed in the reintroduced herd (Williams et al. 2002).

Considerable genetic differentiation was evident among populations within each subset of the data (sources, northern and southern Indiana Fish and Wildlife Areas) using both microsatellite and control region data. One exception was the lack of detectable genetic differentiation among Iowa and Missouri source populations using control region data. It may be that the Iowa population was not

sampled thoroughly enough to detect haplotypes which are different between the two populations. The effect of insufficient sample size would indeed be more evident in control region data, because there are fewer overall types than with microsatellites. The magnitude of pairwise F_{ST} values among populations did not clearly reflect the relationships among populations that would have been expected based on records of reintroduction sources used for each population (Table 4, Figure 2). This was likely a consequence of founder effects at the time of reintroduction, genetic drift, and some limited gene flow among populations. Nonetheless, patterns of genetic diversity were not related to geographic distance either, and the results of Mantel tests did not support the isolation-by-distance model of genetic structure for these reintroduced Indiana turkey populations.

Initially, the genetic attributes of reintroduced populations are a direct function of both the genetic diversity of the source populations used and of the level of sampling effort within each source. Subsequent to the reintroduction event, rates of population growth, gene flow and genetic drift influence rates of change in gene frequencies within populations. In northern Indiana, we expected that the genetic attributes of sources would still be evident within reintroduced populations, primarily due to the rapid establishment of these populations and the reduced probability of gene flow among re-established populations in northern Indiana. With one exception (KI), we found that most turkeys in reintroduced populations in northern Indiana Fish and Wildlife Areas could be reliably assigned back to their source populations, and that the genome of each turkey was still highly characteristic of its source population.

Assignments of individuals in KI were particularly confounding. Although reintroduction records reported that KI was founded using turkeys from Crane, 64% of individuals in this population were assigned to Iowa, indicating a substantial genetic contribution from Iowa. To explore the potential reasons underlying this discrepancy, wildlife biologists for the state of Indiana were contacted for more information. This investigation indicated that turkeys likely were present in KI prior to the 1991 release of Crane turkeys, and that the release was considered to be a supplement rather than a population establishment (S. Backs, pers. commun.). Nearby populations established prior to 1991 included JP, WI, and the Menominee State Wetlands Area in Plymouth, Indiana. All three of these populations were established in 1987 using Iowa turkeys. Despite the high degree of habitat fragmentation in northern Indiana, migration corridors between JP and KI (the Kankakee River) and between Menominee and KI (continuous forest habitat) do exist, making these the only likely source populations for turkeys in KI prior to 1991, and thus providing a plausible explanation for the large genetic influence of Iowa in this population.

In southern Indiana, we predicted that the connectivity of reintroduced populations would encourage gene flow among them. If gene flow occurred among reintroduced populations within a region, it would obscure the initial genetic relationships caused by the genetic contributions of the founding populations, and genetic differentiation would eventually follow an isolation-by-distance model. This process would be accelerated in regions where populations are in close proximity and in continuous habitats where gene flow is not limited by habitat barriers. Following our expectations, the overall number and confidence of correct assignments was somewhat lower for southern Indiana Fish and Wildlife Areas than for northern Fish and Wildlife Areas. However, in 3 of the 5 cases (MI, SR, and CS), the genetic contributions of the source populations were still relatively clear.

In the GL population in southern Indiana, the initial reintroduction of Iowa birds represented only 12% of the total founding population; however, 41% of individuals were assigned to Iowa. In this case, dispersal from nearby is an unlikely explanation, because no Iowa-sourced reintroductions were performed into this area. The large proportion of GL individuals that were assigned to Iowa suggests that although few in number (2), these initial founders had a relatively large effect on the genetic characteristics of the current population.

In HL, approximately the same number of individuals was assigned to the Crane source population (44%) as was assigned to Missouri (41%). Upon inspection of the reintroduction records for sites proximate to the HL Fish and Wildlife Area, it was ascertained that a large reintroduction of turkeys from Missouri was made less than 14 miles from HL in the same year as the

HL reintroduction (into Harmonie State Park in New Harmony, Indiana in 1989). It seems likely that the assignments for individuals in HL reflect genetic contributions by both the original founders and dispersing individuals from Harmonie State Park.

The mixtures of sources assigned for many of the southern Indiana Fish and Wildlife Areas suggest that there may be some gene flow occurring among reintroduced populations that is obscuring patterns of genetic structure caused by the reintroduction events. Certainly, founder effects and genetic drift could be contributing to the ambiguity of assignments for turkeys from southern Indiana Fish and Wildlife Areas; however, such processes do not seem to have substantially obscured the genetic legacy of reintroduced turkey populations in northern Indiana. It seems clear that there has been some movement of turkeys among populations in both northern and southern Indiana following the reintroduction events. The KI and HL populations serve as excellent examples wherein gene flow from neighboring populations has obscured the genetic relationship between reintroduced populations and their documented sources. The reduced Q values for assignments of turkeys in southern Indiana compared to those in northern portions of the state also support the notion that gene flow is occurring more often among reintroduced populations in southern Indiana than among populations in northern parts of the state. However, despite the evidence of gene flow among populations, the magnitude of genetic interchange has not been sufficient to obscure the genetic signature of the initial reintroduction event in most populations.

The approaches used in this study help us to understand the expected extent of the genetic legacy of reintroductions. Although a goal of many reintroduction programs is to create a network of populations connected by gene flow, dispersal among reintroduced populations may be limited even in areas where populations are in close proximity. This study has implications for both wildlife management and conservation biology, as the development of optimal reintroduction strategies for many species will depend on data collected from post-reintroduction monitoring. North American game species with a history of intense management, such as the wild turkey, represent a unique opportunity to employ genetic techniques to address such questions of immediate conservation concern.

Acknowledgements

The authors are grateful for the technical assistance provided by Courtney Shattuck and Rochelle Jacques. We are indebted to Steve Backs from the Indiana Department of Natural Resources for his expertise in turkey biology and the management history of turkeys in Indiana, and to the numerous check station operators and wildlife biologists throughout our sampling area who helped us collect samples. We also would like to thank Jeff Glaubitz, Paul Leberg, Andrew DeWoody, and George Parker for helpful comments on earlier versions of the manuscript. We appreciate funding provided by Purdue University, the National Wild Turkey Federation, and the John S. Wright Endowment to the Department of Forestry and Natural Resources, Purdue University.

References

Allendorf FW (1983) Isolation, gene flow, and genetic differentiation among populations In: Genetics and conservation: a reference for managing wild animal and plant populations. (eds. Schonewald-Cox CM, Chambers SM, MacBryde B, Thomas L), pp. 51–65. Benjamin/Cummings, Menlo Park, CA.

Backs SE, Eisfelder CH (1990) Criteria and guidelines for wild turkey release priorities in Indiana. *Proceedings of the National Wild Turkey Symposium*, **6**, 134–143.

Baker AJ, Moeed A (1987) Rapid genetic differentiation and founder effect in colonizing populations of common mynahs (*Acridotheres tristis*). *Evolution*, **41**, 525–538.

Baker AJ (1992) Genetic and morphometric divergence in ancestral European and descendent New Zealand populations of chaffinches (*Fringella coelebs*). *Evolution*, **46**, 1784–1800.

Depaulis F, Veuille M (1998) Neutrality tests based on the distribution of haplotypes under an infinite-site model. *Mol. Biol. Evol.*, **15**, 1788–1790.

Fitzsimmons NN, Buskirk SW, Smith MH (1997) Genetic changes in reintroduced Rocky Mountain bighorn sheep populations. *J. Wildlife Manag.*, **61**, 863–872.

Fuerst PA, Maruyama T (1986) Considerations on the conservation of alleles and of genic heterozygosity in small managed populations. *Zoo Biol.*, **5**, 171–179.

Glaubitz JC (2004) CONVERT (version 1.2): A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Mol. Ecol. Notes*, **4**, 309–310.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol. Ecol. Notes*, **2**, 618–620.

Holm S (1979) A simple sequentially rejective multiple test procedure. *Scand. J. Statist.*, **6**, 65–70.

Huang HB, Song YQ, Hsel M, Zahorchak R, Chiu J, Teuscher C, Smith EJ (1999) Development and characterization of genetic mapping resources for the turkey (*Meleagris gallopavo*). *J. Hered.*, **90**, 240–242.

Hurlbert SH (1971) The nonconcept of species diversity: a critique and alternative parameters. *Ecology*, **52**, 577–586.

Latch EK, Smith EJ, Rhodes OE (2002) Isolation and characterization of microsatellite loci in wild and domestic turkeys (*Meleagris gallopavo*). *Mol. Ecol. Notes*, **2**, 176–178.

Latch EK (2004) *Population genetics of reintroduced wild turkeys: insights into hybridization, gene flow, and social structure*. Dissertation, Purdue University, West Lafayette, IN.

Latch EK, King JS, Harveson LA, Hobson MD, Rhodes OE (2005) Assessing hybridization in wildlife populations using molecular markers: A case study in wild turkeys. *J. Wildlife Manag.*, in press.

Leberg PL (1991) Effects of bottlenecks on genetic divergence in populations of the wild turkey. *Conserv. Biol.*, **5**, 522–530.

Leberg PL, Stangel PW, Hillestad HO, Marchinton RL, Smith MH (1994) Genetic structure of reintroduced wild turkey and white-tailed deer populations. *J. Wildlife Manag.*, **58**, 698–711.

Leberg PL, Ellsworth DL (1999) Further evaluation of the genetic consequences of translocations on southeastern white-tailed deer populations. *J. Wildlife Manag.*, **63**, 327–334.

Lewis PO, Zaykin D (1999) Genetic data analysis: a computer program for the analysis of allelic data, version 1.1. Available at http://lewis.eeb.uconn.edu/lewishome/software.html.

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998a) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *J. Hered.*, **89**, 238–247.

Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998b) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. *Mol. Ecol.*, **7**, 963–974.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res.*, **27**, 209–220.

Maruyama T, Fuerst PA (1985) Population bottlenecks and nonequilibrium models in population genetics. 2. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics*, **111**, 675–689.

Merila J, Bjorklund M, Baker A (1996) The successful founder: genetics of introduced Caruelis chloris (greenfinch) populations in New Zealand. *Heredity*, **77**, 410–422.

Mock KM, Latch EK, Rhodes OE (2004) Assessing losses of genetic diversity due to translocations: long-term case histories in Merriam's turkey (*Meleagris gallopavo merriami*). *Conserv. Genet.*, **5**, 631–645.

Nei M, Li W-H (1973) Linkage disequilibrium in subdivided populations. *Genetics*, **75**, 213–219.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

Nei M (1987) *Molecular Evolutionary Genetics*, Columbia University Press, New York.

Page RDM (1996) TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.*, **12**, 357–358.

Pearce J, Fields RL, Scribner KT (1997) Nest materials as a source of genetic data for avian behavioral studies. J. Field Ornithol., **68**, 471–481.

Perez T, Albornoz J, Nores C, Dominguez A (1998) Evaluation of genetic variability in introduced populations of red deer (*Cervus elaphus*) using DNA fingerprinting. *Hereditas*, **129**, 85–89.

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.*, **12**, 844–855.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): Population-genetics software for exact tests and ecumenicism. *J. Hered.*, **86**, 248–249.

Rhodes OE, Buford DJ, Miller MS, Lutz RS (1995) Genetic structure of reintroduced Rio Grande wild turkeys in Kansas. *J. Wildlife Manag.*, **59**, 771–775.

Rhodes OE, Reat EP, Heffelfinger JR, DeVos JC (2001) Analysis of reintroduced pronghorn populations in Arizona using mitochondrial DNA markers. *Proceedings of the Biennial Pronghorn Antelope Workshop*, **19**, 45–54.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Rowe GT, Beebee JC, Burke T (1998) Phylogeography of the natterjack toad Bufo calamita in Britain: genetic differentiation of native and translocated populations. *Mol. Ecol.*, **7**, 751–760.

Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, **19**, 2496–2497.

Scribner KT, Stuwe M (1994) Genetic relationships among alpine ibex Capra ibex populations re-established from a common ancestral source. *Biol. Conserv.*, **69**, 137–143.

Slatkin M (1993) Isolation by distance in equilibrium and nonequilibrium populations. *Evolution*, **47**, 264–279.

Sokal RR, Wartenberg DE (1983) A test of spatial auto-correlation analysis using an isolation-by-distance model. *Genetics*, **105**, 219–237.

Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetics*, **105**, 437–460.

Weir BS, Cockerham CC (1984) Estimating F-Statistics for analysis of population structure. *Evolution*, **38**, 1358–1370.

Williams RN, Rhodes OE, Serfass TL (2000) Assessment of genetic variance among source and reintroduced fisher populations. *J. Mammal.*, **81**, 895–907.

Williams CL, Serfass TL, Cogan R, Rhodes OE (2002) Microsatellite variation in the reintroduced Pennsylvania elk herd. *Mol. Ecol.*, **11**, 1299–1310.

Wright S (1978) *Evolution and the genetics of populations, Vol. 4: variability within and among natural populations*, University of Chicago Press, Chicago, IL.