Isolation and characterization of microsatellite loci in wild and domestic turkeys (*Meleagris gallopavo*)

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Abstract

We describe the isolation, development and application of seven microsatellite loci in the eastern wild turkey, *Meleagris gallopavo silvestris*, as well as their amplification and levels of polymorphism in the domestic turkey. The number of alleles per locus ranged from 5 to 15 and average heterozygosity was high for almost all loci. Domestic turkeys showed significantly reduced numbers of alleles per locus and overall heterozygosities when compared to eastern wild turkeys. The high variability in these markers should provide the level of resolution required to continue studies of wild turkey population genetics.

Keywords: domestication, *Meleagris gallopavo*, microsatellite, reintroduction, translocation, wild turkey

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The wild turkey (*Meleagris gallopavo*) was effectively eliminated from almost all of its original range through overhunting and habitat loss by the early 1900s. Extensive efforts to bring this species back from the brink of extinction have primarily involved translocations of thousands of birds throughout the United States and a multitude of different stocking strategies. The success of these translocation efforts is unparalleled; numbers of wild turkeys have risen from approximately 30 000 to over 5.6 million within the last 60 years, and today there is a huntable population of wild turkeys in every state except Alaska (Kennamer et al. 1992). Wild turkey hunting has important economic implications, and thus wild turkeys remain heavily managed. As such, any indicators of population viability, particularly those facets that may not be obvious from direct observations, will be valuable not only in directing conservation and management efforts in re-established wild turkey populations, but also in identifying healthy source populations that could be used for future translocations. One measure of cryptic population viability is genetic variability. Populations that retain large amounts of genetic diversity are thought to have a better chance of survival over a given period of time; hence, levels of genetic variability within and among re-established wild turkey populations can be used to design optimal management strategies (Frankham 1995).

Genomic DNA from a male wild turkey blood sample was extracted using standard phenol : chloroform techniques (Sambrook and Russell 2001) and was sonicated to create fragments, which were then cloned into the pJCP-1 cloning vector. This genomic library was enriched for TG, GAT and CCT repeats following the procedures outlined in Polido & Duyk (1994). Nine hundred and twenty-four independent clones were sequenced at the Iowa State University Nucleic Acids Facility using an ABI 373 sequencer. Primers were designed for 102 clones containing tandem repeats of a length longer than four using the program primer version 0.5 (Daly et al. 1991) to amplify segments from 100 to 400 base pairs in length. We attempted amplification in a small sample (*n* = 12) of the eastern wild turkey (*M. g. silvestris*). Initial polymerase chain reactions (PCRs) were performed using 10 µL reactions: 10 ng DNA (extracted using standard phenol : chloroform techniques; Sambrook and Russell 2001), 0.2 mM of each dNTP, 0.5 µM forward and reverse primers, and 0.65 units *Taq* DNA polymerase (Promega) in PCR buffer containing 1.5 mM MgCl₂ (Promega). Amplifications were performed in a PTC-225 Gradient Cycler (MJ Research) and the reaction profile was 95 °C for 2 min, followed by 30 cycles of 95 °C
for 30 s, $T_a$ °C (annealing temperature) for 30 s and 72 °C for 30 s, then 72 °C for 10 min (Table 1). PCR products were visualized on 1% agarose gels stained with ethidium bromide.

Seventy of the 102 loci amplified in the expected size range in the eastern wild turkey. These 70 loci were screened on an ABI Prism 377-XL DNA Analyser (Applied Biosystems) to assess polymorphism. Reactions mimicked initial conditions with the addition of 1 µM fluorescent-labelled nucleotide (Applied Biosystems) per reaction. Seventy of the 70 loci (10%) were polymorphic in the eastern wild turkey, which was quite surprising given the high degree of relatedness between wild and domestic turkeys. These seven loci were surveyed for levels of polymorphism in 12 individuals from each of three eastern wild turkey populations and in 12 domestic turkeys. PCR amplifications were performed as described above, but fluorescent-labelled primers were used in place of incorporated fluorescent-labelled nucleotides (Table 1). Alleles were sized using an internal lane size standard. We used GENETIC DATA ANALYSIS version 1.0 d15 (Lewis & Zaykin 1999) to calculate observed ($H_o$) and expected ($H_e$) heterozygosities for both wild and domestic turkeys.

All seven loci exhibited substantial polymorphism (5–15 alleles per locus) and high levels of heterozygosity, except locus WT77-2 (Table 1). Observed heterozygosities were less than expected at almost all loci (Table 1). This is not surprising, given that our sample set included several different populations in order to increase the probability of identifying polymorphic loci. Domestic turkeys exhibited fewer alleles per locus than wild turkeys at all seven loci and slightly lower multilocus heterozygosity ($H = 0.523$ wild, $H = 0.417$ domestic; Table 1). Despite reduced variability in domestic turkey populations, the overall levels of multilocus heterozygosity in turkeys based on microsatellite loci ($H = 0.520$) were approximately tenfold higher than those based on allozymes (0.052 in Boone & Rhodes 1996; 0.067 in Rhodes et al. 1995). These new microsatellite loci provide a level of resolution that makes them valuable for any population genetic application, and should provide sufficient variability to assess the genetic consequences of wild turkey translocation programmes.

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References
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