PATTERNS OF GENETIC DIVERSITY IN BLACK BEARS (*URSUS AMERICANUS*) DURING A RANGE EXPANSION INTO OKLAHOMA

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CHAPTER I

PATTERNS OF GENETIC DIVERSITY IN BLACK BEARS (*URSUS AMERICANUS*) DURING A RANGE EXPANSION INTO OKLAHOMA

Abstract

By the early 1900s, black bears (*Ursus americanus*) were extirpated in Oklahoma and nearly extirpated in Arkansas. The translocation of 254 individuals from Minnesota and Canada in 1958–1968 led to the restoration of black bears in Arkansas by the mid-1990s. Black bears are currently dispersing west across ridges of the Ouachita Mountains from Arkansas into areas in southeastern Oklahoma devoid of bears since 1915. It is rare that quality habitat is within dispersal range of a large population of bears due to increasing loss of suitable habitat. Natural recolonization during range expansion is valuable as a potential tool for management of wildlife populations in the absence of adequate funding and public support. Genetic monitoring of this event provides an opportunity to examine the unique characteristics of expanding populations. We collected hair samples noninvasively from 128 hair snares set at a frequency of 1 trap per 23 km^2 across the 3,420 km^2 study area. We collected 1,166 hair samples during 24 weeks in June–August 2004, 2005, and 2006. Of the 498 (42.7%) samples from which we were able to extract DNA, 332 (66.7%) were sexed and genotyped at ≥ 7 of 10 microsatellite loci. Of those, 161 (48.5%) were unique individuals with a male-biased sex ratio of 1.7:1, and 52 (32.3%) were recaptures. The remaining 119 (35.8%) were duplicate samples. Genetic diversity in this population (H_E = 0.82) was similar to its source population in Arkansas (~0.75) and other large black bear populations (~0.79). No negative effects from inbreeding or reduced population size were evident, and maintenance of gene flow from

the source population in Arkansas should preserve high levels of genetic diversity. Hardy-Weinberg and linkage disequilibrium in the sample suggested recent admixture of dispersing bears with individuals representing other gene pools. Bears were aggregated in the southeastern portion of the study area close to the source population and colonized a larger portion of the southern (Kiamichi) mountain ridge than the northern (Ouachita) mountain ridge. Incorporation of genetic and demographic data is critical to an understanding of population status and development of a successful management plan for this large carnivore species.

Keywords: black bear, microsatellites, noninvasive DNA, Oklahoma, population genetics, range expansion, recolonization, *Ursus americanus*

Introduction

Due to anthropogenic forces such as habitat fragmentation and unregulated hunting, many large carnivores in North America experienced local extirpation during the latter half of the 19th century. For example, black bears (*Ursus americanus*) now inhabit approximately 30% of their historic range (Laliberte & Ripple 2004). More specifically, black bears in the southeastern U.S. exist in small, geographically isolated populations (Schoen 1990; Servheen 1990; Freedman et al. 2003), occupying only 5–10% of their historic range (Dobey et al. 2005), but they are slowly expanding into areas of their former range. Recolonizations by large carnivores are rare (Forbes & Boyd 1996; Hellgren et al. 2005), and their genetic and demographic ramifications are not well understood (Vernesi et al. 2003; Excoffier 2004). Recolonizing populations often possess unique genetic characteristics relative to permanent populations (Forbes & Boyd 1997). For example, admixture of remnant and introduced individuals may provide a

source of novel genetic variation to existing populations (Williams et al. 2002), metapopulations (Gaggiotti et al. 2004), or species (Williams et al. 2002). However, in many cases, recolonization events lead to heterozygote deficiency and reduced allelic diversity because they result from founder events that may profoundly affect genetic characteristics for hundreds of generations (Nichols & Hewitt 1994). Because levels of heterozygosity are hypothesized to have direct consequences for individual and population fitness (Mitton & Grant 1984; Allendorf & Leary 1986; Frankham et al. 2002; Hansson and Westerberg 2002) and population sustainability (Hansson and Westerberg 2002), genetic information can be useful to wildlife biologists working to successfully restore native carnivore populations to previously occupied ranges.

Field-based evidence (Skeen 1997a; 1997b; Bales et al. 2005) indicates that black bears are expanding their range westward from Arkansas into areas of southeastern Oklahoma devoid of bears since the early 1900s (Tyler 1989). This range expansion is the result of an aggressive reintroduction effort initiated by the Arkansas Game and Fish Commission in response to the near extirpation of black bears from Arkansas by the 1940s (Smith & Clark 1994). The Arkansas population rapidly grew to > 2,500 individuals (Smith & Clark 1994) and began westward expansion into areas of southeastern Oklahoma by the late 1980s (Skeen 1997a; 1997b; Smith et al. 1990; Bales et al. 2005). The Ouachita Mountains in southeastern Oklahoma run in an east-west direction, and bears likely disperse west across corridors provided by mountain ridges (as in Virginia, Lee & Vaughan 2003), because roads and farms serve as barriers to north-south dispersal in this area (Clark & Smith 1994).

Long-distance dispersal promotes genetic subdivision in expanding populations via small colonization events and may result in admixture of genetically distinct individuals (Nichols & Hewitt 1994). Long-distance dispersers of both sexes can promote gene flow on the periphery of an expanding population's range. Range expansions also may occur via founder events (Excoffier 2004) or via slow range expansion involving an expansion front of several individuals (Swenson et al. 1998). As a consequence, clusters of individuals ahead of the expansion front may be characterized by altered genetic characteristics (Ibrahim et al. 1996), much like the colonization pattern observed in the gray wolf (*Canis lupis*; Valière et al. 2003). Brown bears (*U. arctos*) in Sweden are exhibiting the latter type of range expansion (Swenson et al. 1998).

Dispersal in black bears is male-biased (Bunnell & Tait 1981; Rogers 1987a; Lee & Vaughan 2003; Schwartz & Franzmann 1992), and promotes gene flow (Avise 1995; Dixon et al. 2006) and connectivity (Dixon et al. 2006) via long-distance dispersal (Rogers 1987a). In contrast, female black bears rarely disperse (Rogers 1987a; Schwartz & Franzmann 1992), expanding slowly via the establishment of subadult female home ranges adjacent to their mother's home range (Rogers 1987b), but are capable of long-distance movements (Hellgren et al. 2005).

Advancements in PCR technology now permit genetic analyses to be performed on noninvasive hair samples that may possess only picogram amounts of DNA (Morin et al. 2001; Durnin et al. 2007), and population abundance of bears has been successfully estimated in several studies via noninvasive analysis (Taberlet et al. 1997; Mowat & Strobeck 2000; Poole et al. 2001; Bittner et al. 2002; Boulanger et al. 2002; Boerson et al. 2003; Apps et al. 2004; Lorenzini et al. 2004; Romain-Bondi et al. 2004; Triant et al.

2004; Belant et al. 2005; Solberg et al. 2006). Noninvasive sampling prevents injury to handlers, reduces trauma to animals associated with live-trapping (Waits & Leberg 1999) and results in larger and more even sampling and thus more accurate information about a population (Mace et al. 1994; Banks et al. 2003; Beier et al. 2005; Zhan et al. 2006). Additionally, genetic marks are advantageous over traditional marks, such as eartags, because they are permanent (Palsbøll et al. 1997; Waits & Leberg 1999; 2000; Eggert et al. 2003; Beier et al. 2005).

However, noninvasive sampling is not without its potential problems. Lower concentrations of DNA present in samples may magnify genotyping errors such as allelic dropout (the most common and problematic error; Smith et al. 2000; Miller et al. 2002; Frantz et al. 2003; Sefc et al. 2003; Flagstad et al. 2004; McKelvey & Schwartz 2004a; Roon et al. 2005) or false alleles (Callen et al. 1993; Foucault et al. 1996; Taberlet et al. 1996; Goossens et al. 1998; Taberlet & Luikart 1999; Morin et al. 2001; Miller et al. 2002) that can result in overestimation of population abundance (Mills et al. 2000; Waits & Leberg 2000; Creel et al. 2003; McKelvey & Schwartz 2004a). For example, errors such as allelic dropout or amplification failure can lead to overestimation of the population if the same individual is counted ≥ 1 time (Paetkau 2004). Studies using noninvasive sampling of hair have reported allelic dropout rates from 0% to 31.3% (Valiére et al. 2007).

We used recent advances in PCR technology to characterize a black bear population that is expanding into areas of its former range. Specifically, our objectives were to: 1) genotype individuals using nuclear microsatellite and sexing loci to provide a minimum estimate of unique genotypes; 2) elucidate genetic characteristics (e.g., levels

of diversity, relatedness, and population substructure) of black bears in southeastern Oklahoma; and 3) use these data to provide information to wildlife managers regarding the status of black bears currently recolonizing Oklahoma.

Methods

Study Area. This study was conducted in southeastern Oklahoma in approximately 3,420 km^2 that included the Ouachita National Forest (ONF) and the Ouachita and Kiamichi Mountains and spanned 3 counties: Latimer, LeFlore, and Pushmataha (Fig. 1). This area contained mountain ridges oriented in an east-west direction, resulting in different vegetation communities on north-facing slopes relative to south-facing slopes (Clark et al. 1987). Overall, the area was characterized as pine forest (Clark et al. 1994), with oak species (*Quercus alba, Q. rubra,* and *Q. velutina*) dominating north-facing slopes and shortleaf pine (*Pinus echinata*) and blackjack oak (*Q. marilandica*) dominating south-facing slopes (Clark et al. 1998). East-west mountain ridges were separated by valleys, with elevations of 300–800 m (Clark et al. 1987).

Hair Capture. Hair trapping occurred for 24 weeks in June–August 2004, 2005, and 2006. Permanent baited barbed-wire hair traps (Woods et al. 1999; Bittner et al. 2002; Boerson et al. 2003; Belant et al. 2005) were set at a frequency of approximately 1 trap/23 km^2 (n = 128) by dividing the study area into a grid of squares measuring 4.8 km x 4.8 km (Fig. 1). Female home ranges, conservatively estimated at 21 km^2 (95% adaptive kernel; Bales et al. 2005), indicated that 0.91 traps were located within each female bear home range. UTM coordinates were recorded for each site using a handheld GPS unit (GPS 12; Garmin International Incorporated, Olathe, KS). Hairtraps were designed using the Woods et al. (1999) model. Hair samples with attached follicles were

collected once every 7 to 10 days to prevent possible loss of samples related to weather or other unpredictable factors (Foran et al. 1997; Taberlet et al. 1999; McKelvey & Schwartz 2004a; Buchan et al. 2005). Hair samples were removed from barbed wire with tweezers, sealed in an envelope, marked with the date, time, site of capture, and barb number, and subsequently stored at -20° C until extraction of DNA (Mowat & Strobeck 2000) (within 1 to 6 months; Roon et al. 2003). Barbs were subjected to flame to prevent contamination of future samples.

DNA Extraction from Hair Samples. DNA was extracted from hair samples via the Chelex® forensic method (Walsh et al. 1991) in a room separate from tissue extraction or PCR amplification (Taberlet & Luikart 1999; Fernando et al. 2003; Frantz et al. 2003; Bonin et al. 2004; Waits & Paetkau 2005). Hairs were handled with forceps and examined under a dissecting microscope for intact follicles. All equipment contacting hair samples was washed between each sample with 95% ethanol and rinsed with distilled deionized water (Gagneux et al. 1997). Depending on the quality of the sample, 5 to 14 follicles, preferably from guard hairs, were selected to increase DNA concentration (Gagneux et al. 1997; Goossens et al. 1998; Taberlet et al. 1999; Poole et al. 2001; Goossens et al. 2002; Triant et al. 2004) and aid in successful amplification (Bittner et al. 2002). Hairs were rinsed briefly in distilled deionized water to remove dirt and other contaminants. The follicle end of the hair shaft was cut as close as possible to the follicle to avoid the PCR-inhibiting nature of melanin (Gagneux et al. 1997; Taberlet et al. 1999) and placed in 200 µl of a 5% Chelex® (BioRad, Hercules, CA) solution that was incubated overnight at 56° C. Samples were vortexed for 10 sec, incubated for 8 min at 100° C in a heat block, revortexed for 10 sec, and subsequently spun in a

microcentrifuge at 13,000 rpms for 3 min. A negative control, absent of follicles, was included to evaluate if contamination occurred during DNA extraction (Taberlet et al. 1999; Bonin et al. 2004; Broquet & Petit 2004; Waits & Paetkau 2005).

Identification of Individual Genotypes. Individuals were genotyped for 10 $(GT)_n$ dinucleotide microsatellite loci. Primers for 7 of those loci (G1A, G10B, G10C, G1D, G10L, G10M, and G10X) were developed from black bears (Paetkau & Strobeck 1994) and 2 loci (G10J and G10O) from brown bears (*U. arctos*; Paetkau et al. 1998). Primers for CXX20 were developed from the domestic dog (*C. familiaris*; Ostrander et al. 1993). Fluorescent labeling of primers allowed detection and sizing of microsatellite loci on a Perkin-Elmer ABI 3100 Automated DNA Sequencer (Foster City, CA).

PCR amplifications occurred in an MJ Research, Inc. PTC 100® Programmable Thermo Cycler (Bio-Rad Laboratories Inc., Waltham, MA). Reactions were 15 µl and contained 2.5mM $MgCl_2$, 0.1 mM primer pair, 0.5 units Amplitaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA), 0.33 mM dNTPs, and 6.0 µl of the Chelex extraction. DNA was vortexed for 10 sec and then centrifuged for 3 min at 13,000 rpms prior to addition to the PCR (Sloane et al. 2000). The thermal profile was: 95° C for 10 min; 45 cycles of 95° C for 30 sec, 46 ° C (G10J), 49° C (CXX20, G10M, G10O), 50° C (G10X) or 57.5° C (G1A, G10B, G10C, G1D, and G10L) for 30 sec, 72° C for 40 sec; 72° C for 10 min; and 4° C for 2 min. Negative controls were included in PCR amplifications and fragment analyses to monitor contamination (Taberlet et al. 1999; Bonin et al. 2004: Waits & Paetkau 2005; Adams & Waits 2007). To 9.5 µl loading buffer (0.5 µl of GS-400HD ROX size standard and 9.0 µl of formamide), 0.5 µl of the PCR product was added. Individuals were genotyped at each locus using GENESCAN™

software. Alleles were assigned by hand to reduce error (Bonin et al. 2004) using GENOTYPER™ software. If the initial PCR amplification produced no product or a product that could not be scored with confidence, samples were rerun with a 3.0° C reduction in annealing temperature and a 3.0 µl (50%) increase in template (Paetkau 2003).

Sexing of Individuals. Sex was determined via PCR amplification of the amelogenin locus using primers for the SE47 and SE48 loci (Ennis & Gallager 1994). The SE47 primer was labeled fluorescently to allow detection and sizing of amplified products. Reactions of 20 µl contained 1.4 mM $MgCl_2$, 1.6 units Amplitaq Gold® DNA polymerase, 0.2 mM dNTPs, and 2.0 µl of the Chelex® extraction. The thermal profile was: 97° C for 3 min; 35 cycles of 94° C for 60 sec, 60° C for 60 sec, 72° C for 60 sec; and 72° C for 10 min. To 9.5 µl loading buffer (0.5 µl of GS-400HD ROX size standard and 9.0 µl of formamide) was added 0.5 µl of the PCR product. Individuals were genotyped at each locus using GENESCAN™ software. Product sizes were viewed using GENOTYPER™ software and were 186 base pairs for males and 242 base pairs for females.

Genotyping Errors. After initial amplification and 2 repeat amplifications of 10 microsatellite loci and the amelogenin locus, all samples that failed to amplify at ≥ 7 loci (i.e., problematic samples) and at the sexing loci were removed from further analyses (Paetkau 2003; Bonin et al. 2004). We used GENECAP 1.1 software (Wilberg & Dreher 2004) to examine genotypes differing at between 1–3 loci (Paetkau 2004). Mismatch curves were produced: 1) all amplified genotypes; 2) post-culling genotypes; and 3) the final dataset of unique genotypes (Paetkau 2003; 2004). Additional tests (the examining

bimodality (EB) and difference in capture history (DCH) tests) were conducted using DROPOUT software (McKelvey & Schwartz 2004a; 2004b; 2005) to determine the overall effect of genotyping errors on the elucidation of unique genotypes. Both tests were run for 3 datasets: 1) all amplified genotypes; 2) all useable genotypes; and 3) all unique genotypes.

Probability of Identity (PI, Paetkau & Strobeck 1994), and PI*sibs* (Evett & Weir 1998; Taberlet & Luikart 1999; Waits et al. 2001), a more conservative estimate of PI incorporating the likelihood that 2 individuals visiting the same trap may be related (Donnelly 1995; Taberlet & Luikart 1999; Waits et al. 2001), were calculated using GENECAP 1.1 software (Wilberg & Dreher 2004). Locus-specific PI*sibs* was estimated using GENECAP 1.1 (Wilberg & Dreher 2004). Use of few (6 to 10; Waits 1999), highly polymorphic loci (those with the lowest PI calculation) provides lowest levels of error while retaining power to identify unique individuals (Taberlet & Luikart 1999; Waits & Leberg 2000; Waits et al. 2001). Following recommendations by several studies to screen all individuals at a single, robust locus, individuals not amplifying at the locus with the lowest PI*sibs* were culled from the dataset (Sloane et al. 2000; Paetkau 2003; Scandura et al. 2006).

The conditional probability that siblings will have the same genotype at > 4 loci (P*sib*; Woods et al. 1999) was calculated with the computer program GENECAP 1.1 (Wilberg & Dreher 2004). Similar to other noninvasive studies, the threshold of P*sib* < 0.05 was accepted as an indicator of unique genotypes (Woods et al. 1999; Mowat & Strobeck 2000; Boulanger & McLellan 2001; Poole et al. 2001; Mowat & Paetkau

2002; Boerson et al. 2003; Mowat et al. 2005) because mean litter size in our study area was previously estimated as 2 individuals (Bales et al. 2005).

Each locus was evaluated for the presence of null alleles, scoring errors, and allelic dropout using MICRO-CHECKER 2.2.3 software (Van Oosterhout et al. 2004; 2005). Estimation of error rate resulted from comparison between multiple genotypes of duplicated samples and recaptured individuals to calculate a per locus and an overall genotyping error rate for the study (Palsbøll et al. 1997; Björklund 2005; Hoffman & Amos 2005). To produce an unbiased estimation of observed heterozygosity, loci with high null allele frequencies were adjusted by removing 1 allele of homozygous individuals (Lebas 2001) identified by MICRO-CHECKER 2.2.3 software (Van Oosterhout et al. 2004; 2005).

Human error (scoring, data input errors, etc.) can be a primary source of error in studies such as this (Fernando et al. 2003; Bonin et al. 2004; Hoffman & Amos 2005), but it can be reduced by adequate training of personnel and strict guidelines for scoring (Fernando et al. 2003). MICRO-CHECKER 2.2.3 software (Van Oosterhout et al. 2004) examined loci that possessed lower than expected heterozygosity for scoring errors involving allelic stutter (Smith et al. 1995; Shinde et al. 2003), which is often a factor in scoring errors (Hoffman & Amos 2005). Repeated scoring of alleles produced familiarity with specific patterns within loci, and all loci were rescored a minimum of 3 times to ensure that scoring error associated with initial training was not a factor.

Elucidation of Unique Genotypes. We genotyped individuals using 10 highly variable loci (Taberlet et al. 1997; Ernest et al. 2000). Pairwise comparisons of genotypes were performed using Microsoft Access (Redmond, WA), and 3 lists were

produced: 1) samples that matched at ≥ 7 loci; 2) samples that matched at 6 loci plus 1 locus that did not amplify; and 3) samples that matched at 5 loci plus 2 loci that did not amplify. Those matching were compared for sex, sample collection location, and date. Sexing of pairwise identical genotypes with different sexes was performed in duplicate to further reduce error (Hung et al. 2004), although amplification of the Y chromosome amelogenin locus using noninvasive samples was nearly error-free (Bradley et al. 2001; Durnin et al. 2007).

Genotypes that represented identical individuals were input into GIMLET 1.0.1 (Valiére 2002) to produce consensus genotypes. Duplicate samples (more than 1 sample from the same individual collected from the same trap during the same sampling period) were eliminated, and remaining pairs of identical genotypes were deemed recaptures. A minimum census size of unique number of genotypes was identified in the study (Pallsbøll et al. 1997; Roon et al. 2005). A Chi-square analysis was performed to determine if the sex ratio differed from 1:1.

Analysis of Population Genetic Characteristics. Genetic diversity was measured for unique genotypes by calculating total and mean number of alleles per locus, expected (H_E) and observed (H_O) heterozygosity, conformity of each locus to Hardy-Weinberg equilibrium (Guo and Thompson 1992), and testing each locus for linkage disequilibrium, in ARLEQUIN 2.0 software (Schneider et al. 2000) for all unique genotypes. A sequential Bonferroni correction was performed on all tests of Hardy-Weinberg equilibrium (Rice 1989) to determine significant differences between H_O and H_E at each locus and tests of linkage equilibrium to determine significantly linked loci at $p < 0.05$.

The pairwise relatedness coefficient (*R*; Queller & Goodnight 1989) was estimated using SPAGeDi (Hardy & Vekemans 2002). We tested for population bottlenecks using the results of the Wilcoxon Test 2-Phase Model (Barker 2005) in BOTTLENECK 1.2 software (Cornuet & Luikart 1996; Piry et al. 1999). Data input files for the programs ARLEQUIN 2.0, GIMLET 1.0.1, and BOTTLENECK 1.2 were created using CONVERT 1.3 software (Glaubitz 2004). All analyses were performed on datasets corrected for null alleles.

Analysis of Population Genetic Structure. Because the study area was characterized by 2 mountain ridges separated by a valley, we hypothesized that cryptic population structure based on geographic barriers to gene flow existed in the sample. An analysis of molecular variance (AMOVA, Cockerham & Weir 1993; Weir 1996) was performed to test for differences between individuals trapped on the Ouachita mountain ridge versus the Kiamichi mountain ridge using ARLEQUIN 2.0 software (Schneider et al. 2000).

We used several Bayesian approaches to explore population subdivision by using existing allele frequency data to calculate prior distributions of allele frequencies (Ellison 1996; Weir 1996). Preliminary exploration of cryptic population structure was evaluated using the program STRUCTURE 2.1 (Pritchard et al. 2000; Falush et al. 2003). This program assigns genotypes to *K* numbers of clusters based on allele frequencies (Pritchard et al. 2000). Although we hypothesized that $K = 2$ based upon the 2 mountain ridges in our study area, we evaluated results for $K = 1$ to $K = 10$ with 10 repetitions, a burnin period of 50,000, and MCMC lengths of 100,000 using the admixture model (Falush et al. 2003) to produce an unbiased estimate of *K*. Runs were conducted with no

prior population delineation and population delineation defined by the mountain ridge where individuals were captured. The ΔK statistic (Evanno et al. 2005) was used to determine number of clusters in the sample. Separate runs using the same parameters were also conducted for males and females. Data input files for the program STRUCTURE 2.1 were created using CONVERT 1.3 software (Glaubitz 2004).

A Markov Chain Monte Carlo (MCMC) algorithm approach was employed by GENELAND 1.0.8 (Guillot et al. 2005a; 2005b) and R 2.4.1 software (Ihaka and Gentleman 1996) to detect genetic discontinuities along the study-area landscape by incorporating specific geographic coordinates of each genotype into the analysis. The MCMC analysis was run 5 times without *a priori* knowledge of population subdivision. The MCMC algorithm was run for 100,000 iterations with no uncertainty of geographic coordinates, minimum $K = 1$, maximum $K = 10$, using the Dirichlet distribution model of independent allele frequencies (Guillot et al. 2005a; 2005b). A second MCMC algorithm was run 10 times for 100,000 iterations with a fixed number of populations that equaled the mode found for the previous 5 runs using the Dirichlet distribution model of independent allele frequencies. MCMC algorithms using the same protocols were also run for males and females separately.

A third Bayesian analysis was conducted using TESS 1.0.1 that also incorporates geographical information into clustering methods by using hidden Markov random fields (HMRFs) to examine allele frequencies across a geographical scale (Francois et al. 2006). Analyses were run for all individuals and for females and males only. For the sample with all individuals, we evaluated results for K_{max} from 1 to 10 with 10 repetitions of the HMRF with no prior population information, and $\psi = 0.9$. For the sample of females and

males only, we set $K_{max} = 5$ with 10 repetitions and $\psi = 0.9$. All analyses were run with 30,000 sweeps and a burnin period of 20,000.

Further investigation of gene flow was evaluated using the protocol outlined in Frantz et al. (2006). Matrices were created using unique genotypes in SPAGeDi 1.2 (Hardy & Vekemans 2002): 1) a matrix of pairwise kinship coefficients (Loiselle et al. 1995) and 2) a matrix of the natural log of pairwise Euclidean distances. Additionally, binary matrices were created by allocating 0 values to individuals sharing sex or mountain ridge, respectively, and values of 1 to pairs of individuals of opposite sex or different mountain ridge to test for demographic or physical effects on gene flow in this sample (Frantz et al. 2006). Simple and partial Mantel tests were conducted on all possible combinations of matrices using program ZT (Bonnet & Van de Peer 2002).

To test for isolation by distance, we examined the relationship between the pairwise kinship coefficient and the natural log of pairwise Euclidean distance. Distance classes were chosen to provide equal sample sizes between classes (Aspi et al. 2006). Mean kinship coefficients for each distance class were compared with the overall population mean to determine significant differences at each distance class (Aspi et al. 2006) and for males and females. To examine spatial patterns based upon allele frequencies (Manel et al. 2003), a principal components analysis was conducted on individual genotypes using PCA-GEN 1.2 software (http://www2.unil.ch/popgen/softwares/pcagen.htm) for the entire sample, females only, and males only.

Results

Hair Capture, Extraction, and Amplification. There were 2,766 sampling opportunities for hair capture (99 traps x 9 sampling sessions [2004], 125 traps x 7 sampling sessions [2005], and 125 traps x 8 sampling sessions [2006]). We collected 1,166 hair samples from 94 different traps during the study; 314 (26.9%) were collected from 59 of 99 traps (59.6%) in June–August 2004, 336 (28.8%) were collected from 60 of 125 traps (48%) in June–August 2005, and 516 (44.3%) were collected from 80 of 125 traps (64%) in June–August 2006. Of the 1,166 collected samples, 668 (57.3%) excluded samples possessed ≤ 4 follicles, and 498 (42.7%) produced DNA. A total of 166 (33.3%) culled samples failed to meet controls established for the study. Of the remaining 332 (66.7%) extracted samples, 130 (39.2%) amplified at all 10 microsatellite loci and the sexing loci, 116 samples (34.9%) amplified at 9 loci and the sexing loci, 53 samples (16%) amplified at 8 loci and the sexing loci, and 33 samples (9.9%) amplified at 7 loci and the sexing loci.

Estimation of Error Rate. Examination of 1-MM through 4-MM pairs produced 3 mismatch curves (Fig. 2). Analyses in DROPOUT software (Schwartz et al. 2006) indicated error in the dataset for all amplified genotypes (n = 464) and all useable genotypes (n = 237), but not for the dataset containing all unique genotypes (n = 161) (Fig. 3). When examining all 3 datasets, the DCH test indicated no significant addition of individuals to the sample as loci were added, an indicator that error rates were equal at all loci.

Calculation of PI*sibs* revealed that the minimum number of loci used to elucidate individual genotypes was 7 (Fig. 4). At 7 loci, this study had a PI*sibs* < 0.0001 and a

PI = 2.42 X 10^{-10}. Single-locus PI*sibs* for the 7 loci were 0.33–0.36 (Table 1). A sufficiently low PI (PI < 0.0001–0.001; Waits et al. 2001) indicates that markers used are powerful enough to elucidate unique genotypes. To eliminate addition of erroneous duplicate genotypes, the result of poor-quality samples (Mills et al. 2000), the 3 loci with the highest PI*sibs* calculation (G1A, G10B, and G10O) were removed from the final dataset (Waits et al. 2001). Individuals not amplifying at the 2 loci with the lowest PI*sibs* (G10J (0.314) and G10X (0.315)) were culled from the dataset because the PI*sibs* statistics of the 2 loci were not different from each other. All unique genotypes possessed P*sib* < 0.004.

Null alleles were found at 4 loci (G1D, G10J, G10L, and G10X) and no incidence of false alleles or allelic dropout was detected by MICRO-CHECKER 2.2.3 software (Van Oosterhout et al. 2004; 2005). Lack of adjacent-allele heterozygotes, an indicator of scoring error due to stuttering, was found at 1 locus (G10L). Per locus error rates calculated using pairwise comparisons of all duplicated (e.g., both duplicate samples and recaptures) genotypes were 1.7% for allelic dropout, 0.9% for stutter, and 0.3% for false alleles, resulting in an overall per locus error rate of 2.9% for the study (n = 148).

Population Characteristics. To ensure that the sexing protocol was appropriate when applied to black bears, we correctly determined sex for 36 hair samples collected from captured individuals of known sex. Of 275 useable genotypes, 161 (58.6%) were unique genotypes. Of 114 identical genotypes, 52 (45.6%) were recaptures and 62 (54.4%) were duplicate samples. Unique genotypes captured each season were: 1) 74 (46%) during summer 2004 (44 M: 30 F; 4 recaptures) captured at 32 (32.3%) of 99 traps; 2) 58 (36%) during summer 2005 (39 M: 19 F; 22 recaptures) captured at 33

(26.4%) of 125 traps; and 3) 29 (18%) during summer 2006 (19M: 10 F; 26 recaptures) captured at 26 (20%) of 125 traps. The 161 unique genotypes were represented by a male-biased sex ratio of 1.7:1 ($\chi^2 = 11.48$; df = 1; $p < 0.005$; 102 M: 59 F). Of those, 100 (62.1%) were captured initially on the Kiamichi mountain ridge and possessed a male-biased sex ratio of 1.6:1 ($\chi^2 = 5.76$; df = 1; $p < 0.025$; 62 M: 38 F), whereas the remaining 61 unique genotypes (37.9%) initially captured on the Ouachita mountain ridge possessed a male-biased sex ratio of 2.9:1 ($\chi^2 = 12.52$; df = 1; $p < 0.005$; 38 M: 14 F).

Population Genetic Characteristics. To minimize errors in assignment, all subsequent genetic analyses were conducted using 161 unique genotypes and the 7 most powerful loci (Table 1; Fig. 4) corrected for null alleles. Mean observed heterozygosity (H_O) (± SD) was 0.81 (± 0.039) and ranged from 0.766 to 0.868, and mean expected heterozygosity (H_E) (± SD) was 0.82 (± 0.022) and ranged from 0.78 to 0.843 (Table 1). Allelic diversity (A) was 11.3 ± 1.8 alleles per locus, with a range of 9 to 15 alleles per locus, with 79 total alleles in the sample (Table 1). Using an adjusted $\alpha = 0.007$ as a cutoff for significance, H_O was less than H_E for 3 loci (G10C, G1D, and G10L), greater than expected for 2 loci (G10M and G10X), and as expected for 2 loci (CXX20 and G10J) (Table 1). The overall sample was in Hardy-Weinberg equilibrium ($\chi^2 = 0.007$; df = 8; $p > 0.05$). At the adjusted $\alpha = 0.002$, linkage disequilibrium was present in 13 of 21 pairs (61.9%) of loci (CXX20, G10C; CXX20, G1D; G10C, G10L; G10J, G10L; G1D, G10J; CXX20, G10M; G10L, G10M; G1D, G10M; CXX20, G10X; G10J, G10M; G10J, G10X; G10L, G10X; and G10M, G10X).

Overall mean pairwise coefficient of relatedness (*R*; Queller & Goodnight 1989) (± 95% CI) for the sample was -0.001 ± 0.004. Mean pairwise *R* (± 95% CI) for females

(-0.018 ± 0.012) was not different than that of males (-0.013 ± 0.007; t_{calc} = 0.628; df = 2823; p = 0.265). The mean ln(pairwise Euclidean distance) (± 95% CI) of females (9.15 ± 2.18; 9.41 ± 0.01 km) was lower than that of males (9.51 ± 1.88; 13.49 ± 0.01 km; t_{calc} = 5.998, df = 2604; p < 0.001). Overall mean ln(pairwise Euclidean distance) (± 95% CI) for the sample was 9.39 ± 0.03 (12.02 ± 0.001 km). Results of partial and simple Mantel tests indicated that bears closer together were more related to each other (r = -0.016; p < 0.001; Fig. 5), and females possessed nearly twice as strong a negative relationship (r = -0.09; p < 0.001) when compared to males (r = -0.04; p < 0.002).

The ln(pairwise Euclidean distance) of bears on the Ouachita mountain ridge (9.03 ± 0.10; 8.37 ± 0.001 km) was larger than that of bears on the Kiamichi mountain ridge (8.8 ± 0.8; 6.63 ± 0.002 km; t_{calc} = 3.898, df = 4402, p < 0.001). Mean pairwise R did not differ between the Ouachita mountain ridge (-0.019 ± 0.011) and the Kiamichi mountain ridge (-0.009 ± 0.006; t_{calc} =1.544, df = 3128, p = 0.061). Recent bottlenecks were detected for the overall sample (p < 0.008), females (p < 0.04), males (p < 0.02), and individuals trapped initially on the Kiamichi mountain ridge (p < 0.004), but not for individuals trapped initially on the Ouachita mountain ridge (p = 0.344).

The mean pairwise kinship coefficient of individuals captured close together (0.011; 0–6.7 km apart) was higher than the population mean (0; t_{calc} = 3.316; df = 1819; p < 0.001), whereas the mean pairwise kinship coefficient of individuals captured 9.2–11.2 km apart (-0.009) was lower than the population mean (t_{calc} = 1.934; df = 1826; p = 0.027), and the mean pairwise kinship coefficient of individuals captured 18.2–37.9 km apart (-0.009) was lower than the population mean (t_{calc} = 2.016; df = 1470; p = 0.022) (Fig. 5). The mean pairwise kinship coefficient of females (0.025;

t_{calc} = 3.062; df = 352; p < 0.001) and males (0.015; t_{calc} = 2.617; df = 558; p < 0.005) caught closer together (0–6.7 km apart) were higher than the overall population mean.

Population Genetic Structure. Results of the AMOVA indicated the highest source of variation (99.49%) was within populations. Calculation of Δ*K* indicated a possible mode at *K* = 2 for the run with no prior population information (Fig. 6). For *K* = 2 populations, mean *q*-intervals for individuals resulted in no definitive population assignments (Manel et al. 2004). Calculated mean *q*-intervals for individuals over the 10 runs at *K* = 3 clusters using mountain ridge of original capture for each individual resulted in 94 of 100 individuals assigned correctly with > 95% Ln PD (X | *K*) to the Kiamichi mountain ridge, with 54 of 61 individuals assigned correctly > 96% Ln PD (X | *K*) to the Ouachita mountain ridge, and 6 remaining unassigned. The remaining 6 individuals were assigned using the leave-one-out procedure in GENECLASS2 software (Piry et al. 2004) with the partial Bayesian approach of Rannala & Mountain (1997) and exclusion probabilities calculated via the method of Paetkau et al. (2004) simulating 1000 genotypes and a threshold of p ≤ 0.001. This analysis produced no definitive assignments for the 6 genotypes. Calculation of Δ*K* for females indicated a possible mode at *K* = 2 clusters, but mean *q*-intervals resulted in no definitive assignment of any genotypes.

Results of GENELAND 1.0.8 indicated a mode at *K* = 1 for the initial runs when number of populations was not set (e.g. *K* = 1 to *K* = 10). Males also possessed a mode at *K* = 1. For females, a mode of *K* = 2 was found in 2 of 5 runs when the initial number of populations was not set (e.g. *K* = 1 to *K* = 5). We then ran the MCMC algorithm an additional 10 times each with a fixed *K* = 2 for the dataset and compared the runs for

similarity. For females, the mean log posterior probability was -1430.78 (range of -1430.5–-1431.1), but no landscape heterogeneity was detected. TESS 1.0.1 indicated $K = 4$ when all individuals were included (Fig. 7(a)), $K = 3$ for females only (Fig. 7(b)), and $K = 4$ for males only (Fig. 7(c)).

Some evidence for barriers to gene flow based upon demographics was found because pairs of individuals on each mountain ridge were more related to each other than to individuals on opposite mountain ridges ($r = 0.015$; $p < 0.05$) and pairs of individuals sharing sex were more related than pairs of individuals of opposite sex ($r = 0.015$; $p < 0.05$). Pairs of females were more related to one another than to males ($r = 0.742$; $p < 0.001$).

The PCA returned a random spatial pattern for the entire sample (37.6% of the variation explained by the first 2 axes), for females only (52.2% of the variation explained by the first 2 axes), and for males only (41.4% of the variation explained by the first 2 axes).

Discussion

Elucidation of Unique Genotypes. Error was minimized by using microsatellites ≤ 205 base pairs in length (Hare et al. 1996; Wattier et al. 1998; Taberlet et al. 1999; Woods et al. 1999; Banks et al. 2002; Palomares et al. 2002; Buchan et al. 2005; Hoffman & Amos 2005; Petit et al. 2005; Scandura et al. 2006; Broquet et al. 2007) and by using only hair samples with ≥ 5 follicles (Miller et al. 2002; Triant et al. 2004). However, errors are nonrandomly distributed among samples and loci (Taberlet et al. 1996; Gagneux et al. 1997; Goossens et al. 1998; Morin et al. 2001; Björklund 2005; Buchan et al. 2005: Pompanon et al. 2005; DeWoody et al. 2006; Broquet et al. 2007;

Hausknecht et al. 2007). For example, Lorenzini et al. (2004) reported an error rate of 23% in the CXX20 locus in an Italian brown bear population. In our study, 2 of 7 loci accounted for 49.9% of the calculated allelic dropout error (CXX20 (28.8%) and G10X (21.8%)), but G10X possessed the 2nd lowest and CXX20 the 4th lowest PI*sibs* calculation (Table 1), and therefore both were deemed necessary to elucidate unique genotypes in this population. The duplicated genotypes (n = 148; unique n = 40) used to estimate error may therefore have represented a nonrandom sample of genotypes (Sloane et al. 2000; Parsons 2001; Lucchini et al. 2002; Hedmark et al. 2004; Scandura 2005; Scandura et al. 2006; Soulsbury et al 2007) that produced a biased estimation of the error rate (Scandura et al. 2006). The majority of errors associated with these duplicated genotypes were excluded from the study by manual determination and inclusion of their consensus genotypes in the study.

Tests in the software DROPOUT and mismatch curves, both of which are highly reliable methods of determining the incidence of duplicate genotypes incorrectly considered unique (Paetkau 2003; 2004; McKelvey & Schwartz 2004a; 2004b; 2005; Schwartz et al. 2006), clearly indicated no such phenomenon. These tests show that our conservative protocols virtually eliminated any significant scoring error. The assumption that genotypes identified as matching at ≥ 7 loci and at the sexing loci were identical provided a conservative means of elucidating unique genotypes (Palsbøll et al. 1997; Paetkau 2003; Hoffman & Amos 2005; Waits & Paetkau 2005).

This study possessed a low false allele error rate; this is not surprising because false alleles are rarely an issue in PCR amplifications using noninvasive samples (Hedmark & Ellegren 2005), and actually may be higher in studies using traditional blood

and tissue samples (Fernando et al. 2003). Use of dinucleotide microsatellites can potentially increase incidence of false alleles because these markers are more prone to slippage during PCR amplification (Goosssens et al. 1998; Fernando et al. 2003). Moreover, use of a large number of highly polymorphic loci may compound error rates (Hoffman & Amos 2005). However, false alleles also likely would contribute to a pattern of overall excess heterozygosity but this was found in only 2 of 7 loci in our study and analyses using MICRO-CHECKER 2.2.3 software (Van Oosterhout et al. 2004) indicated no false alleles when data from all individuals were included in the estimate.

Heterozygote deficiencies found at several loci in the present study also could indicate genotyping error (e.g., Xu et al. 2002). Goossens et al. (1998) used noninvasive samples from Alpine marmots (*Marmota marmota*) to estimate error rates due to allelic dropout, which decreased from 14% when using 1 hair follicle to 0.3% when using 10 hair follicles. However, rates as high as 13% were found when using DNA extracted from blood samples (Jeffery et al. 2001), and Flagstad et al. (1999) found no difference between allelic dropout rates of blood and fecal samples of sheep, indicating that allelic dropout is not a problem unique to noninvasive studies. Ideally, per locus error rates should be ≤ 0.01 (Roon et al. 2005). Errors due to allelic dropout affect only a few loci (Björklund 2005), and a significant heterozygote deficit would be caused only by a large amount of this type of error in our sample (Paetkau 2003). Error estimation involving repeat amplification of 5% of random samples (e.g. Bellemain & Taberlet 2004; Bonin et al. 2004) may have provided a less biased estimation of error rate, but a truly random sampling was impossible in this study due to the limited volume (200 µl) of initial sample.

The male-biased sex ratio is not surprising, considering dispersal of black bears is male-biased and usually involves subadult males that may travel great distances (up to 200 km; Elowe & Dodge 1989) to establish a home range (Rogers 1987a; Schwartz & Franzmann 1992; Beckmann & Berger 2003) and that male-biased sex ratios are common in studies of black bears (e.g., LeCount 1982; Doan-Crider and Hellgren 1996; Beckmann & Berger 2003). Traps set at a frequency of 1 per female home range could also lead to a capture bias skewed toward males (Clarke et al. 2000). Females with cubs may possess lower capture probabilities because cubs limit a female's movement (Boulanger et al. 2004), further compounding the effect. Although a higher density of traps would have been ideal (e.g., Boerson et al. 2003), limited human and material resources precluded this in our study (e.g., Bittner et al. 2002).

Previous genetic studies have revealed capture heterogeneities between samples based on quality and/or quantity of DNA. Environmental conditions, including heat, moisture, and UV radiation, can contribute to degradation of hair samples before collection and extraction (Foran et al. 1997; McKelvey & Schwartz 2004a; Buchan et al. 2005; Ball et al. 2007; Jeffery et al. 2007). Certain individuals may leave hair samples on traps based purely upon biological factors affecting rate of hair loss (Creel et al. 2003) or differences in amount of tissue shed (Lukacs & Burnham 2005b), which may depend on stage of hair growth (Jeffery et al. 2007). Larger individuals may shed larger follicles that result in a higher concentration of DNA (Woods et al. 1999). Individuals also can differ in number of hair samples deposited (Piggott et al. 2006). Finally, differences in sizes and number of hair follicles extracted cause variation in sample DNA concentration (Goossens et al. 1998).

Comparison of genotypes to DNA extracted from ear tissue of 22 known bear captures in 2004–2006 produced only 3 (13.6%) matching individuals, indicating that more bears exist than were sampled in the study area (e.g., Triant et al. 2004). The census size of 161 (102 M:59 F) provides a minimum conservative estimate of unique genotypes (Palsbøll et al. 1997; Roon et al. 2005; Kalinowski et al 2006; Hedmark & Ellegren *In Press*) for the study area, given the strict protocols used and limited duration of sampling. Although overestimation of unique genotypes was a concern, per locus error rates of 2.9% were well below several reported values for studies using hair extraction (0–31.3%, Valiére et al. 2007) and also below the threshold of 5% that is implicated by simulation models to overestimate populations by >10% (Waits 2004).

New methods proposed are specifically designed to provide population estimates from genotypic data elucidated from noninvasive samples (Lukacs & Burnham 2005a; Miller et al. 2005; Petit & Valiére 2006; Solberg et al. 2006). Capture-mark-recapture methods can be applied to these data that are more robust to violations of assumptions of equal catchability because black bear demographic structure and the nature of noninvasive samples violate such assumptions. Because this process is prone to error, we recommend such methods be used in the future to accurately estimate abundance and other demographic parameters of this population (Lukacs & Burnham 2005b). Sound and informed management decisions should be based on reliable estimates of demographic characteristics of the population, including size, age structure, and rate of growth.

Genetic Characteristics of the Population. This expanding population of black bears exhibited high levels of genetic (ours 0.81; ~0.79, Paetkau & Strobeck 1998) and

allelic (ours 11.3; A = 7.8, Paetkau & Strobeck 1998) diversity typical of large, stable black bear populations (Waits 1999; Garner et al. 2005). The source population in Arkansas exhibited a similar level of heterozygosity in 2 independent studies (0.73, Warrillow et al. 2001; 0.75, Csiki et al. 2003). More specifically, for the 2 loci shared by the studies (G10C and G10L), H_E = 0.796 in the source population (Csiki et al. 2003) was comparable to H_E = 0.82 in the Oklahoma sample. Allelic diversity (A) was much lower in the source population (3.6, Csiki et al. 2003) than in the present sample (11.3). Specifically, our sample shared 37.5% of G10C alleles and 27–36.4% of G10L alleles found in the source population (Warrillow et al. 2001; Csiki et al. 2003). This discrepancy could simply be the result of large differences in sample size that caused lower allelic diversity in the Arkansas sample because rare alleles would not be represented in a small sample size, but also can indicate admixture in the sample.

Because Csiki et al. (2003) concluded that populations in Arkansas were derived from source bears in northeastern Minnesota, further investigation of the genetic diversity in that population is warranted. Their study found A = 5.4 and H_E = 0.77 in the Minnesota population, and H_E = 0.65 for the 2 loci shared by the 2 studies (n = 10, Csiki et al. 2003). Warrillow et al. (2001) also found an overall H_E = 0.77 in the Minnesota population (n = 36). Levels of diversity in the present study are therefore similar to both hypothesized source populations. Gray wolves naturally recolonizing areas of Montana and the Italian Alps retained levels of genetic diversity similar to that of their source populations (Forbes & Boyd 1996; Lucchini et al. 2002), which was attributed to high levels of dispersal preventing a bottleneck or founder events (Forbes & Boyd 1996).

No physical linkage exists between loci used in this study (Paetkau & Strobeck 1994, 1995; Paetkau et al. 1999). Therefore, the significant linkage disequilibrium found in 61.9% of pairs of loci and significant heterozygote deficiencies at 42.9% of loci may indicate that inbreeding levels are higher than expected by random. However, large levels of inbreeding likely would produce excess homozygosity at all 7 loci (Triant et al. 2004), and therefore effects of nonrandom mating were not strong in this study. Rather, the observed heterozygote deficiency could be result of undetected null alleles. MICRO-CHECKER calculates null allele frequencies by using only genotypes that are not missing any data, thus only 49.1% of genotypes (n = 79) provided a representative sample that may have been biased toward high-quality samples.

Single or multiple founder events could have occurred in Oklahoma, given patterns of colonization in bear populations that may involve a few solitary individuals establishing home ranges in an area (Smith & Clark 1994; Kojola & Laitala 2000). Founder events can result in genetic bottlenecks that, depending upon number of generations perpetuated, can promote inbreeding depression and genetic drift. Such founder events are supported by the detection of ≥ 1 recent bottleneck, significant linkage disequilibrium (Houlden et al. 1996), and significant heterozygosity excess (Forbes & Boyd 1996) found at 2 loci. This range expansion by a large population of black bears in Arkansas should not be as vulnerable to inbreeding depression and genetic drift as smaller, isolated populations (Frankham et al. 2002). Levels of allelic diversity provide evidence against any recent bottlenecks because allelic diversity is reduced more quickly during such events than levels of heterozygosity (Allendorf 1986; Cornuet & Luikart 1996; Leberg 2002). The L-shaped distribution of allele frequencies found in all tests for

bottlenecks indicates presence of many rare alleles that result from rapid population expansion (DeYoung et al. 2003; Vernesi et al. 2003) or admixture of individuals from different gene pools.

Hardy-Weinberg disequilibrium, linkage disequilibrium (Slatkin 1994; Falush et al. 2003), and high levels of allelic diversity may indicate that the sample contains individuals from > 1 gene pool (Hansen et al. 2001; Lance et al. 2003). This area of range expansion may therefore contain individuals resulting from the admixture of the gene pool of the Arkansas population, which began its rapid expansion into Oklahoma in the 1980s, and bears that established residency in Oklahoma via individual dispersal events believed to have begun sometime in the 1960s (McCarley 1961). Warrillow et al. (2001) concluded that the population of black bears in the Ouachita Mountains of Arkansas was an intergrade population between the American black bear and the Louisiana black bear (*U. a. luteolus*); the source population could therefore represent an admixture of different gene pools of the black bear. Additionally, bears from the northern Arkansas (Ozark) population may have contributed genes to the sample. Male and female black bears are both capable of long-distance dispersal across many habitat types, even a seemingly inhospitable matrix (Beckmann & Lackey 2004; Proctor et al. 2004; Hellgren et al. 2005), and dispersing black bears follow corridors provided by natural landscape features (Lee & Vaughan 2003).

Swenson et al. (1998) found that an expanding brown bear population in Sweden was characterized by a female-biased core area and an expansion front consisting mainly of natal males. Our findings provided support for a female-biased core area because ~53% of females were trapped at approximately 6% of traps (n = 8) located in the

southeastern corner of the study area. These same traps provided ~31% of male captures and 38.5% of total captures for the study. Bales et al. (2005) concluded that a large proportion of the expanding population resided in the southeastern portion of the study area because it represented the highest-quality available habitat in the region.

The Oklahoma population shared several characteristics with Swenson et al.'s (1998) expanding brown bear population in Sweden. For example, the 1:1 sex ratio ($\chi^2 = 0.016$; df = 1; $p < 0.75$; 32 M: 31 F) in the hypothesized core area contrasted with an overall male-biased sex ratio in the sample. Rapidly expanding bear populations are expected to possess a female-biased sex ratio, which would be expected if males were dispersing from natal areas (White et al. 2000); therefore, the southeastern corner of the study area may be transforming to a region possessing a female-biased sex ratio. The Oklahoma population also appears to be rapidly expanding west across the Kiamichi mountain ridge from this area, as indicated by the smaller geographic distance between pairs of individuals on this ridge (6.63 km) than on the Ouachita mountain ridge (8.10 km; $t_{calc} = 4.18$; df=4447; $p < 0.001$). Also, 69% of captures during the study (147 of 213) occurred on the Kiamichi mountain ridge. Finally, the Ouachita mountain ridge possessed a male-biased sex ratio of 2.9:1, nearly double the number of males as that within the sample or those trapped on the Kiamichi mountain ridge, a strong indicator that males existed in a population expansion front.

Fortunately, the present sample showed little evidence of negative effects often associated with range expansions. Spatial distribution could encourage inbreeding (Garnier et al. 2001) in this population where the majority of individuals (~57%) were trapped within 15 km of the Arkansas state line. Black bears in Oklahoma are

characterized by a 14% annual growth rate (Bales et al. 2005), and rapid rates of growth combined with population expansion may reduce effects of genetic drift (Vernesi et al. 2003; Zenger et al. 2003). Polyandry in black bears could increase the effective size of a population (Sugg & Chesser 1994), thus contributing to higher levels of heterozygosity in founding populations (Spencer et al. 2000; Winters & Waser 2003).

Bears, like other large carnivores, are a highly mobile species and therefore should not be sensitive to landscape features (e.g., Geffen et al. 2004). However, isolation by distance may occur as bears disperse west across the study area. Isolation by distance is expected in recolonizing populations that stem from a single source population (Pamilo 2004) and can indicate fine-scale spatial genetic structure (Vekemans & Hardy 2004) of socially distinct groups of individuals (Sacks et al. 2005). In the present study, pairs of males trapped 0–6.8 km apart were more related than random pairs of individuals, whereas related females were often captured at the same traps (data not shown). In addition, at the closest geographic distance class (0–6.8 km apart), 3rd distance class (9.2–11.2 km apart), and 8th distance class (28.2–32.9 km apart), the mean pairwise kinship coefficients of individuals were different from that of individuals in the entire population (Aspi et al. 2006). Isolation by distance also was indicated by Ln Pr (X | K) values reaching an asymptote (Fig. 5; Frantz et al. 2006). These results indicate that related bears of both sexes are spatially aggregated, but pairs of females are closer together (9.90 km) than pairs of males (13.36 km; $t_{calc} = 5.998$; df = 2604; $p < 0.001$).

Former habitats are colonized slowly because successful recolonization of requires resident females (Onorato et al. 2004), and female dispersal is generally a slow

process. Moreover, expanding populations likely exhibit very different patterns of dispersal than stable, continuous populations and may include dispersal of a larger number of females than expected (Swenson et al. 1998; Støen et al. 2006). Gene flow is promoted between areas when related males disperse and unrelated males immigrate (Rogers 1987a; LeCount 1991). Isolation by distance in female black bears is expected to result from female philopatry (Campbell & Strobeck 2006), but is surprising in males, and could indicate a nonrandom spatial pattern. The majority of both female and male movement in this population appears to be incremental as shown by the high kinship coefficients at spatially proximal traps and also because 50% of recaptures occurred at the same trap, with 54% of these within the same season.

Values of relatedness of bears in Oklahoma were near 0, indicating high levels of gene flow from unrelated immigrants into the area (Onorato & Hellgren 2001). Adequate habitat corridors (such as mountain ridges) can promote male immigration from proximal populations to new areas (White et al. 2000; Dixon et al. 2006). In high-density areas, males may disperse shorter distances to lower the possibility of inbreeding and competing with close relatives (Proctor et al. 2004; Støen et al. 2006). Subadult male black bears in Virginia (yearlings to three year olds) moved an average of 11.6 to 15.7 km from their natal ranges, presumably large enough distances to avoid inbreeding and competition with kin (Lee & Vaughan 2003), although the authors suggest 80 km as the upper boundary for the distance of natal male dispersal in the eastern states. However, male movement alone may not prevent differences in allele frequencies between areas (Purdue et al. 2000) and actually can contribute to demographic autonomy when combined with female philopatry (Prout 1981; Chesser 1991; Avise 1995).

Management Implications. Bears in Oklahoma exhibit high levels of genetic diversity, which are hypothesized to help populations persist when changes in the environment are brought about by anthropogenic forces or stochastic events (Frankham et al. 2002; Hansson and Westerberg 2002). Because Arkansas black bears serve as the source population for the individuals currently occupying southeastern Oklahoma (Smith & Clark 1994; Bales et al. 2005), we believe that adequate gene flow to support long-term population viability is not of concern for bears in Oklahoma.

It is important to note that there is a distinct possibility that this study did not sample cubs, and genetic data does not provide information on age structure. Concurrent live trapping in different portions of the study area has taken place and will continue in summer 2007, but ages are yet to be determined for these individuals. Bales et al. (2005) found that a large portion (~40%) of their trapped individuals in southeastern Oklahoma were 1–3 years of age, indicating that many individuals are not yet sexually mature (Rogers 1987b) and therefore are not contributing to the gene pool. Several males and 2 females captured in the western portions of the study area may be subadults incapable of reproduction, but indicate potential for population growth in western areas that would promote expansion into unoccupied bear habitat in the future.

Expansion of Oklahoma black bears throughout former ranges will be limited only by habitat and anthropogenic effects such as land use and human behavior (Pyare et al. 2004). The study area holds the potential to support a higher density of bears, but as bear presence increases in the region, the potential for nuisance activity will increase (Bales et al. 2005). The 2 mountain ridges represent the highest-quality habitat for black bears in the area (Hellgren et al. 1998) but are separated by valleys consisting of private

land, much of which is dedicated to agricultural processes. Suitable habitat is a limiting factor for this geographic region, and therefore increasing density will result in increased contact with humans (Spiker & Bittner 2004) in southeastern Oklahoma. In general, public attitudes are positive when bear populations are low and grow increasingly negative as bear sightings and human-bear interactions increase due to increasing bear density (e.g., Clark et al. 1991; Bowman et al. 2001).

Oklahoma is unique in that 95% of its land is privately-owned, a factor that could exacerbate negative public attitudes toward bears if nuisance activity increases as it has in the southeastern portion of the state (Bales et al. 2005). Fall foods are critical to several aspects of bear population dynamics, including reproduction, and fluctuations in fall food availability will drive bears from traditional habitat areas into areas of human occupancy, increasing nuisance activity (Clark et al. 1987). The potential for nuisance activity to increase could be intensified by the presence of 11 apiaries with > 143 honeybee (*Apis* species) colonies within the counties encompassed by the study area (M. Woods, Department of Agricultural Economics, Oklahoma State University, *personal communication*). Landowners may change their attitude toward bears after a negative experience with a nuisance bear (Clark et al. 1991).

Management practices should therefore promote a reproductively viable black bear population with appropriate densities that are high enough to sustain bear populations yet low enough to ensure limited human-bear conflicts (e.g., cultural carrying capacity; Davidson 1999). Cultural carrying capacity is defined as "the maximum number of bears in an area that is acceptable to the human population" relative to the

conflict between human tolerance versus benefits from bears (Virginia Department of Game and Inland Fisheries 2002).

Our study illustrates the importance of incorporating both genetic and demographic data into an understanding of population status and a successful management plan for a large carnivore species. Genetic data can contribute a deeper understanding of management units (Moritz 1994; Michaux et al. 2004; Allendorf & Luikart 2007; Pallsbøll et al. 2007) and spatial dynamics of a population (Manel et al. 2003; 2004; Scribner et al. 2005). Continued genetic monitoring of this unique recolonization event will permit detection of intensified isolation by distance, which can contribute to differentiation via genetic drift and increased levels of inbreeding (Hardy & Vekemans 1999). In this capacity and others, our genotypes provide permanent genetic marks that can be compared with future data to track individual and population trends and movement.

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Table 1. Levels of genetic diversity at 7 microsatellite loci used to genotype black bears in Oklahoma. Shown are number of individuals genotyped at each locus (*n*), alleles per locus, observed heterozygosity (H_O), and unbiased expected heterozygosity (H_E). *P*-values less than the corrected $\alpha = 0.0071$ indicate that H_O is different from H_E.

Locus	*n*	Alleles	PI*sibs*	H_O	H_E	p-value
CXX20	138	12	0.344	0.7681	0.7801	0.040
G1OC	140	9	0.343	0.8165	0.8297	<0.001
G1D	111	10	0.339	0.7895	0.8077	0.005
G10J	118	10	0.325	0.8136	0.8373	0.008
G10L	126	12	0.324	0.7540	0.8068	<0.001
G10M	144	11	0.329	0.8681	0.8300	<0.001
G10X	150	15	0.339	0.8500	0.8428	<0.001
Mean	132.4	11.3	0.335	0.8102	0.8195	>0.050

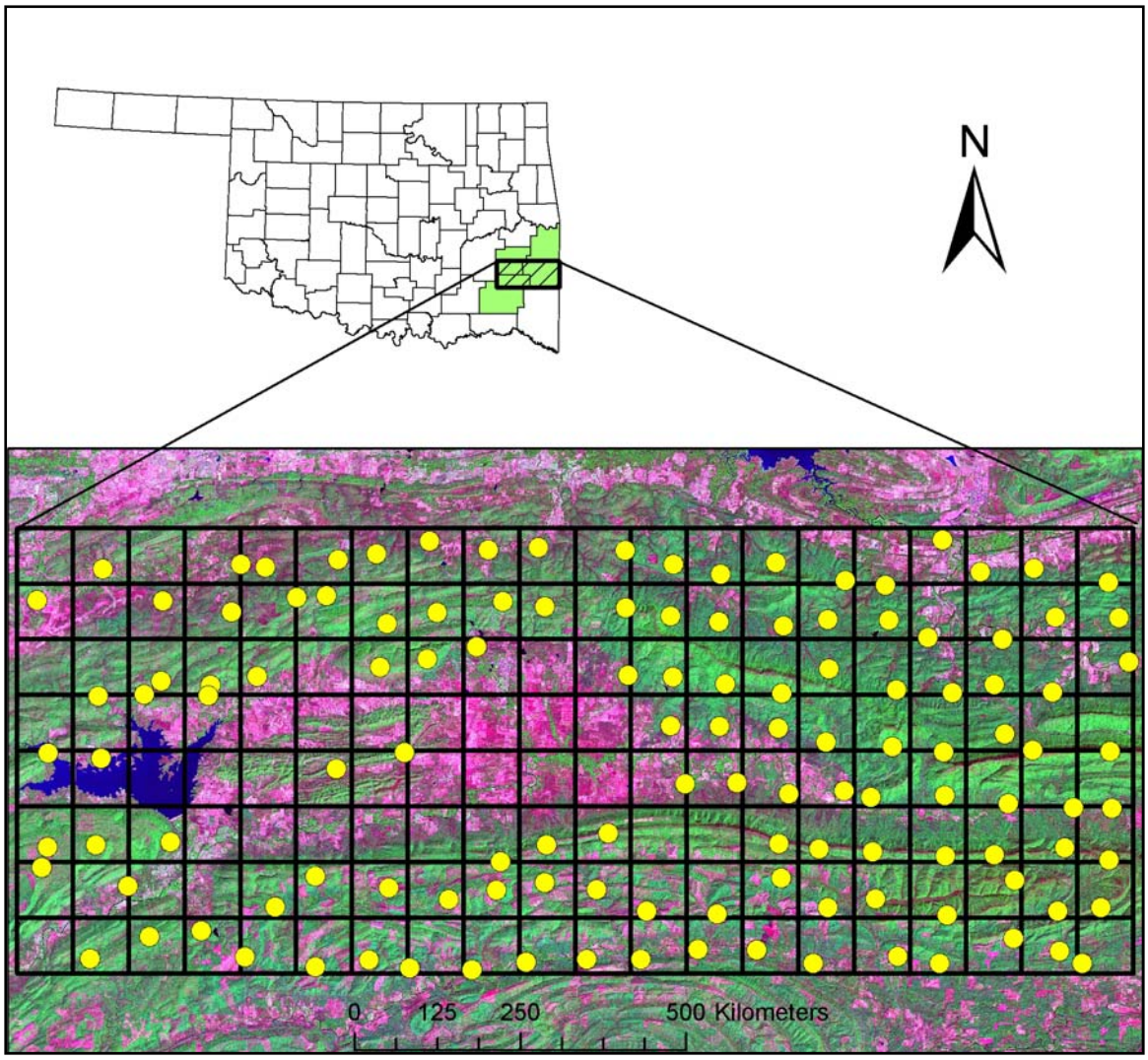

Figure 1. Study area currently being recolonized by black bears dispersing west into Oklahoma from Arkansas. This area spanned 3 Oklahoma counties (Latimer, Leflore, and Pushmataha), and encompasses approximately 3,420 km^2 that was divided into a 4.8 x 4.8 km grid.

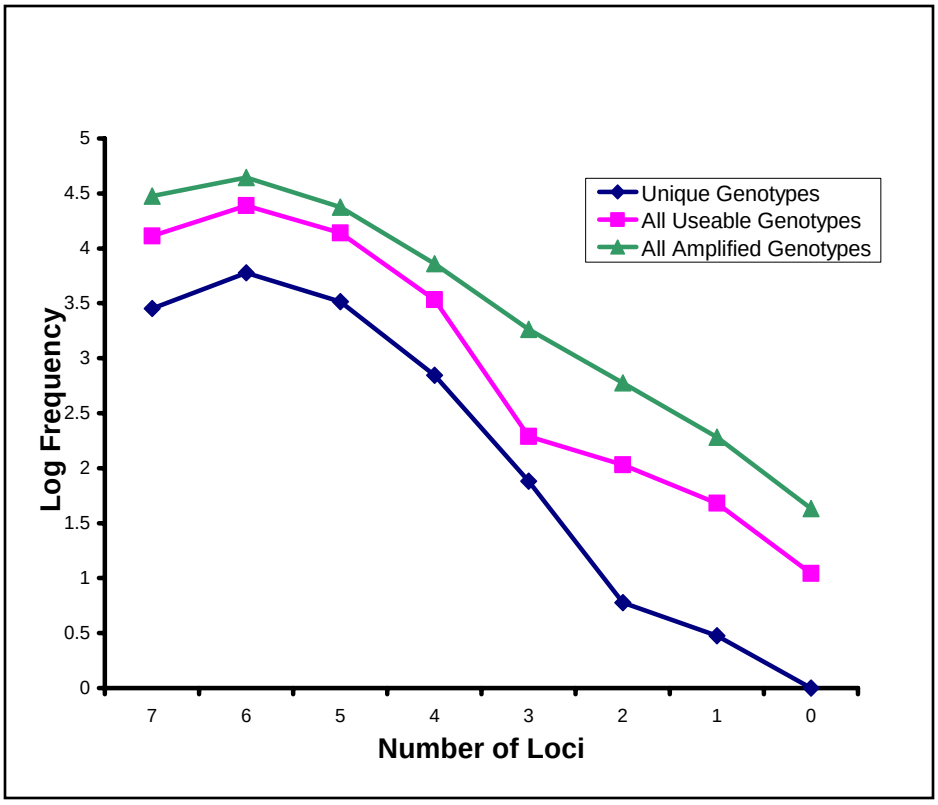

Figure 2. Mismatch distribution for black bear genotypes consisting of 10 microsatellite loci. Distributions illustrated include: 1) all amplified genotypes (n = 464); 2) all useable genotypes (n = 237); and 3) all unique genotypes (n = 161).

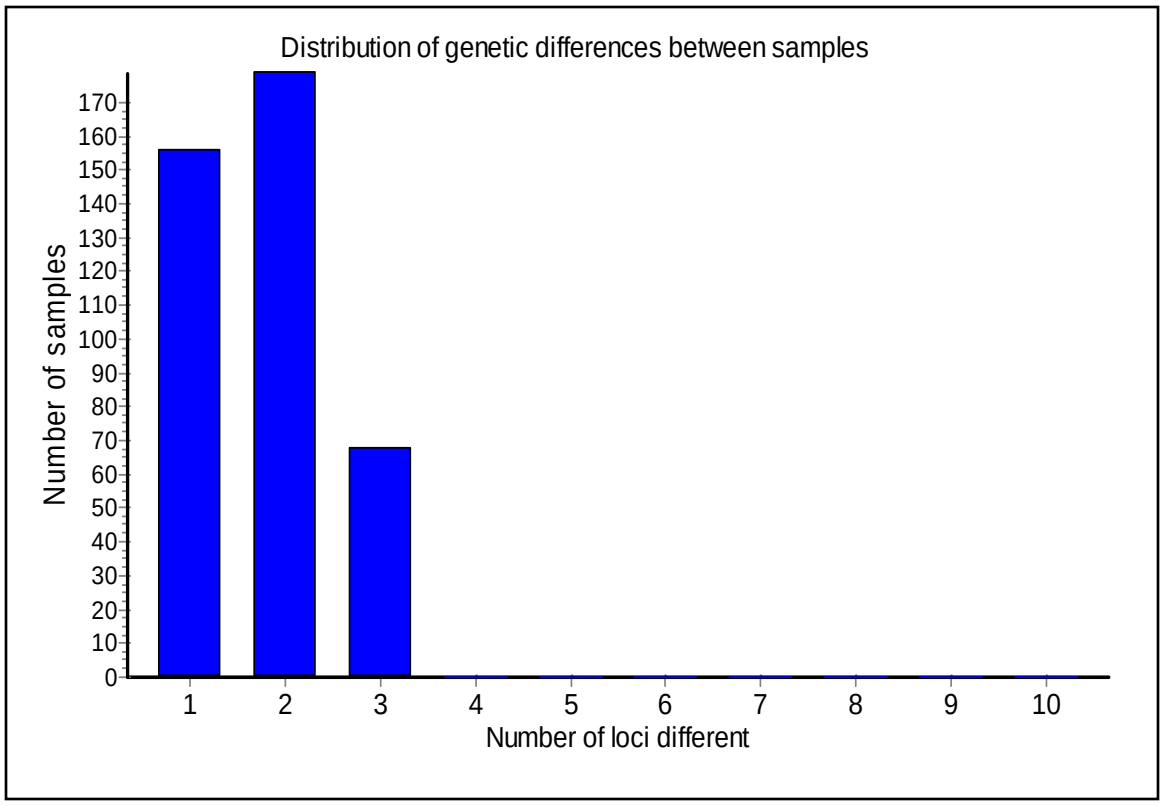

Figure 3(a). Distribution of the minimum pairwise differences between black bear genotypes consisting of 10 microsatellite loci produced using DROPOUT software for all amplified genotypes (n = 464). Number of samples (y-axis) containing differences in number of loci (x-axis) are plotted to determine addition of unique genotypes to the study that are actually duplicate genotypes.

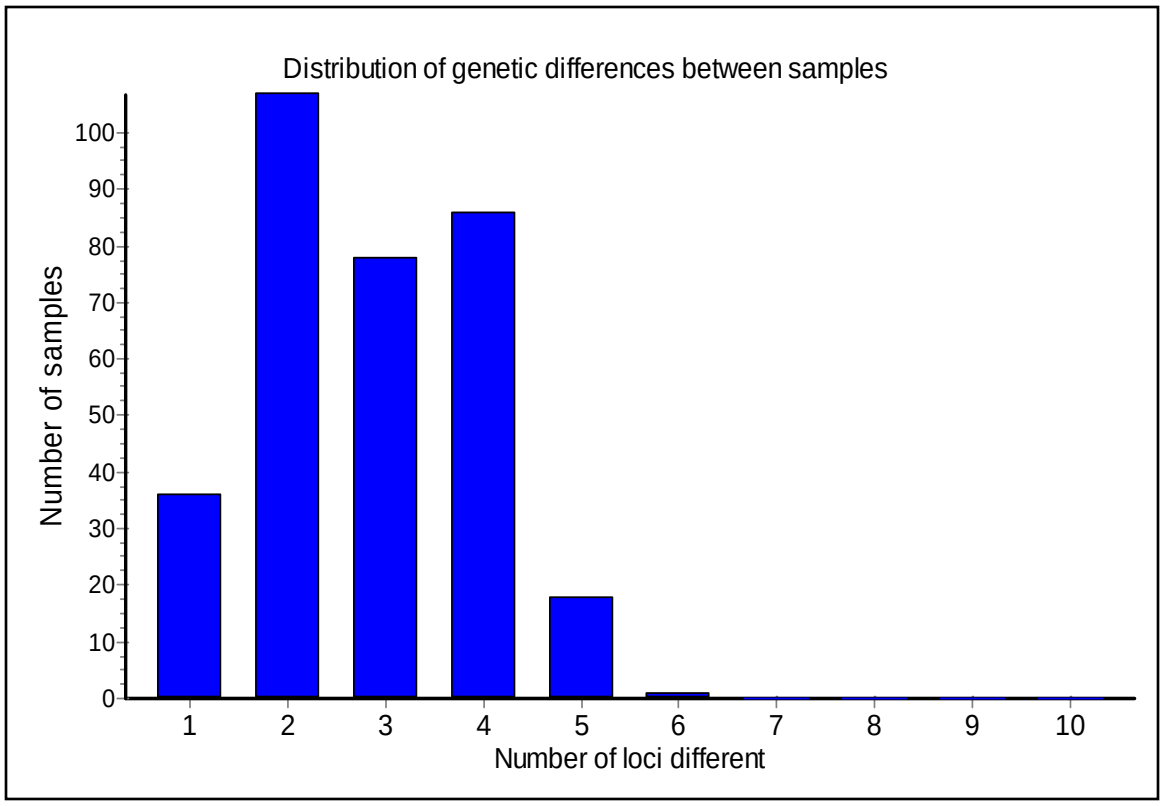

Figure 3(b). Distribution of the minimum pairwise differences between black bear genotypes consisting of 10 microsatellite loci produced using DROPOUT software. for all useable genotypes (n = 237). Number of samples (y-axis) containing differences in number of loci (x-axis) are plotted to determine addition of unique genotypes to the study that are actually duplicate genotypes.

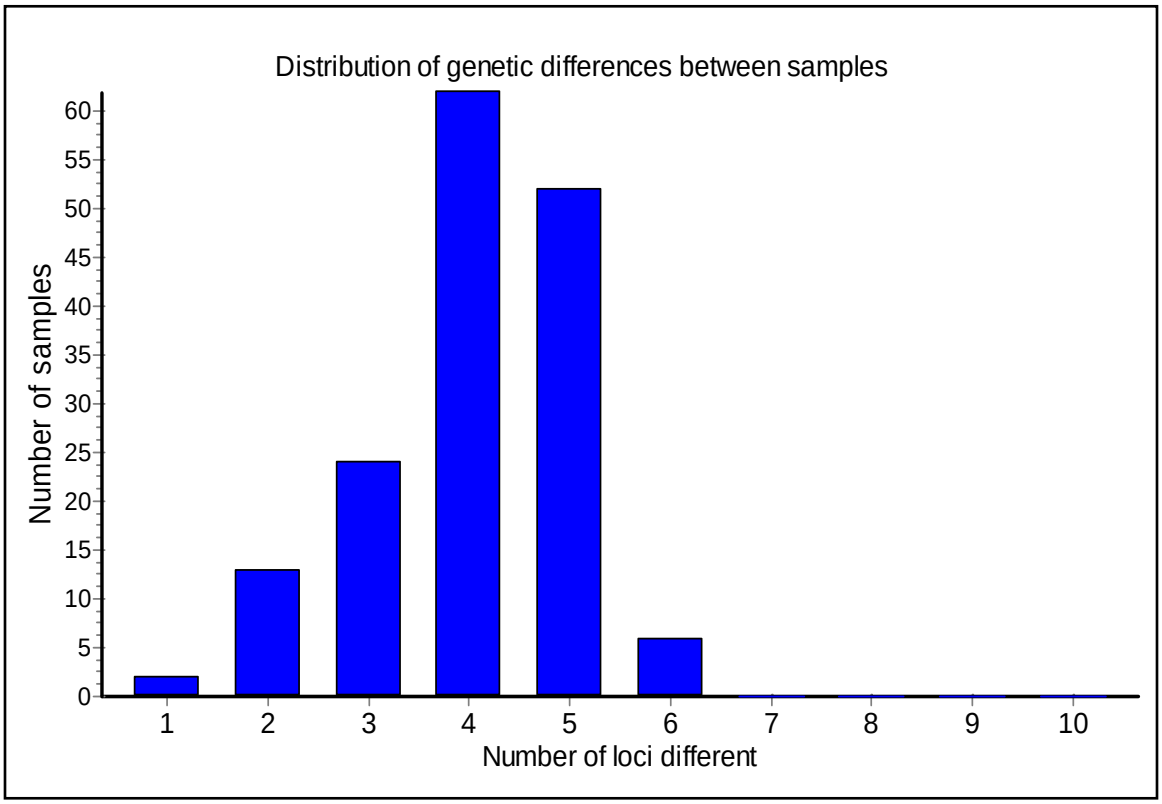

Figure 3(c). Distribution of the minimum pairwise differences between black bear genotypes consisting of 10 microsatellite loci produced using DROPOUT softwarefor all unique genotypes (n = 161). Number of samples (y-axis) containing differences in number of loci (x-axis) are plotted to determine addition of unique genotypes to the study that are actually duplicate genotypes.

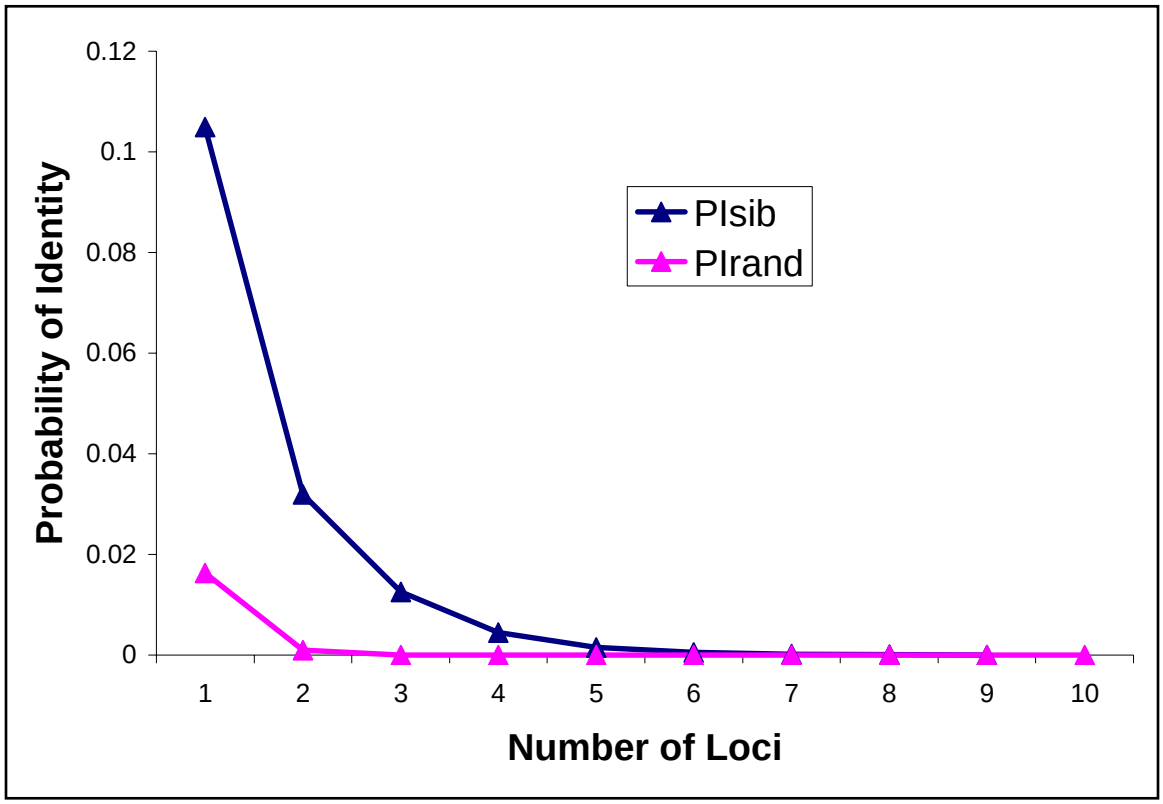

Figure 4. Hardy-Weinberg probability of identity (PI*rand*) and probability of identity taking into account related individuals visiting hairtraps together (PI*sibs*).

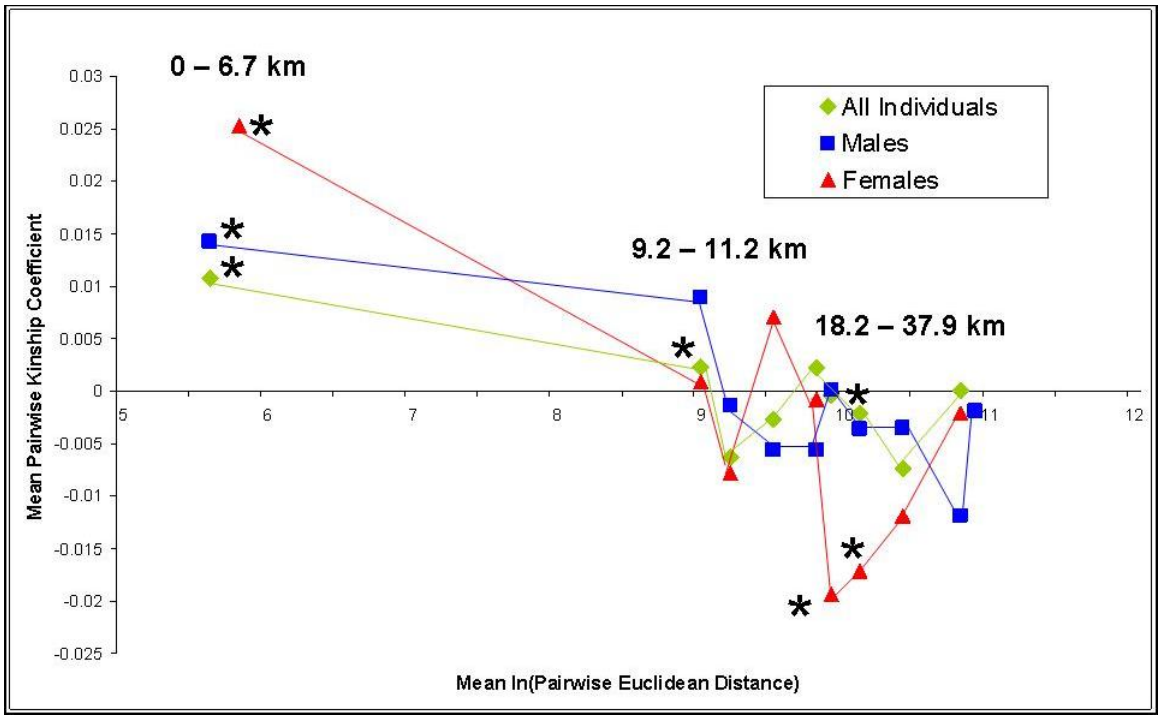

Figure 5. Plot of the relationship between the mean natural log of pairwise Euclidean distance and the mean pairwise kinship coefficient of pairs of individuals in distance classes for all individuals, males, and females. Values significantly different from the mean pairwise kinship of random individuals are marked with an asterisk (*).

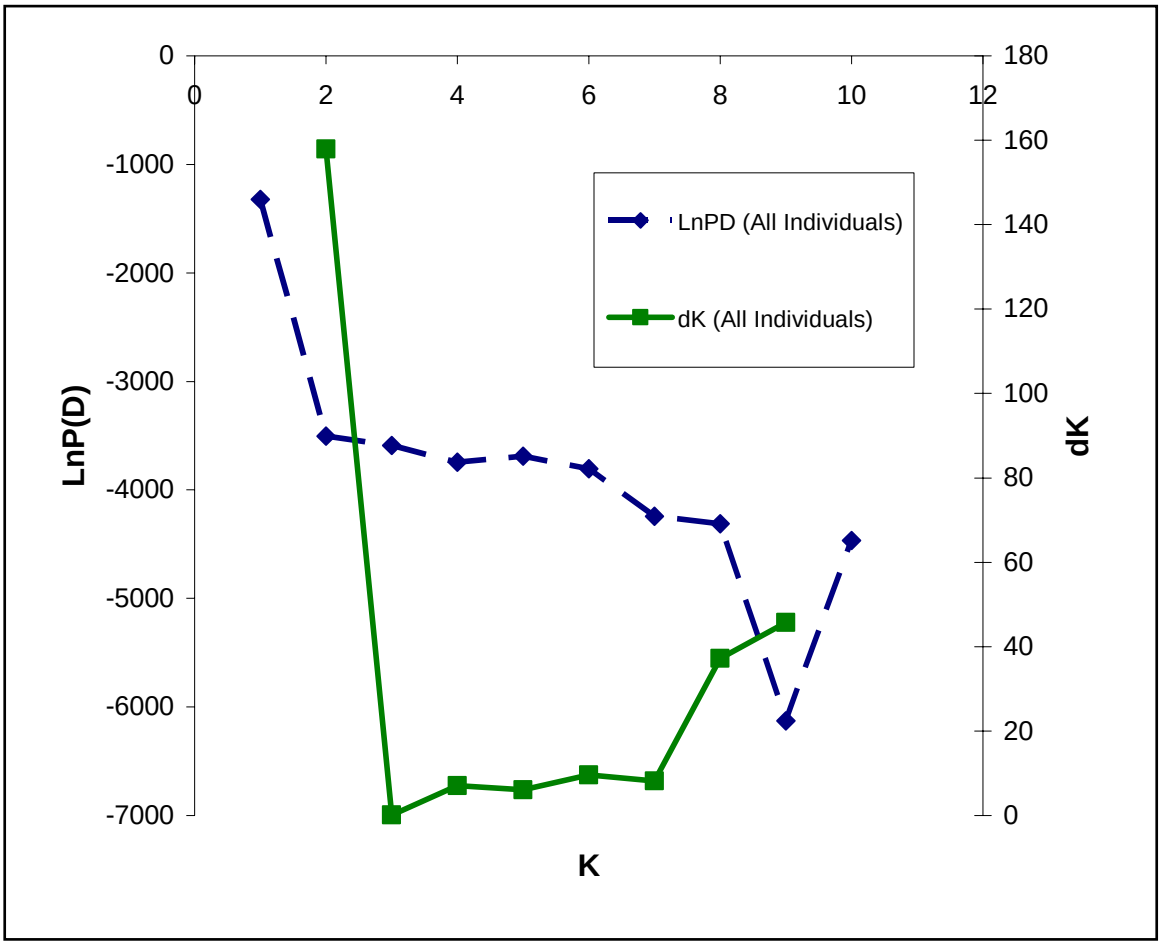

Figure 6. Results of population genetic structure analysis in STRUCTURE 2.0 indicating *K* numbers of clusters without prior knowledge of area of capture. The highest value of the posterior probability distribution (e.g. prior distributions) of allele frequencies (LnP(D)) indicated *K*=1 clusters, while Evanno et al.'s (2005) Δ*K* indicated *K=2* clusters of individuals.

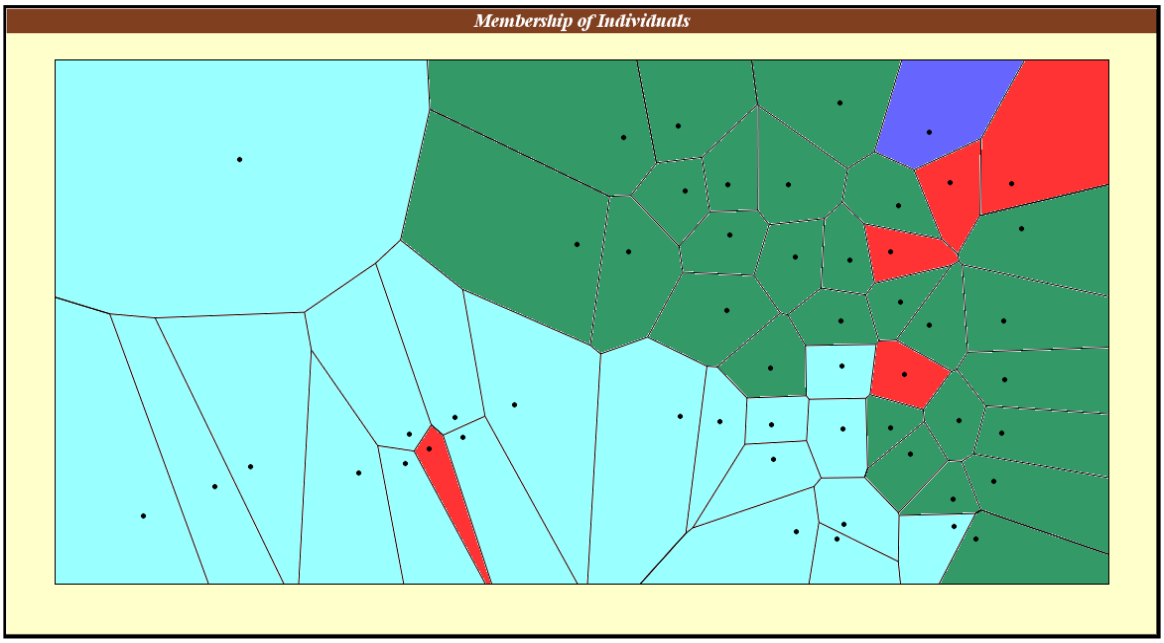

Figure 7(a). Results of population genetic structure analysis in TESS 1.0.1 for the entire sample (n = 161). Different-colored polygons indicate clusters based on prior distribution of allele frequencies in the sample.

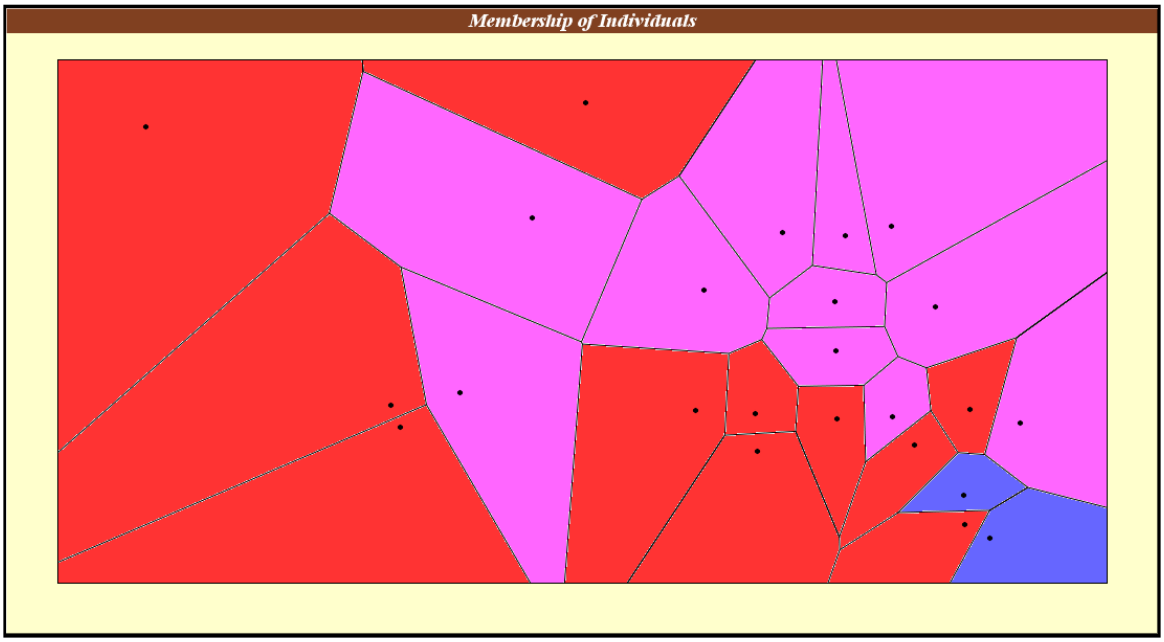

Figure 7(b). Results of population genetic structure analysis in TESS 1.0.1 for females only (n = 59). Different-colored polygons indicate clusters based on prior distribution of allele frequencies in the sample.

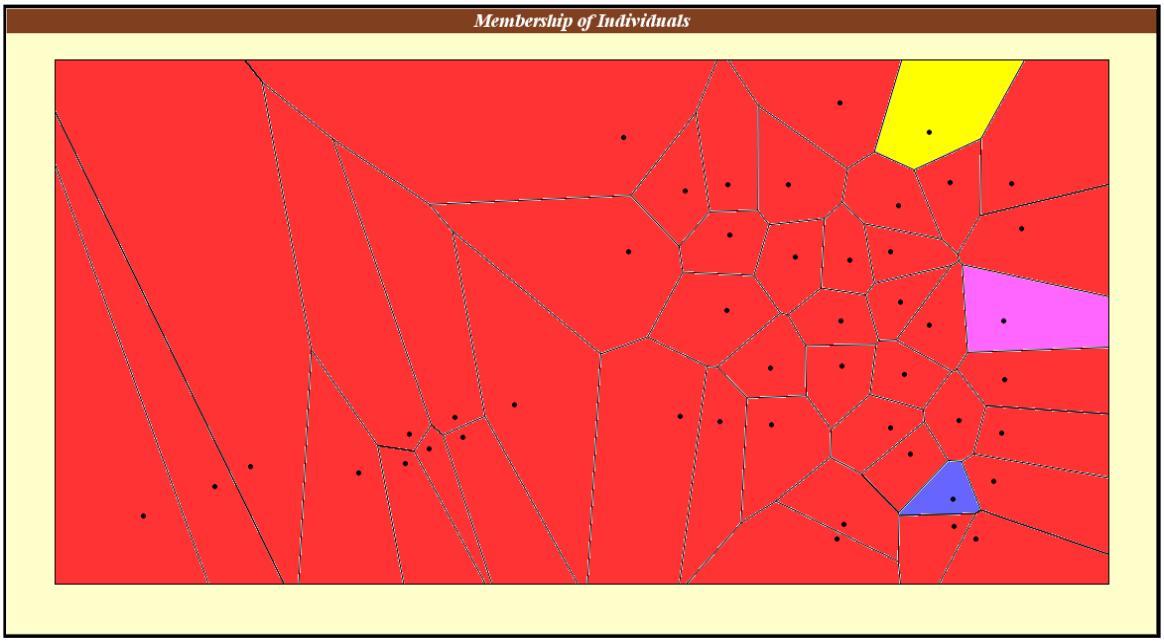

Figure 7(c). Results of population genetic structure analysis in TESS 1.0.1 for males only (n = 102). Different-colored polygons indicate clusters based on prior distribution of allele frequencies in the sample.

VITA

Lynne Carol Gardner-Santana

Candidate for the Degree of

Master of Science

Thesis: PATTERNS OF GENETIC DIVERSITY IN BLACK BEARS (*URSUS AMERICANUS*) DURING A RANGE EXPANSION INTO OKLAHOMA

Major Field: Conservation Science, Wildlife Ecology Emphasis

Education: Bachelor of Science in Biology *cum laude* and Bachelor of Science in Microbiology *cum laude* University of Arkansas, Fayetteville, Arkansas, August 2003. Completed the requirements for the Master of Science degree with a major in Conservation Science with an emphasis in Wildlife Ecology at Oklahoma State University in July 2007.

Research Experience: As undergraduate, awarded a grant to study a viral gene that allows a herpesvirus of poultry to emerge from latency and conducted undergraduate research collecting body temperature data on gravid and nongravid timber rattlesnakes to study differences in thermoregulation. Lived and worked on a swine research facility. Employed as a teaching and research assistant at Oklahoma State University 2003–2006. Employed as a research specialist collecting data on diseases and population genetics of wild city rats and predator-prey models incorporating disease status of prey at Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD 2006–present

Professional Memberships: Phi Kappa Phi Honor Society, Golden Key International Honor Society, National Scholars Honor Society, National Society of Collegiate Scholars, American Society of Mammalogists, International Association for Bear Research and Management, The Wildlife Society, Oklahoma State University Zoology Graduate Student Society, Oklahoma Academy of Science

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Name: Gardner-Santana, Lynne Date of Degree: July, 2007

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: PATTERNS OF GENETIC DIVERSITY IN BLACK BEARS (*URSUS AMERICANUS*) DURING A RANGE EXPANSION INTO OKLAHOMA

Pages in Study: 71 Candidate for the Degree of Master of Science

Major Field: Conservation Science, Wildlife Ecology Emphasis

Scope and Method of Study: Successful translocation of black bears (*Ursus americanus*) into Arkansas led to their restoration into former ranges and expansion of bears into Oklahoma, populating areas devoid of bears since 1915. Genetic monitoring of this event provides an opportunity to examine its unique characteristics and to gather valuable information for wildlife managers.

Findings and Conclusions: We collected 1,166 hair samples from 128 hair snares in June–August 2004–2006. Of the 498 (42.7%) samples from which DNA was extracted, 332 (66.7%) were genotyped, 161 (48.5%) of which were unique individuals with 52 (32.3%) recaptures. Maintenance of gene flow from the source population should preserve high levels of genetic diversity typical of large black bear populations in North America. Recent admixture of dispersing bears with individuals representing other gene pools may have occurred. Incorporation of genetic and demographic data is essential to understanding population status and to develop a successful management plan for this large carnivore.

Advisor's Approval: _____Ronald A. Van Den Bussche_____