



Research Article

Rapid Growth and Genetic Diversity Retention in an Isolated Reintroduced Black Bear Population in the Central Appalachians

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ABSTRACT Animal reintroductions are important tools of wildlife management to restore species to their historical range, and they can also create unique opportunities to study population dynamics and genetics from founder events. We used non-invasive hair sampling in a systematic, closed-population capture-mark-recapture (CMR) study design at the Big South Fork (BSF) area in Kentucky during 2010 and Tennessee during 2012 to estimate the demographic and genetic characteristics of the black bear (*Ursus americanus*) population that resulted from a reintroduced founding population of 18 bears in 1998. We estimated 38 (95% CI: 31–66) and 190 (95% CI: 170–219) bears on the Kentucky and Tennessee study areas, respectively. Based on the Tennessee abundance estimate alone, the mean annual growth rate was 18.3% (95% CI: 17.4–19.5%) from 1998 to 2012. We also compared the genetic characteristics of bears sampled during 2010–2012 to bears in the population during 2000–2002, 2–4 years following reintroduction, and to the source population. We found that the level of genetic diversity since reintroduction as indicated by expected heterozygosity (H_E) remained relatively constant ($H_{E(\text{source}, 2004)} = 0.763$, $H_{E(\text{BSF}, 2000-2002)} = 0.729$, $H_{E(\text{BSF}, 2010-2012)} = 0.712$) and the effective number of breeders (N_B) remained low but had increased since reintroduction in the absence of sufficient immigration ($N_{B(\text{BSF}, 2000-2002)} = 12$, $N_{B(\text{BSF}, 2010-2012)} = 35$). This bear population appears to be genetically isolated, but contrary to our expectations, we did not find evidence of genetic diversity loss or other deleterious genetic effects typically observed from small founder groups. We attribute that to high initial genetic diversity in the founder group combined with overlapping generations and rapid population growth. Although the population remains relatively small, the reintroduction using a small founder group appears to be demographically and genetically sustainable. © 2015 The Wildlife Society.

KEY WORDS Appalachia, black bear, demographics, founder event, iteroparous, Kentucky, population genetics, reintroduction, Tennessee, *Ursus americanus*.

Reintroduction has been successfully used to restore many animal species to their historical ranges, typically with the goal of establishing self sustaining, viable populations (Griffith et al. 1989, Seddon 1999, Swaisgood 2010). In general, the probability of reintroduction success is improved with large founder population size, high genetic variation in the founder group, availability of high quality habitat, high initial population growth rates, low mortality, and low intraspecific competition (Griffith et al. 1989, Earnhardt 1999, Seddon 1999, Frankham

2009b). Therefore, reintroduction efforts with small founder population sizes should establish intensive monitoring programs during and beyond the initial reintroduction phase to determine if further management intervention is necessary to ensure long-term population viability (Frankham 2009b, De Barba et al. 2010). Nonetheless, many reintroduction programs have failed to determine whether the criteria for success were met, not only during the focal reintroduction phase, but also at any time post-reintroduction (Beck et al. 1994, Sarrazin and Barbault 1996, Clark et al. 2002). Ultimately, a lack of monitoring can limit science-based assessment and management of often time- and cost-intensive efforts, and risk damage to species conservation programs and public confidence in such activities (Seddon et al. 2007, Armstrong and Seddon 2008, Seignobosc et al. 2011).

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The American black bear (*Ursus americanus*) has been subjected to anthropogenic exploitation and habitat destruction throughout North America. Consequently, bear populations in the eastern United States reached historical lows by the early 1900s (Hall 1981, Pelton 2001). Although habitat recovery and population expansion occurred in some areas over the last half century, a number of smaller black bear populations in the eastern United States remained isolated within fragmented landscapes (Hellgren and Maehr 1992, Pelton and van Manen 1997, Clark et al. 2002, Dixon et al. 2007, O'Connell-Goode et al. 2014). The popularity of the black bear as a game species and the recognition of its important ecological and cultural roles have led to multiple reintroduction efforts within its historical range (Alt 1995, Comly-Gericke and Vaughn 1997, Clark et al. 2002, Wear et al. 2005, Benson and Chamberlain 2007). Perhaps the most successful black bear reintroduction occurred in Arkansas where 254 individuals were translocated from Minnesota and Canada, creating a population of >2,500 within 20 years (Smith and Clark 1994). However, the fate of most black bear reintroductions in the United States remains unknown because of the lack of post-release monitoring, and few black bear reintroduction successes have been documented to date (Clark et al. 2002, Clark 2009).

In the late 1990s, a limited black bear reintroduction took place in an attempt to re-establish the species in a portion of its historical range in the central Appalachians (Eastridge 2000, Eastridge and Clark 2001). During 1996 and 1997, 14 adult female black bears with 13 cubs were captured at Great Smoky Mountains National Park (hereafter referred to as GSMNP) in Tennessee, and were released in the Big South Fork National River and Recreation Area (hereafter referred to as BSF) along the Kentucky-Tennessee border (11 adult females in Tennessee and 3 in Kentucky), a translocation distance of more than 150 km (Eastridge and Clark 2001). No effort was made to maximize genetic diversity of the original founder group, but all of the founders were sourced from different sites within GSMNP (J. Clark, United States Geological Survey, personal communication). By 1998, 6 adult founders had either left the area or died (Eastridge 2000), and adult female survival including emigration was estimated at 0.66 (SE = 0.12; Eastridge and Clark 2001). A recommended supplementation to this population during 2000 was disallowed because of public opposition, and no supplementation occurred at any time following the initial release of founders. Approximately 18 bears (4 subadult females, 6 subadult males, and 8 adult females) resided in and around the BSF by 1998 (J. Clark, unpublished data), and Eastridge and Clark (2001) estimated that the population had a 24% chance of extinction within 100 years. Persistent observations of adult female bears with cubs in the study area from 1999 to 2009 suggested that either individuals from the original founder population or their descendants remained at BSF, or the area had been colonized by bears from relatively distant (>70 km) neighboring populations.

Hast (2010) investigated the genetic structure of black bears at BSF and other areas of southeast Kentucky by

analyzing hair samples collected from hair traps and live captures during 2006–2009. Whereas bears in the neighboring southeast Kentucky population were primarily an admixture of genotypes from Virginia and West Virginia, Hast (2010) determined that bears in the BSF population were comprised almost entirely of genetic material identical to bears in the GSMNP source population, a phenomenon observed in other reintroduced bear populations (Puckett et al. 2014). Hast (2010) identified only 2 migrants in the BSF population; both individuals were males with genotypes similar to the neighboring southeast Kentucky population located approximately 70 km to the east, possibly the result of a nuisance translocation (J. Plaxico, Kentucky Department of Fish & Wildlife Resources, personal communication). Additionally, none of the 146 bears that were radio collared in the nearest southeast Kentucky population during 2000–2014 were known to have successfully traveled to the BSF area (S. Murphy and J. Cox, University of Kentucky, unpublished data). Collectively, this evidence suggests that bears at BSF primarily are descendants of the original translocated founders with minimal gene flow having occurred between BSF and other central Appalachian bear populations since reintroduction.

The winter soft-release method employed by Eastridge and Clark (2001) to initiate the BSF population focuses on the translocation of adult females with cubs to avoid emigration from the release area and to increase settling rates. Although effective (Alt 1995, Eastridge and Clark 2001, Wear et al. 2005, Benson and Chamberlain 2007), the method results in an initially skewed sex ratio and, because it is labor intensive, the number of founders is typically low. A consequence of such reintroductions that rely on small numbers of founder individuals is an increased likelihood of population bottlenecks (Jaimeson 2010) characterized by heightened vulnerability to demographic and environmental stochasticity (Lande 1993, Lande et al. 2003), and the deleterious genetic effects of inbreeding depression, genetic drift, and loss of genetic diversity (Allendorf and Luikart 2007, Brook 2008). Additionally, without immigration from nearby populations, the male component of the population consists of cubs born to founding females, thereby enhancing the prospects for inbreeding. Although bears typically avoid inbreeding because subadult males disperse (Rogers 1987, Clevenger and Pelton 1990, Lee and Vaughn 2004), without unrelated sexually mature males present in the population to replace these offspring, inbreeding can occur (Kasbohm et al. 1994, Zedrosser et al. 2007). Collectively, these inherent consequences of small founder sizes can lead to the loss of fitness and adaptive potential, and an increased risk of population extinction.

Given the lack of critical biological information for the BSF bear population, our goals were to evaluate its demographic and genetic status to provide wildlife and land managers with information for the establishment of management efforts for bears in this area, and to provide further insight into the genetic consequences of reintroducing a large mammal based on a small founder group size. Therefore, we used DNA obtained with non-invasive hair

sampling in a systematic, closed-population mark-recapture study design to 1) estimate population abundance and growth rate, 2) determine the potential for deleterious genetic effects resulting from the low number of original founders, and 3) evaluate reintroduction success (i.e., establishment of a self-sustaining population (Griffith et al. 1989) of black bears at BSF.

STUDY AREA

The 1,915-km² study area was located on the western edge of the Cumberland Plateau physiographic region of the Appalachian Plateau physiographic province in the Central Appalachian ecoregion (Thornbury 1965, Smalley 1986, Woods et al. 2002). Study area boundaries included the entirety of the Big South Fork National River and Recreation Area in south-central Kentucky and north-central Tennessee, the Stearns Ranger District of the Daniel Boone National Forest, a portion of Pickett State Park in Tennessee, a portion of Cumberland Falls State Park in Kentucky, and a matrix of forested and agricultural private lands in McCreary, Whitley, and Wayne counties, Kentucky, and Scott, Fentress, Pickett, and Morgan counties, Tennessee. The area was topographically characterized as a plateau, dissected by deep, narrow ravines cut by multiple rivers and streams, such as the Big South Fork of the Cumberland River (Kleber 1992). Elevations in the study area ranged from 220 m to 659 m above sea level. Vegetation was predominantly mixed-mesophytic forest characterized as having up to 30 co-dominant canopy tree species (Braun 1950, Safley 1970). Forest composition included several species of oak (*Quercus* spp.), hickory (*Carya* spp.), ash (*Fraxinus* spp.), and pine (*Pinus* spp.), as well as American beech (*Fagus grandifolia*), cucumber magnolia (*Magnolia acuminata*), and eastern hemlock (*Tsuga canadensis*). Climate was hot and humid in summer and mild during winter with a typical mean annual temperature of 13°C, and 133 cm of average annual precipitation (Shaw and Wofford 2003). The Daniel Boone National Forest was managed for multiple uses, including timber, water, wildlife, fish, minerals, and recreational activities (United States Forest Service 2009). Active management plans commonly implemented included prescribed burning and timber harvesting. Forests in Big South Fork National River and Recreation Area were managed for multiple uses as well, primarily recreation and conservation but did not include timber harvesting (National Park Service 2005). Removal of timber was permitted only for development of public and administrative facilities by the National Park Service. Prescribed burning, however, was permitted and used as a management tool. Bear harvests did not occur in the study area following reintroduction or during this study. Human population density averaged 15 per km² across the entire study area.

METHODS

Study Design

Black bears are elusive, cryptic, and occur at low density, which introduces difficulty in collecting demographic data

(Mowat and Strobeck 2000, Coster et al. 2011). As a result, live trapping often yields sample sizes too small for reasonably precise demographic estimates (Coster et al. 2011, Marucco et al. 2011). Non-invasive genetic capture-mark-recapture (CMR) methods have become increasingly used among bear researchers and managers as practical and economic alternatives for estimating demographic and genetic characteristics of populations (Woods et al. 1999, Waits and Paetkau 2005, Clark et al. 2010). These methods can increase trapping efficiency and capture probabilities, reduce bias, and mitigate the loss of marks common in live-trapping studies (Woods et al. 1999). The advent of highly variable molecular markers, such as microsatellites, and the ability to amplify the DNA in small quantities of tissue, such as hair, have further increased the applicability and usefulness of non-invasive genetic sampling methods (Taberlet and Luikart 1999, Waits and Paetkau 2005).

Our CMR dataset was the result of 2 independent studies conducted in Kentucky (2010) and Tennessee (2012). We used non-invasive hair traps randomly placed in systematic grids to collect black bear hair for microsatellite DNA analysis. In Kentucky, we established a 1,260-km² sampling grid composed of 126 contiguous 10-km² cells that covered all of McCreary County, Kentucky, and included the Kentucky portion of Big South Fork National River and Recreation Area. We established a single baited, barbed wire hair trap in each sampling cell to collect black bear hair for microsatellite DNA analysis (Fig. 1; Woods et al. 1999). Hair trap placement was restricted to >100 m from roads and trails, and >500 m from campgrounds, picnic areas, and residential areas. A hair trap consisted of 1–2 barbed wire strands wrapped around 3–5 perimeter trees to create an enclosure about 25 m² in size (Woods et al. 1999). We secured wire 65 cm above the ground but added a second wire to some traps at a height of 35 cm above the ground. Laufenberg and Clark (2014) identified no captures of cubs based on backdating captures of bears >1 year of age from subsequent years using these wire heights, so our population estimates probably did not include cubs. Each trap was baited with a combination of sardines and pastries suspended between 2 trees using a string secured approximately 3 m above the ground. From May 2010 to July 2010, we checked and re-baited all hair traps every 7 days for 7 consecutive sampling sessions. We did not move traps between or during any sampling sessions.

In Tennessee, we created a hair-trap sampling grid composed of 107 contiguous sampling cells that covered a 645-km² area. We used a grid cell size of 6.7 km² instead of the 10-km² spacing used in Kentucky in an effort to increase capture probabilities and minimize capture heterogeneity (Settlage et al. 2008). As in Kentucky, we constructed a single baited, barbed wire hair trap within each sampling cell to collect black bear hair and adhered to the same placement restrictions (Fig. 1). We constructed all hair traps on the Tennessee study area using 2 wires placed 35 cm and 65 cm above the ground; we wrapped each around 3–5 perimeter trees to form an enclosed polygon. We again baited with sardines and pastries and checked traps weekly for 8

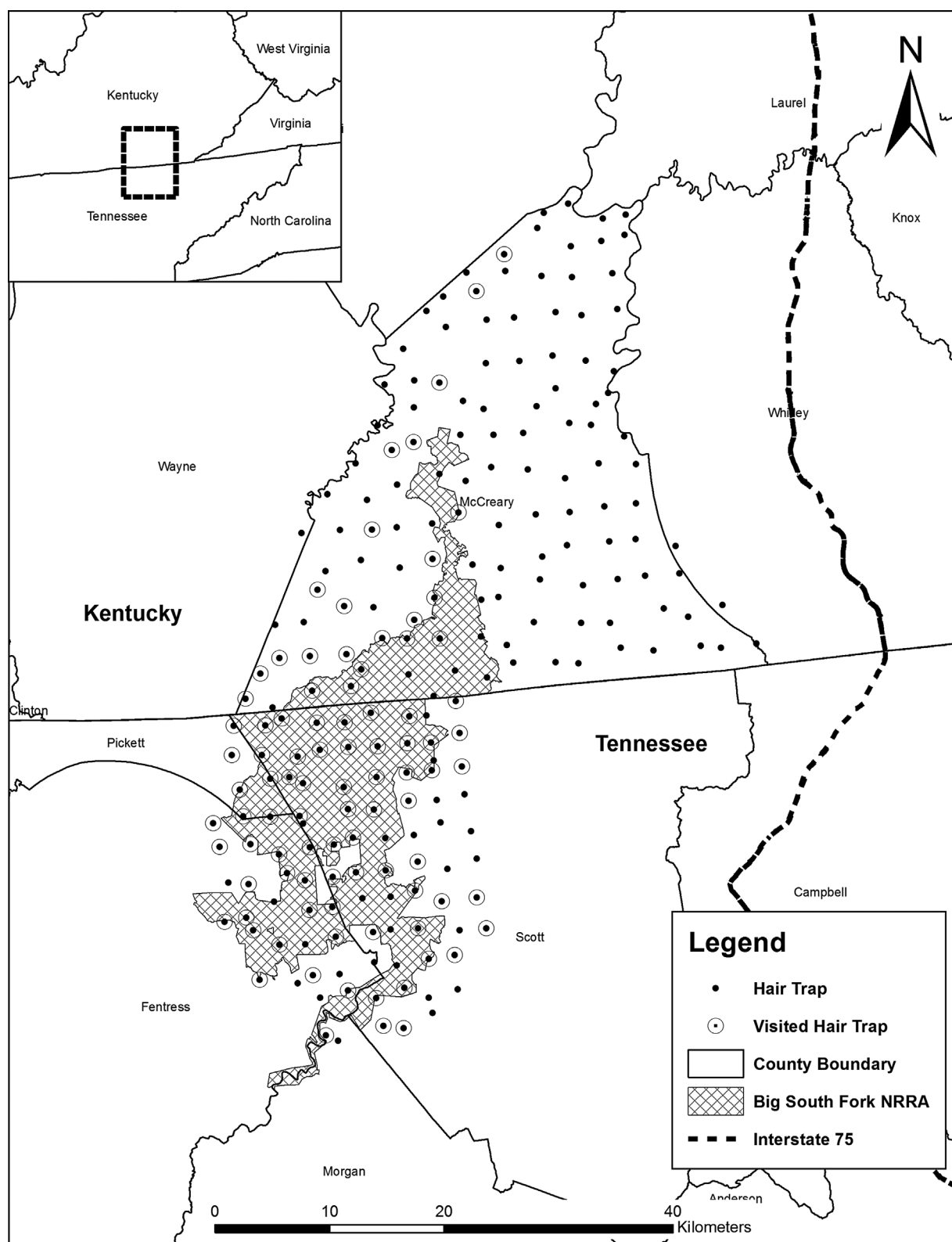


Figure 1. Locations of 233 black bear hair traps and 106 visited hair traps where we collected hair samples at the Big South Fork study area in Kentucky (2010), and Tennessee (2012), USA. Hair trap density was 1 trap/10 km² in Kentucky and 1 trap/6.7 km² in Tennessee.

consecutive weeks from May 2012 to July 2012. Again, we did not move traps between or during any sampling sessions. For both studies, we treated each barb on the barbed wire as an individual sample, and removed hairs using tweezers that

we sterilized between collections. Procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee (Protocol #626A2003). We placed collected hair samples in individually labeled paper coin envelopes that

were stored at room temperature. We used a flame to sterilize barbs following individual sample collections to prevent cross contamination of samples.

Although no genetic samples were available from the original founders at BSF, we acquired a dataset consisting of black bear hair samples that were collected during 2000–2002 (i.e., 2–4 years post-reintroduction) from bears at BSF. We used these samples to investigate potential changes in genetic characteristics of the BSF population since reintroduction. These samples were collected opportunistically year round at randomly placed non-invasive hair traps in the Kentucky portion of BSF by Kentucky Department of Fish & Wildlife Resources and National Park Service personnel. The frequency of trap checks for this dataset was variable; otherwise, hair traps and collection methods were similar to those used for the 2010–2012 CMR samples.

We also acquired an additional dataset of black bear hair samples from the GSMNP source population that were collected during 2004 by University of Tennessee researchers as part of a mark-recapture study (Settlage et al. 2008). Although these samples were collected 6 years after the BSF reintroduction, to our knowledge the GSMNP population has not endured any severe reductions in population size in recent history that would alter the estimates of genetic parameters for this source population. Furthermore, Hast (2010) determined that the genotypes of BSF bears and GSMNP bears remained nearly identical. Therefore, we considered these samples representative of the genetic characteristics of the GSMNP source population that the original BSF founders originated from.

Laboratory Analyses

Following sample collection, we sent all hair samples to Wildlife Genetics International (WGI; Nelson, British Columbia) for DNA extraction and amplification. We shipped samples to WGI within 1 month of collection, and data quality was managed by WGI using the methodology described in Paetkau (2003). All samples collected during 2004 at GSMNP, 2000–2002 at BSF, and 2010 in Kentucky were selected for genotyping. However, because a large number of samples were collected in Tennessee during 2012, we established a subsampling protocol to mitigate the financial costs of genotyping. We selected 1 sample per hair trap per week for genotyping (Settlage et al. 2008, Laufenberg 2010) to reduce the number of duplicate samples analyzed from an individual trap site while maximizing the success rate of genotyped samples. Personnel at WGI randomized samples from each site week and selected the first sample encountered containing 5 guard hair roots or ≥ 20 underfur hairs. If no samples at a site week met this threshold, they chose the best available sample using a threshold of 1 guard hair root or 5 underfur hairs. If none of the samples at a site week met the more lenient threshold, they did not genotype any hair samples for that site during that capture session (D. Paetkau, WGI, personal communication).

Following the protocols described by Woods et al. (1999) and Paetkau (2003), DNA was extracted using QIAGEN

DNeasy Blood and Tissue spin columns (Qiagen, Valencia, CA). Seven black bear-specific microsatellite loci (G10B, G10H, G10J, G10L, G10M, G10P, and MU23) were used to identify individual black bears using the polymerase chain reaction (PCR) and genotyping methods described by Paetkau and Strobek (1994). A sex marker was used to delineate sex of identified individuals (Ennis and Gallagher 1994). To minimize genotyping error and mitigate incorrect identification of individuals, WGI discarded samples that failed at >3 markers on the first pass of amplification. Additionally, samples with 1–3 misidentified pairs were reanalyzed, and samples without complete genotypes for all markers were discarded. Finally, error checking was completed by reanalyzing pairs of samples with genotypes matching at all-but-one (1-MM pairs) or all-but-two markers (2-MM pairs) to determine if differences existed at each locus (D. Paetkau, personal communication). This process effectively ensured that the number of individuals identified had not been inflated through undetected genotyping error (Paetkau 2003).

Population Genetics

We tested Hardy–Weinberg equilibrium (HWE) between genotypes with Program Genepop 4.2 using the complete enumeration method and a Markov Chain sampling process (Louis and Dempster 1987, Guo and Thompson 1992, Raymond and Rousset 1995). Linkage disequilibrium, the failure of alleles at 2 loci to be statistically independent, was investigated using the linkage disequilibrium test in Genepop 4.2 (Raymond and Rousset 1995) with P values adjusted using a Bonferroni sequential correction (Rice 1989). We estimated genetic diversity as expected heterozygosity (H_E) using the allele identity method in Genepop 4.2 (Raymond and Rousset 1995). We used the R statistical program (R Core Team 2012) package *diversity* to estimate allelic richness (R_A) using a rarefaction technique (Hurlbert 1971, Keenan et al. 2013). We then compared R_A and H_E of the BSF population during 2000–2002 to that during 2010–2012 with a non-parametric Mann–Whitney U test in R to investigate whether or not observed changes of these measures of genetic health were the result of differing sample sizes (i.e., the null hypothesis asserts that the medians of the 2 samples are identical; Zar 2010).

Effective population size (N_E) is the size of an ideal population (i.e., a population with an equal sex ratio of reproducing individuals that are equally likely to reproduce, that exhibits random mating, and in which the number of reproducing individuals is constant among generations) that experiences the same rate of random genetic change over time as the population under consideration (Wright 1931, 1938), and is one of the most important parameters to estimate for conservation and management of wildlife populations (Frankham 2005). This is especially true for reintroduced populations because they are typically vulnerable to deleterious genetic effects, such as founder effect, genetic drift, and a loss of diversity due to small population size (Frankham et al. 2003). Estimating the effective population size and deriving an estimate of the ratio of

N_E to abundance (N) can help researchers understand the relative risk that genetic factors may pose for population fitness and persistence (Frankham 2009a, Luikart et al. 2010, Palstra and Fraser 2012).

Multiple methods exist to estimate N_E , all of which must be appropriately applied. The standard genetic drift method is temporally based, requiring estimates of allelic or genetic diversity at 2 time points (Kempthorne 1957). Although this method appears to produce precise estimates of N_E for semelparous species, it often biases estimates high for iteroparous species that have overlapping generations such as bears (Palstra and Fraser 2012). An ecological method to estimate N_E developed by Nunney and Elam (1994) performs well for iteroparous organisms (De Barba et al. 2010) but requires long-term demographic data that were not available for the BSF population. In contrast, the linkage disequilibrium method (Hill 1981) requires only a single sample of genetics data, and when used for iteroparous species, it provides an estimate of the number of breeding individuals that have genetically contributed to the population, thereby reflecting N_E of the current generation (Waples 2005, Antao et al. 2011, Palstra and Fraser 2012). Therefore, we estimated effective population size as the number of breeding individuals that have contributed to the population (hereafter referred to as N_B ; Waples and Teel 1990) using the linkage disequilibrium method in NeEstimator v2.01 (Ovenden et al. 2007, Do et al. 2014) for the 2000–2002 and 2010–2012 microsatellite data from BSF, as well as the 2004 microsatellite data from GSMNP.

Demographic Estimates

To estimate abundance (N), we employed closed-population models in Program MARK for the 2010–2012 CMR data (White and Burnham 1999). Closed-population models are based on the assumptions of 1) demographic and geographic closure (i.e., no births, deaths, immigration, or emigration during sampling), 2) animals do not lose their marks during sampling, 3) marks are recognized and recorded correctly, and 4) all animals have an equal opportunity of being captured during each sampling session. Closed-population models assume equal capture probability (p ; assumption 4 above), but variation often exists (Otis et al. 1978). To address sources of variation in p , models that account for temporal variation (time), behavioral response to capture ($p \neq c$; where c is the recapture probability), and individual heterogeneity (π) have been developed (Otis et al. 1978, Huggins 1989, Pledger 2000).

However, in the presence of missing data introduced by processes such as subsampling hair samples and DNA amplification failure, models that include a behavioral response to capture can sometimes produce biased high N estimates because subsequent captures can erroneously be identified as first captures (Augustine et al. 2014). Alternatively, excluding a behavioral response to capture when there is indeed a positive behavioral response to capture leads to an overestimation of p and therefore an underestimation of N . Methods have recently been developed to fit behavioral response models in the presence of missing data

(Augustine et al. 2014), but the methodology has not yet been extended to the subsampling protocol we used in this study for the 2012 Tennessee CMR dataset (i.e., select 1 sample per trap per capture occasion). Therefore, we chose to exclude the behavioral response to capture and time effects for the 2012 Tennessee CMR dataset to produce conservative estimates of N and λ .

We used a method to account for individual heterogeneity by modeling capture probability as coming from a 2-class mixture with different capture probabilities for each mixture (Pledger 2000). We did not use individual covariates to model capture heterogeneity (Huggins 1989). For the Kentucky dataset, we considered various combinations of models accounting for individual heterogeneity with a 2-class mixture, sex, behavioral response to capture, and time. For the Tennessee dataset, we considered similar models as those used for the Kentucky dataset but excluded a behavioral response to capture and a time effect. We evaluated models with Akaike's Information Criterion corrected for small sample size (AIC_c ; Burnham and Anderson 2002). We averaged models with $\leq 4 \Delta AIC_c$ values (i.e., no subsetting) according to the methods outlined in Burnham et al. (2011) to produce final, model-averaged parameter estimates. Because a small number of bears were released in suitable habitat (van Manen and Pelton 1997) that was likely void of resident bears prior to the reintroduction (Eastridge 2000, Eastridge and Clark 2001), we used the exponential growth equation from Gotelli (2008) to estimate the mean annual population growth rate since 1998.

RESULTS

During 2000–2002 at BSF, 83 hair samples were collected, 64 of which assigned to 19 individuals (13M:6F). Twelve (15%) samples failed during genetic analysis and 7 (8%) samples lacked sufficient DNA for analysis. All individuals sampled were successfully genotyped for 7 microsatellites with no 1-MM or 2-MM pairs in the dataset. The sample met the criterion for HWE ($\chi^2_{14} = 6.82$, $P = 0.98$), although 4 of 21 loci pairings (19% of total) for the 7 markers were in linkage disequilibrium ($P < 0.05$) following Bonferroni sequential correction. Allelic richness (R_A) from the 7 markers was 6.30 (SE = 0.22), genetic diversity as indicated by expected heterozygosity (H_E) was 0.729 (SE = 0.033), and observed heterozygosity (H_O) was 0.822 (SE = 0.023; Table 1). The estimated number of breeding individuals that had genetically contributed to the population (N_B) for the 2000–2002 sample was 12 bears (95% CI: 7–18) following 696 independent comparisons (Table 1).

We acquired data from 39 hair samples from bears at GSMNP during 2004, 24 of which assigned to 22 individuals (10M:12F). Thirteen (33%) samples failed during genetic analysis and 2 (5%) samples lacked sufficient DNA for analysis. All individuals sampled were successfully genotyped for 7 microsatellites with no 1-MM or 2-MM pairs in the dataset. The sample met the criterion for HWE ($\chi^2_{14} = 13.3$, $P = 0.65$) and had no signs of linkage disequilibrium ($P < 0.05$) following Bonferroni sequential correction. Allelic richness (R_A) from the 7 markers was 6.58

Table 1. Estimated genetic parameters of 3 independent datasets comprised of black bear hair collected at Big South Fork in Kentucky (2000–2002), USA, Great Smoky Mountains National Park in Tennessee (2004), USA, and Big South Fork in Kentucky and Tennessee (2010–2012), USA. We estimated allelic richness (R_A), observed heterozygosity (H_O), expected heterozygosity (H_E), and the effective number of breeders (N_B) for each dataset using 7 microsatellite markers.

Location	Year	n^a	R_A	H_O	H_E	N_B
Big South Fork	2000–2002	19	6.30	0.822	0.729	12
Great Smoky Mountains	2004	22	6.57	0.787	0.763	142
Big South Fork	2010–2012	153	7.81	0.739	0.712	35

^a Number of individual bears in sample.

(SE = 0.014), genetic diversity as indicated by expected heterozygosity (H_E) was 0.763 (SE = 0.026), and observed heterozygosity (H_O) was 0.787 (SE = 0.018; Table 1). The estimated number of breeding individuals that had genetically contributed to the population (N_B) for the 2004 GSMNP sample was 142 bears (95% CI: 49–225) following 1,131 independent comparisons (Table 1).

During summer 2010, we collected 156 bear hair samples in Kentucky from 23 individual hair trap sites (mean = 3.3 visited sites/sampling session). We captured only males ≥ 15 km from the original BSF reintroduction area (Fig. 1). Of those hair samples, 25 (16%) lacked sufficient DNA for analysis, and 44 (28%) failed during genetic analysis. The laboratory extracted DNA from the remaining 87 (56%) samples, which produced successful identities for 29 (16M:13F) individual bears. During summer 2012, we collected 1,508 bear hair samples from 83 sample sites (mean = 10.4 visited sites/sampling session) in Tennessee (Fig. 1). Of those samples, 1,491 met our predetermined selection criteria to produce a genotype and we selected 295 samples for extraction and genotyping. Technicians at WGI then selected an additional 34 samples for genotyping, beginning with sites with the most multiple samples. A total of 307 (93%) genotyped samples were assigned to 124 (60M:64F) individuals. In sum, 20.3% of collected samples produced an individual identification for the Tennessee dataset. Two male bears (M516 and M510) were sampled during both 2010 and 2012, and 1 male (M610) was sampled in Kentucky during 2009 by Hast (2010) and also during 2012 in Tennessee. No bears sampled during 2000–2002 were resampled during 2010–2012 in either Kentucky or Tennessee.

All individuals sampled in Kentucky and Tennessee during 2010–2012 ($n = 153$) were successfully genotyped for 7 microsatellites with no 1-MM or 2-MM pairs in the dataset. The pooled sample ($n = 153$) met the criterion for HWE ($\chi^2_{14} = 19.1$, $P = 0.16$); however, 1 individual locus (G10L) deviated from HWE ($P \leq 0.001$). Of 21 loci pairings for 7 markers, 11 pairs ($>51\%$ of total) were in linkage disequilibrium ($P < 0.05$) following Bonferroni sequential correction. Allelic richness (R_A) from the 7 markers was 7.81 (SE = 0.53), genetic diversity as indicated by expected heterozygosity (H_E) was 0.712 (SE = 0.025), and observed heterozygosity (H_O) was 0.739 (SE = 0.033; Table 1). When we compared these results with those from BSF during 2000–2002, we did not detect differences (R_A : $U = 23.0$, $Z = 0.893$, 1-tailed $P = 0.187$; H_E : $U = 29.5$, $Z = 0.210$,

1-tailed $P = 0.417$). The estimated number of breeding individuals that had genetically contributed to the population (N_B) for the 2010–2012 sample was 35 bears (95% CI: 26–47) after 1,062 independent comparisons (Table 1).

Sex-specific, closed-population CMR models without some form of heterogeneity in capture probability received essentially no support, which conforms to what has typically been the case for most non-invasive bear CMR studies (Boulanger et al. 2004). Therefore, we excluded non-heterogeneity models from model averaging. The top model for the Kentucky (2010) dataset included a mixture effect on capture probability and a behavioral response to capture that varied by mixture group, whereas the top model for the Tennessee (2012) dataset included a mixture effect on capture probability with mixture proportions varying by sex (Table 2). Model-averaged abundance estimates for males and females in Kentucky were 21 (SE = 5.79, 95% CI: 17–42) and 17 (SE = 9.84, 95% CI: 14–42), respectively, or 38 in total (95% CI: 31–66). Model-averaged abundance estimates in Tennessee were 103 (SE = 39.2, 95% CI: 73–202) and 87 (SE = 45.2, 95% CI: 71–142) for males and females, respectively, or a total of 190 (95% CI: 170–219). Beginning with 18 bears in 1998 and using only the Tennessee total abundance estimate, the estimated annual population growth rate was 18.3% (95% CI: 17.4–19.5%).

DISCUSSION

Our findings conservatively indicated that bears at BSF exhibited very robust population growth that averaged 18.3% across the 14-year period since reintroduction (1998–2012). This rate is conservative because, although possible, the cub component of the population was not likely captured using our hair trap design (Laufenberg and Clark 2014), and we did not include the 2010 estimate from Kentucky. Moreover, the estimate of abundance in Kentucky may have been negatively biased because the trap site density was lower than that used in Tennessee, which can result in undetected heterogeneity (Settlage et al. 2008). Furthermore, the exclusion of a behavioral response to capture in Tennessee probably resulted in a negative bias (Augustine et al. 2014). Although our estimated rate of population growth is one of the highest reported, especially over such an extended period of time, it is within the biological capabilities of black bears (Bunnell and Tait 1981). The relatively low initial growth rate of 2.6% estimated for this population by Eastridge and Clark (2001) was substantially exceeded over subsequent years possibly because male cubs became reproductively

Table 2. Closed population sex-specific models used to estimate abundance (N) of the Big South Fork black bear population in Kentucky (2010), and Tennessee (2012), USA. We modeled individual heterogeneity with a 2-class mixture (π), capture probability (p), and abundance (N) as functions of sex (sex), a mixture effect (mixture), a null effect (~ 1), and a time effect (time; Kentucky (2010) only). We accounted for a behavioral response to capture ($p \neq c$) only for the Kentucky (2010) dataset by using additive and interaction effects of the recapture probability (c).

Model	K^a	AIC_c^b	ΔAIC_c^c	w_i^d	Deviance
Kentucky (2010)					
$\pi (\sim 1)p(\sim \text{mixture} \times c)N(\sim \text{sex})$	7	126.13	0.00	0.42	81.32
$\pi (\sim 1)p(\sim \text{mixture})N(\sim \text{sex})$	5	127.49	1.36	0.22	86.95
$\pi (\sim \text{sex})p(\sim \text{mixture} \times c)N(\sim \text{sex})$	8	127.50	1.37	0.21	80.52
$\pi (\sim 1)p(\sim \text{mixture} \times \text{sex})N(\sim \text{sex})$	7	129.94	3.81	0.06	85.13
$\pi (\sim 1)p(\sim \text{mixture} \times \text{sex} + \text{time})N(\sim \text{sex})$	13	130.55	4.41	0.05	72.39
$\pi (\sim \text{sex})p(\sim \text{mixture} \times \text{sex})N(\sim \text{sex})$	8	132.08	5.94	0.02	85.10
Tennessee (2012)					
$\pi (\sim \text{sex})p(\sim \text{mixture})N(\sim \text{sex})$	6	304.34	0.00	0.33	261.09
$\pi (\sim 1)p(\sim \text{mixture} + \text{sex})N(\sim \text{sex})$	6	304.43	0.09	0.31	261.18
$\pi (\sim \text{sex})p(\sim \text{mixture} + \text{sex})N(\sim \text{sex})$	7	305.88	1.54	0.15	260.61
$\pi (\sim 1)p(\sim \text{mixture} \times \text{sex})N(\sim \text{sex})$	7	306.45	2.11	0.11	261.18
$\pi (\sim \text{sex})p(\sim \text{mixture} \times \text{sex})N(\sim \text{sex})$	8	307.88	3.54	0.06	260.57
$\pi (\sim 1)p(\sim \text{mixture})N(\sim \text{sex})$	5	308.43	4.09	0.04	267.21

^a Number of model parameters.

^b Akaike's Information Criterion corrected for small sample size.

^c Relative difference between AIC_c of model and the highest ranked model.

^d Model weight.

mature, and because bears became settled and more accustomed to the location of resources within the large forested area. Additionally, no bear harvests were permitted at BSF during the 14-year period following reintroduction. Perhaps the most critical component to population growth at BSF was the availability of a large, contiguous area of state and federally managed forested lands that reached high productivity soon after the bear reintroduction; van Manen and Pelton (1997) predicted 49% of all hard mast producing tree species at BSF would reach the optimal production age within 10 years of the 1996–1997 bear reintroduction. Griffith et al. (1989) posited the availability of sufficient habitat quality and quantity was more critical to reintroduction success than supplementation strategies. Assuming the predictions of van Manen and Pelton (1997) are valid, success of the BSF bear reintroduction supports that postulation.

The decision to soft release adult females with cubs during the winter (Eastridge and Clark 2001), which suppresses homing behavior common in bears and conversely increases settling rates (Clark et al. 2002), likely promoted successful establishment of resident bears at BSF during the initial reintroduction phase. Although most of the bears released during summer either died or left the reintroduction area, the majority of bears released during winter remained at BSF (Eastridge and Clark 2001). The winter soft-release technique using adult females with cubs has been successfully used for other bear reintroductions (Clark et al. 2002, Wear et al. 2005, Benson and Chamberlain 2007), and results from our study indicate it is an effective technique to establish resident bears and promote subsequent population growth.

Biologists generally agree that reintroductions should be initiated with a large founder population that exhibits high genetic diversity to facilitate resiliency to environmental stochasticity and to avoid deleterious genetic effects (Griffith et al. 1989, Earnhardt 1999, Frankham 2009b). Although

genetic diversity of the founders was probably high based on our estimate from the 2004 GSMNP dataset ($H_E = 0.763$), the size of the founder group of the BSF bear population declined to as few as 18 individuals by 1998. Our estimate of the effective number of breeders for the 2000–2002 BSF dataset ($N_{B(\text{BSF}, 2000-2002)} = 12$) supports the presumption of a small founder size. Yet, contrary to traditional population genetics theory, genetic diversity of the BSF population remained relatively constant since reintroduction and compared to the source population, and N_B increased at BSF ($N_{B(\text{BSF}, 2010-2012)} = 35$; Table 1).

Although it is widely accepted that small founder groups are at an increased risk of diversity loss (Allendorf and Luikart 2007), recent studies have posited that this may not always be the case for iteroparous mammals because the loss of alleles may be reduced because of generation overlap (Kaeuffer et al. 2007, Kekkonen et al. 2012, Nyman et al. 2014). Additionally, because offspring diversity (i.e., heterozygosity) depends on the genetic composition of parents, and 2 homozygous parents can produce heterozygous offspring, repetitive selection for heterozygotes could increase diversity in reintroduced iteroparous species (Kaeuffer et al. 2007). For example, Kekkonen et al. (2012) conducted a genetics study of an introduced white-tailed deer (*Odocoileus virginianus*) population in Finland sourced from a founder group size of 4 individuals. They discovered that even though the population experienced a historical bottleneck and no supplementation, high levels of genetic diversity were retained in this isolated population more than 75 years after the founder event. Kekkonen et al. (2012), attributed this to overlapping generations, but more importantly, also to a high initial population growth rate that caused the population to remain small for a relatively short time period, thereby retaining diversity. Similarly, Kaeuffer et al. (2007) investigated the genetic consequences of an isolated island population of moufflons (*Ovis aries*) founded

by only 2 individuals. They found that genetic diversity increased in the absence of supplementation and attributed this to a high population growth rate along with the natural selection of heterozygotes.

Our results indicate that the deleterious genetic effects commonly observed in reintroduced populations sourced from small founder groups have not occurred in the BSF bear population. Typically, in the absence of supplementation, this would suggest that immigration and resulting gene flow into the population has occurred at a level substantial enough to mitigate these effects. Supporting this is the identification of 2 male migrants during 2009 in BSF by Hast (2010). Yet, the level of immigration estimated by Hast (2010; $N_m = 0.735$) was below the minimum threshold of 1–10 migrants per generation that is needed to effectively mitigate deleterious genetic effects (Mills and Allendorf 1996). Furthermore, even if both migrants identified by Hast (2010) were completely heterozygous, and assuming both individuals successfully reproduced, their genes would likely have had a negligible impact on diversity considering the rapidly growing founding population of bears (Nei et al. 1975, Kekkonen et al. 2012). Though possible because of new allele admixtures from immigration (Hartl 2000, Frankham et al. 2003), the level of linkage disequilibrium we observed in the 2010–2012 dataset (>51% of pairings) would be accompanied by increased H_E and H_O , and the effective number of breeders would likely be higher than what we observed (Nunney 1993, Miller et al. 2009). A low number of breeders at BSF was supported by a parentage analysis conducted by Murphy (2011) during 2009–2010, which indicated that roughly 30% of the sampled individuals ($n = 48$) were descendants of a single father–son pair of male bears.

Although the 2 migrants identified by Hast (2010) could have introduced new alleles into the BSF population thereby increasing R_A , we found no differences between 2000–2002 and 2010–2012. Therefore, similar to Kaeuffer et al. (2007) and Kekkonen et al. (2012), we suggest that the overlapping generations of bears and high population growth rate at BSF, combined with the natural selection of heterozygotes in the absence of adequate gene flow, caused genetic diversity to be retained in the BSF population. Our postulation is further supported by Groombridge et al. (2012) who noted that rapid population growth of iteroparous mammals was associated with no difference in genetic diversity between reintroduced and source populations. More recently, Puckett et al. (2014) discovered that a reintroduced black bear population in Arkansas exhibited relatively constant genetic diversity since reintroduction as well, and posited that this may have been the result of a high population growth rate.

Classical population genetics theory was developed based on semelparous species and, therefore, may not be applicable to iteroparous species that have overlapping generations, such as bears (Johnson 1977). Our findings combined with those of Puckett et al. (2014) support this postulation for bears but also accentuate the interplay of variables in reintroductions, some of which may be beyond management control (i.e., immigration). Additionally, Palstra and Fraser (2012) posited that the incorrect application of methods to

estimate genetic parameters for iteroparous species with overlapping generations has remained a problem in wildlife studies. Methods developed for semelparous species are often used for iteroparous species but can produce biased estimates and inaccurate inferences about the genetic health of iteroparous species, particularly long-lived species such as black bears, which are capable of many litters over a lifetime, often with multiple paternities in a single litter (Schenk and Kovacs 1995). Therefore, we caution that classical population genetics theory was not developed for iteroparous organisms and as a result may not apply to large mammals with overlapping generations.

MANAGEMENT IMPLICATIONS

Success of the BSF bear reintroduction demonstrates the importance of investigating habitat characteristics of the release area prior to reintroduction, the use of species-specific release techniques that suppress emigration, and the release of founders that exhibit high genetic diversity. Although our findings combined with that of Hast (2010) indicate that gene flow into BSF from neighboring populations remains minimal, we do not perceive this issue as necessitating immediate action but instead should be monitored and considered for future management. We, therefore, suggest non-invasive CMR sampling be repeated to evaluate changes in abundance and growth, which would also allow monitoring of population genetics, thereby affording the opportunity to investigate if gene flow into BSF has increased. Based on our findings, we agree with Kekkonen et al. (2012) that future reintroduction programs for iteroparous large mammals should consider prioritizing efforts towards maximizing population growth of founders regardless of founder group size. This may include allocating financial resources to restore or improve habitat, and providing the population with protection from harvest and illegal kills.

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