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## STATUS OF A REINTRODUCED BLACK BEAR POPULATION IN THE BIG SOUTH FORK AREA OF KENTUCKY

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Sean McCarthy Murphy, Student

Dr. John J. Cox, Major Professor

Dr. David B. Wagner, Director of Graduate Studies

## ABSTRACT OF THESIS

### STATUS OF A REINTRODUCED BLACK BEAR POPULATION IN THE BIG SOUTH FORK AREA OF KENTUCKY

Large carnivores have been subjected to overexploitation and extensive habitat loss for centuries. Reintroduction has become an increasingly used tool for recovering and reestablishing large carnivore populations; however, most reintroductions have either failed or resulted in small populations that are vulnerable to deleterious demographic, environmental, and genetic effects that can lead to population loss or extinction. Long-term monitoring of small, reintroduced populations is critical to population persistence and viability. To evaluate long-term reintroduction success and current status of a recently reintroduced, small black bear (*Ursus americanus*) population in the Big South Fork area of Kentucky, I used non-invasive hair sampling in a systematic, closed-population capture-mark-recapture study design. I used  $\geq 20$  microsatellite loci to identify individual bear, quantify genetic diversity, investigate genetic relatedness, estimate population abundance and density, and investigate patterns of range expansion. The Big South Fork population is comprised of closely-related individuals, is small ( $N = 40$ ; 95% CI: 30-113), of low density (0.03 bear/km<sup>2</sup>), has experienced minimal range expansion, and exhibits decreased genetic diversity ( $H_E = 0.698$ ). Because of prolonged isolation from nearby subpopulations, the Big South Fork population remains vulnerable and requires immediate and continued monitoring.

KEYWORDS: Bear, Big South Fork, Kentucky, Reintroduction, Small Populations

Sean McCarthy Murphy

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December 16, 2011

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THESIS

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A thesis submitted in partial fulfillment of the  
requirements for the degree of Master of Science in the  
College of Agriculture  
at the University of Kentucky

By

Sean McCarthy Murphy

Lexington, Kentucky

Co-Directors: Dr. John J. Cox, Adjunct Assistant Professor of Wildlife and  
Conservation Biology  
and Dr. Songlin Fei, Assistant Professor of Forestry

Lexington, Kentucky

2011

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### Dedication

This work is dedicated to my parents, Patrick and Nora Murphy, who instilled in me the desire to seek challenges in life, and the determination to achieve more.

## ACKNOWLEDGEMENTS

First and foremost, I must thank the late Dr. Dave Maehr, who offered me an opportunity to explore, learn, and grow in a profession in which I had absolutely no experience. Dave was an excellent mentor, researcher, and teacher, but above all, Dave was a great friend. I will forever miss Dave, along with his enthusiasm, humor, and wit.

I am deeply indebted to Dr. John Cox for not only hiring me as a graduate student, but for fighting for me and believing that a ruffian from the Appalachian “hollers” could actually do this science gig. Additionally, I want to thank Dr. Songlin Fei and Dr. Paul Kalisz for serving as committee members, and for providing support from beginning to end on this project. I also want to thank Dr. John Cox, Dr. Paul Kalisz, and Dr. Mike Lacki for their guidance throughout my academic career. Thanks must also be given to Steven Dobey and the Kentucky Department of Fish and Wildlife Resources for providing funding and assistance, and to the University of Kentucky, Department of Forestry for providing funding, support, and an avenue for me to progress and learn.

Many thanks must be given to those who assisted with the various aspects of field work and data analysis. John Hast performed exquisite work in the field, taught me the definition of “loci”, perfected wheel-bearing swaps, provided endless comedic mispronunciations of place names, and had a knack for getting us in downward-spiraling situations hours from civilization, but countered with an uncanny ability to somehow ensure we always survived. I would also like to thank Ben Augustine, whose patience, statistical and analytical prowess, and willingness to help on short notice were key to the success of this project and my improvement as a student. I owe much gratitude to Mike Strunk for showing me the ropes in McCreary County, providing excellent assistance

with field work, and for making my time in McCreary County more enjoyable. I also want to thank Joe Metzmeier for offering more support than I could have ever asked for, and for providing much needed cold ones at just the right time. I would like to thank Bill Ridener, George Corder, Dwight Anderson, Earl Brown, and Kyle Burnett at Beaver Creek WMA for their assistance, humor, and ability to fix anything. Additionally, I want to thank Jayson Plaxico for his advice, support, and excellent communication. Many thanks must also be given to Tim Reed, the United States Forest Service, and the Stearns Ranger District of the Daniel Boone National Forest for housing, support, and cooperation, and to the numerous employees who helped check hair traps. I also want to thank Steve Bakaletz, the National Park Service, and the Big South Fork National River and Recreation Area for their assistance and cooperation.

Finally, I want to thank family and friends who provided support and assistance throughout my graduate career. I would like to thank my brother, Brian Murphy, who not only laboriously helped construct hair traps in uncharted territory, but also took on my graduate school-induced debt without question, and provided irreplaceable support and friendship. I must also thank Rachel Rowe for her endless support, unwavering patience and understanding, and for simply making my life better. Additionally, Joe Guthrie's friendship, humor, and advice have been essential to my growth and success as a graduate student and person. I also owe many thanks to my parents, Patrick and Nora Murphy, my grandparents, Ben and Ute Franklin, and my aunt, Mary Molly Flanders, for their unyielding support and words of confidence.



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## CHAPTER 1: INTRODUCTION

### Wildlife reintroductions

The International Union for Conservation of Nature and Natural Resources (IUCN) defined reintroduction as “an attempt to establish a species in an area which was once part of its historical range, but from which it has been extirpated or become extinct” (IUCN 1998). Reintroduction is a frequently used tool for wildlife management that has led to the successful reestablishment of animal species across the globe (Griffith et al. 1989). The majority of wildlife reintroductions in North America have focused on mammal conservation (Fischer and Lindenmayer 2000), the most frequently reintroduced mammal species being top-order predators (Hayward and Somers 2009). More than 28 reintroduction projects have been implemented for six North American large carnivores within the last century (Breitenmoser et al. 2001). For example, the federally-endangered gray wolf (*Canis lupus*) was reintroduced to Yellowstone National Park after a nearly century-long absence (Bangs and Fritts 1996), brown bear (*Ursus arctos*) were reintroduced to the Cabinet Mountains of Montana to supplement an extremely small, isolated population thought functionally extinct (Servheen et al. 1995), and multiple black bear (*Ursus americanus*) reintroduction projects have occurred since the 1930s for a variety of reasons (Clark et al. 2002). Large carnivores have often been considered keystone species, and reestablishment of these species may increase natural biodiversity and allow recovery of ecosystem processes (Seddon 1999).

Ultimately, the goal of any reintroduction is to establish a population that persists without intervention (Seddon 1999). Determining reintroduction success, however, can be difficult with no definitive protocol to aid researchers in the confirmation process

(Seddon 1999, Fischer and Lindenmayer 2000, Gusset 2009). Success of most reintroductions has been evaluated based on the establishment of a self-sustaining population (Swaisgood 2010). For example, the Colorado Division of Wildlife (CDOW) declared the Canada lynx (*Lynx canadensis*) reintroduction a success based on consistent documentation of reproduction of kittens over a 7-year period (CDOW 2010). Currently, the IUCN Species Survival Commission (SSC)/Re-introduction Specialist Group (RSG) requires >1000 mature individuals to be present in a population for a species to be listed as vulnerable or better (IUCN 2001), and Griffith et al. (1989) defined reintroduction success as a self-sustaining population of >500 individuals. Many naturally-occurring wildlife populations, especially large carnivore populations, however, do not meet these criteria, and would require augmentation had populations resulted from reintroductions (Hayward and Somers 2009). Therefore, Gusset (2009) recommended criteria to evaluate reintroduction success, divided into short-term and long-term assessments: 1) short-term success is achieved if the founder generation survives, and reproduction occurs, and 2) long-term success is achieved if the population persists over time. Seddon (1999) provided a definition of reintroduction success similar to Gusset's (2009), but included an additional criterion; reproduction in the founder generation's offspring. While short-term success may be quickly evaluated, determining overall success of reintroductions may take many years (Fischer and Lindenmayer 2000), which reinforces the need for long-term monitoring of reintroduced populations.

Life history traits of reintroduction candidate species can limit the applicability of any success criteria (Seddon 1999). For instance, behavioral and natural history characteristics of species can influence breeding success, dispersal, and settling (Hayward

and Somers 2009). Furthermore, failure to understand single species and community ecology can be fatal to reintroduction programs. For example, if habitat quality and quantity are insufficient, reintroduction failure can quickly occur (Hayward and Somers 2009). Additionally, genetic characteristics of founder populations are important to both short-term and long-term success, especially when reintroductions occur in isolated or fragmented areas (Frankham 2009).

In general, success of reintroductions can be enhanced if a large number of founders with high genetic variability are released in suitable habitat, and exhibit high population growth rate, low mortality, and low intraspecific competition (Griffith et al. 1989, Thatcher et al. 2006). Many reintroduction programs, however, often release a small number of founders. Therefore, long-term monitoring is crucial to assess population status and reintroduction success, and to determine if management intervention is needed (De Barba et al. 2010). Demographic information such as population abundance, growth rate, reproduction, mortality, immigration, and genetic diversity should be monitored at pre-defined time intervals following reintroductions (Seddon 1999, De Barba et al. 2010). Additionally, ecological characteristics, such as patterns of range expansion, dispersal, and connectivity with nearby populations, are critical for management. Many researchers, however, often do not implement long-term monitoring strategies for reintroduced populations, despite the known importance of such programs (Sarrazin and Barbault 1996, De Barba et al. 2010). As a result, numerous reintroductions have either failed or resulted in very small populations (Frankham 2009, Hayward and Somers 2009).

Because most surviving reintroduced populations are small (Frankham 2009), they are vulnerable to demographic and environmental stochasticity, such as density-independent mortality and natural disasters (Lande et al. 2003). Furthermore, small populations are susceptible to deleterious genetic effects, such as inbreeding depression, genetic bottleneck, low effective population size, and genetic drift (Hartl 2000, Brook 2008, Frankham 2009, Johnson et al. 2010). Combined, these factors can ultimately lead to extinction of populations or, in extreme cases, entire species (Brook 2008); however, with proper monitoring, researchers can devise management actions to combat these effects (Frankham 2009). Therefore, long-term monitoring of small, reintroduced populations is critical to afford researchers opportunities to implement timely and well-informed conservation strategies (Gusset 2009).

### **Bear reintroductions**

Bear species worldwide have been targets of anthropogenic exploitation and associated habitat destruction for millennia. Of these threats, habitat loss and overexploitation have been the primary causes of decline of the genus (Clark et al. 2002, Clark 2009). Within the last half-century, bear habitat in many areas has recovered, and most bear species have been put under some form of legal protection to prevent overexploitation (Pelton 2001; 2003, Clark 2009); both factors have allowed many bear species, particularly black bears, to increase in distribution and numbers to increase. Many bear populations, however, remain isolated within fragmented landscapes, which can impede dispersal and recolonization of historic ranges (Dixon et al. 2007, Clark 2009). Furthermore, a number of bear populations have become increasingly threatened by the effects of small population size (Servheen 1998). Consequently, reintroductions

may be required to overcome anthropogenic landscape barriers (van Manen and Pelton 1997) and to reverse deleterious genetic effects (Frankham 2009, Johnson et al. 2010).

Bear reintroductions have been implemented since the 1930s with varying success (Clark et al. 2002). Perhaps the most successful reintroduction occurred in Arkansas where 254 black bear were translocated from Minnesota and Canada from 1958-1968 (Smith and Clark 1994, Clark et al. 2002). Within 20 years, this population increased to >2500 individuals (Smith and Clark 1994). In contrast, a 1938 brown bear reintroduction in Bailowieza, Poland ultimately failed due to overexploitation and illegal poaching (Buchalczyk 1980). More recently, a number of black bear reintroductions have occurred in the eastern and southeastern United States, many with unknown outcomes (Clark et al. 2002).

Similar to other large carnivores, inherent behavioral attributes of bears can pose potential post-reintroduction challenges to restoration programs. Homing behavior (*i.e.* a species' ability to return to its original location following translocation) by bears has been problematic in many reintroduction projects (Eastridge 2000, Clark et al. 2002, Clark 2009), and serves as a formidable obstacle to reintroduction success. Male bear typically have large, but poorly defined home ranges and wide-ranging dispersal, whereas females usually have smaller, more well-defined home ranges and exhibit strong philopatric tendencies (Schwartz and Franzmann 1992, Clark et al. 2002). While homing is not uncommon among male bear, such behavior is generally less likely among females, but has been documented (Eastridge 2000, Eastridge and Clark 2001). Recent studies have investigated multiple techniques to mitigate homing behavior in bear reintroductions. Suggestions have been made for winter soft-release of adult females with cubs to



improve settling rates and reduce the likelihood of homing exhibited by translocated individuals (Eastridge and Clark 2001, Clark et al. 2002, Clark 2009). In addition to problems caused by homing, low reproductive rates (adult females typically produce offspring every other year - Bunnell and Tait 1981) can impede initial establishment and long-term population viability. As such, Clark et al. (2002) described the black bear as a poor colonizer, and suggested supplementation may be required following initial reintroduction to enhance population growth and persistence.

### **Black bear in Kentucky**

The black bear historically inhabited all of Kentucky (Barbour and Davis 1974, Hall 1981), and was so ubiquitous and abundant during European settlement that the state was sometimes referred to as the “The Bear State”. For example, during Dr. Thomas Walker’s exploration of Kentucky in 1750, his party killed 53 bear, among a host of other large mammals, and stated “we might have killed three times as much meat, if we had wanted it” (Walker 1750). By the end of the 19<sup>th</sup> century, however, the black bear was extirpated from Kentucky most likely as a result of a combination of overexploitation, habitat loss, and habitat fragmentation (Barbour and Davis 1974, Pelton 2001, Unger 2007, Hast 2010).

During the 20<sup>th</sup> century, the human population in Kentucky increased, cities expanded, forests were converted to agriculture, and miles of road were created. As of 2010, Kentucky was inhabited by ~ 4.3 million people (United States Census Bureau 2010a), agriculture accounted for approximately 56% of the land area (United States Department of Agriculture 2011), and more than 78,000 miles of roads existed (American Automobile Association 2010). Despite such large-scale anthropogenic impacts,

however, the black bear successfully recolonized a portion of eastern Kentucky prior to the end of the 20<sup>th</sup> century (Unger 2007).

Currently, 2 genetically differentiated black bear subpopulations occur in Kentucky (Hast 2010). One subpopulation, considered most abundant (Frary 2008, Hast 2010), is located in extreme southeastern Kentucky counties along the borders of Virginia, West Virginia, and Tennessee (Figure 1.1). This subpopulation (hereafter referred to as Pine Mountain population; PMP) resulted from a natural recolonization event over the last half-century from the aforementioned border states (Unger 2007, Frary 2008, Hast 2010), and likely forms the western-most extent of a regional metapopulation (Hast 2010). A separate subpopulation (hereafter referred to as Big South Fork population; BSFP), located in McCreary County, Kentucky, along the Tennessee border, resulted from a limited reintroduction into the Big South Fork National River and Recreation Area (BSF) in Kentucky and Tennessee (Figure 1.1).

### **Black bear in the Big South Fork**

Black bear historically inhabited the Big South Fork area of Kentucky and Tennessee, until the last one was reportedly killed during the early 1900s in Scott County, Tennessee (Eastridge 2000 from Smith 1985). Overexploitation and habitat loss were likely causes of the species' demise in the area. By the late 1970s, much of the land area in the region had been acquired by the United States Forest Service (USFS – Daniel Boone National Forest) and the National Park Service (NPS – Big South Fork National River and Recreation Area), and forest maturation was allowed to occur on most of the newly acquired land.

A working group comprised of the Kentucky Department of Fish and Wildlife Resources (KDFWR), Tennessee Wildlife Resources Agency (TWRA), NPS, USFS, United States Fish and Wildlife Services (USFWS), and the University of Tennessee (UT) was formed in 1987 to consider reintroducing black bear into the Big South Fork area (Eastridge 2000). In 1990, researchers performed a habitat suitability analysis in the BSF to evaluate bear habitat quality, and concluded the area could support black bear (van Manen 1990, van Manen and Pelton 1997, Eastridge 2000). Following approval, an experimental reintroduction was implemented to restore black bear in the BSF.

In 1996 and 1997, researchers at UT and NPS reintroduced black bear into the BSF by translocating 14 adult female black bear with 16 cubs from Great Smoky Mountains National Park (GSMNP) (Eastridge 2000, Eastridge and Clark 2001). Of 6 total release sites in the BSF, 3 were located in Scott County, Tennessee, and 3 were located in McCreary County, Kentucky (Eastridge 2000) (Figure 1.2). Assessment of short-term reintroduction success was evaluated by UT and NPS in 1998 and 1999. By November 1999, 3 adult founders had left the BSF and never returned, and an additional 4 founders had died (Eastridge 2000). However, reproduction was documented in 2 of the remaining 7 founder females during winter 1999, with production of 5 total cubs (Eastridge 2000), and adult female survival was estimated at 0.66 (SE = 0.12) (Eastridge 2000, Eastridge and Clark 2001). According to Gusset's (2009) evaluation criteria, the reintroduction could likely be considered a short-term success; however, the fate of black bear in the BSF since those early observations remained unknown.

van Manen and Pelton (1997) had recommended 40 individual bears be released in the BSF over a 6-7 year period to ensure persistence of the population. In 2000, researchers were scheduled to release an additional 12 adult female bears in the BSF. Concerns voiced by the public, however, resulted in the Fentress County, Tennessee Chamber of Commerce passing a resolution that halted this release, and banned all further releases of black bear. Although population modeling suggested extinction would occur without further supplementation of individuals (Eastridge and Clark 2001), no supplementation occurred following the original reintroduction (Eastridge 2000), and no long-term monitoring strategies were devised. As of 2002, biologists at UT were uncertain if the reintroduction was successful at establishing a viable, self-sustaining population (Clark et al. 2002).

Bear were assumed to still be confined within the BSF until recently (M. Strunk, KDFWR, pers. comm.). Since 2004, biologists from KDFWR have received multiple reports of black bear sightings, including adult females with cubs, in areas outside the boundary of the BSF in McCreary County, Kentucky (J. Plaxico and M. Strunk, KDFWR, pers. comm.). Additionally, nuisance complaints and bear-vehicle collisions increased in McCreary County, Kentucky, over the last 6 years. Documented nuisance complaints increased from 0 in 2004 to >100 since 2006, and documented bear-vehicle collisions increased from 0 prior to 2004 to a total of 7 since 2004 (J. Plaxico, KDFWR, pers. comm.). The increase of reported sightings, nuisance complaints, and bear-vehicle collisions in recent years suggests this population may have increased in number, and range expansion occurred.

Long-term monitoring of reintroduced populations is essential to the success of reintroduction projects (Seddon 1999, Fischer and Lindenmayer 2000, Hayward and Somers 2009), and to track changes in small populations (Gusset 2009, De Barba et al. 2010). Fischer and Lindenmayer (2000) suggested evaluating parameters, such as abundance, sex ratio, and population growth rate at predefined time intervals following reintroductions; however, after the initial stages of monitoring, little research was conducted on the BSF population. In 2002, KDFWR conducted a non-systematic, non-invasive genetic hair trap survey as a pilot detectability study in McCreary County, Kentucky. Results identified 16 individual bears, and estimated genetic diversity (*i.e.* expected heterozygosity) at  $H_E = 0.819$  (KDFWR, unpublished data, Hast 2010). In 2009, Hast (2010) conducted a non-invasive genetic hair trap survey and identified 19 individual bears in McCreary County, Kentucky. Interestingly, Hast (2010) found that genetic diversity in the BSF population had decreased to  $H_E = 0.770$  since 2002. Nonetheless, genetic diversity remained relatively high; perhaps suggesting a remnant population of bears may have existed in the Big South Fork area prior to the reintroduction (Hast 2010). Neither the 2002 study nor the 2009 study were designed to produce abundance or density estimates for black bear in the BSF population. To date, no studies have produced estimates of these population parameters for the BSFP, which are fundamental to evaluating success of reintroductions, especially when the number of founders is low, and for management of small wildlife populations (Fischer and Lindenmayer 2000, Gusset 2009, De Barba et al. 2010).

If the BSF population has increased in number and range expansion occurred, these results likely stem from natural processes within the population, not from immigration from nearby source populations (Figure 1.3). While forested lands, and presumably good bear habitat do exist in the area between the known extents of the PMP and BSFP, immigration of individual bears into the BSF population appears to be minimal. Hast (2010) identified only 2 migrants in the BSFP, both sourced from the neighboring Pine Mountain population. Additionally, Hast (2010) performed a STRUCTURE analysis at  $K = 2$  and  $K = 3$  subpopulations, and concluded the BSF population was comprised of a single genotype identical to that of black bear in the GSMNP. These results suggest the BSF population is primarily the product of the original reintroduction, and may be isolated from other black bear subpopulations in the region (Figure 1.3).

Increasing the likelihood of isolation is the fact that the nearest major black bear population beyond the Pine Mountain population is in the Great Smoky Mountains, approximately 132 km away from the BSF (Figure 1.3). Substantial anthropogenic barriers exist between the BSF and GSMNP, including Interstate 75 and the greater Knoxville, Tennessee, area. No other known black bear populations exist within the states of Kentucky or Tennessee to the north or west of the BSF. Therefore, deleterious genetic effects, such as inbreeding depression, could result from prolonged isolation in this presumably small black bear population.

Collectively, observations of black bear in the BSF area since 2002 suggest the population has grown and expanded, but empirical evidence to support this assumption is lacking. Multiple natural resource agencies and the general public have expressed

interest in better understanding the current status of the black bear in the greater Big South Fork area. In addition, there is considerable pressure to expand the Kentucky black bear hunt to include the BSF population in McCreary County (S. Dobey, KDFWR, pers. comm.). In contrast, there is widespread public sentiment in the Commonwealth for protecting black bear in the BSF population and elsewhere in the state. Furthermore, given the potential deleterious impacts that overharvest can have on small bear populations (Clark et al. 2010), estimates of demographic parameters, such as abundance and density, for black bear in the BSF population are much needed.

### **Non-invasive genetic sampling**

Long-term population monitoring is critical for management of reintroduced and small wildlife populations (De Barba et al. 2010), particularly large carnivores, such as black bear, which often are relegated to fragmented or isolated landscapes (Settlage et al. 2008, Clark 2009). Capture-mark-recapture (CMR) is widely-accepted among researchers as a tool for estimating demographic parameters of black bear, such as abundance, density, growth rate, occupancy, and sex ratio (Garshelis and Hristienko 2006). By sampling a portion of a population, CMR modeling can be used to extrapolate indices and provide estimates for the sampled population (Nichols 1992).

Historically, CMR was used with live-trapping data, observational data, or harvest data (Pelton 2003). These approaches, however, often yielded low sample sizes (Coster et al. 2011, Marucco et al. 2011) because bear, in general, are solitary, display wide-ranging movements, often inhabit landscapes at low densities, and exhibit cryptic behavior (Mowat and Strobeck 2000, Coster et al. 2011). Non-invasive genetic CMR methods have become increasingly used (Waits and Paetkau 2005) among bear

researchers and managers as practical and economic alternatives for estimating demographic parameters of populations (Woods et al. 1999, Boersen et al. 2003, Gardner et al. 2010). Non-invasive genetic sampling for bear typically employs a systematic sampling regime using either transects or grids, and the collection of hair or fecal samples via hair traps or scat detection dogs (Long et al. 2007, Long et al. 2008). Such non-invasive sampling methods may reduce negative effects on study animals because researchers do not have to physically observe, handle, or live-capture study animals (Long et al. 2008). Additionally, 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, has further increased the applicability and usefulness of non-invasive genetic sampling methods (Taberlet and Luikart 1999, Waits and Paetkau 2005). Microsatellites are short, tandem repeats that are highly polymorphic and easy to isolate (Waits and Paetkau 2005). Therefore, genetic samples containing DNA (deoxyribonucleic acid), such as hair and feces, can be amplified using the polymerase chain reaction (PCR). These molecular markers are advantageous because DNA amplification is possible even with minute amounts of tissue or by-product samples, such as hair and feces (Taberlet and Luikart 1999).

To investigate the status of black bear in the BSF population, I used non-invasive hair sampling in a capture-mark-recapture study design to estimate abundance and density of this population. I used individual genotypes of black bear to investigate relatedness of extant individuals in the BSFP by employing parentage analysis. I



quantified genetic diversity of black bear in the BSFP by calculating expected heterozygosity ( $H_E$ ). I also investigated range expansion using non-invasive genetic sampling and program ArcMap (Environmental Systems Research Institute - ESRI, Redlands, CA). I used these results to evaluate long-term success of the black bear reintroduction in the BSF, and to provide wildlife managers with data applicable to management of black bear in this area.

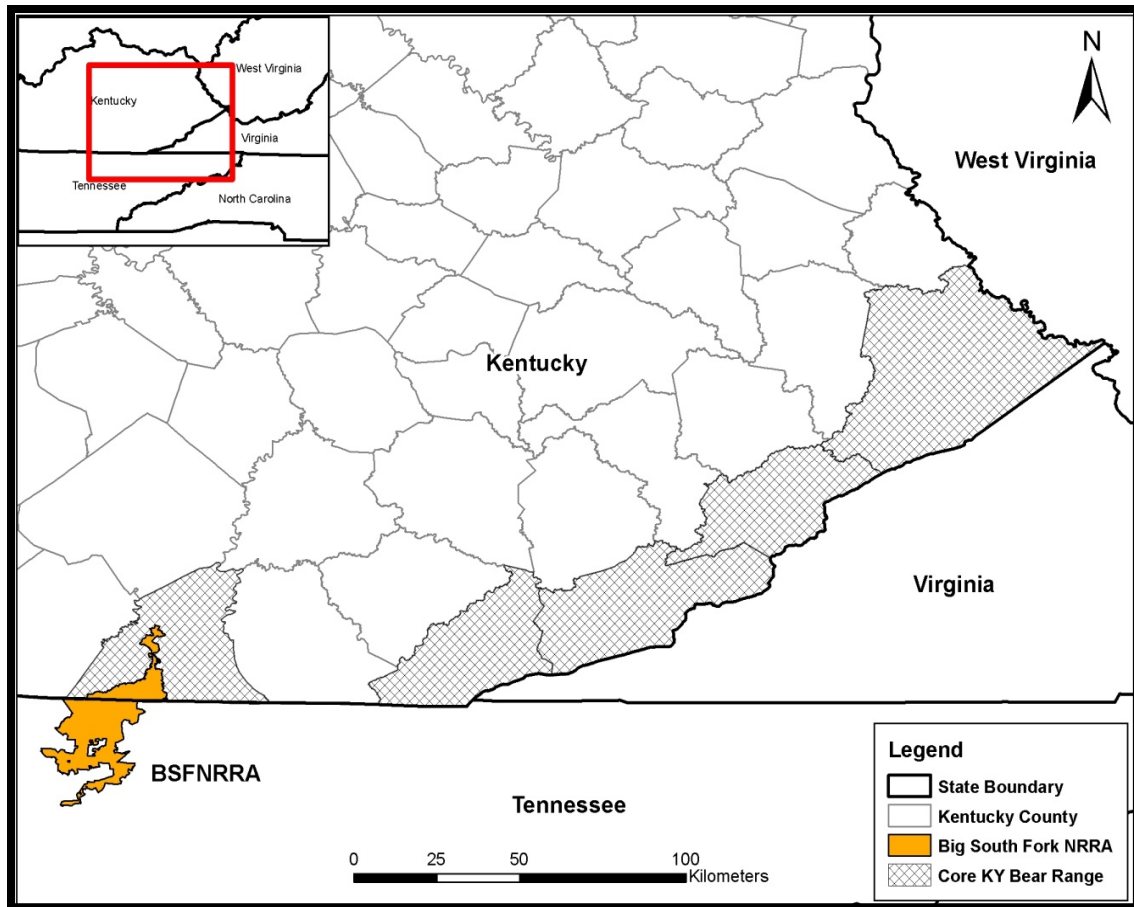


Figure 1.1: Kentucky counties with the highest number of black bear nuisance and observation reports (KDFWR, unpublished data) used to delineate core bear range within the state (1987-2010). The Pine Mountain population core was comprised of the four southeastern-most counties bordering Virginia, West Virginia, and Tennessee. The Big South Fork population included the Big South Fork National River and Recreation Area, McCreary County, Kentucky, and Scott County, Tennessee. From Hast (2010).

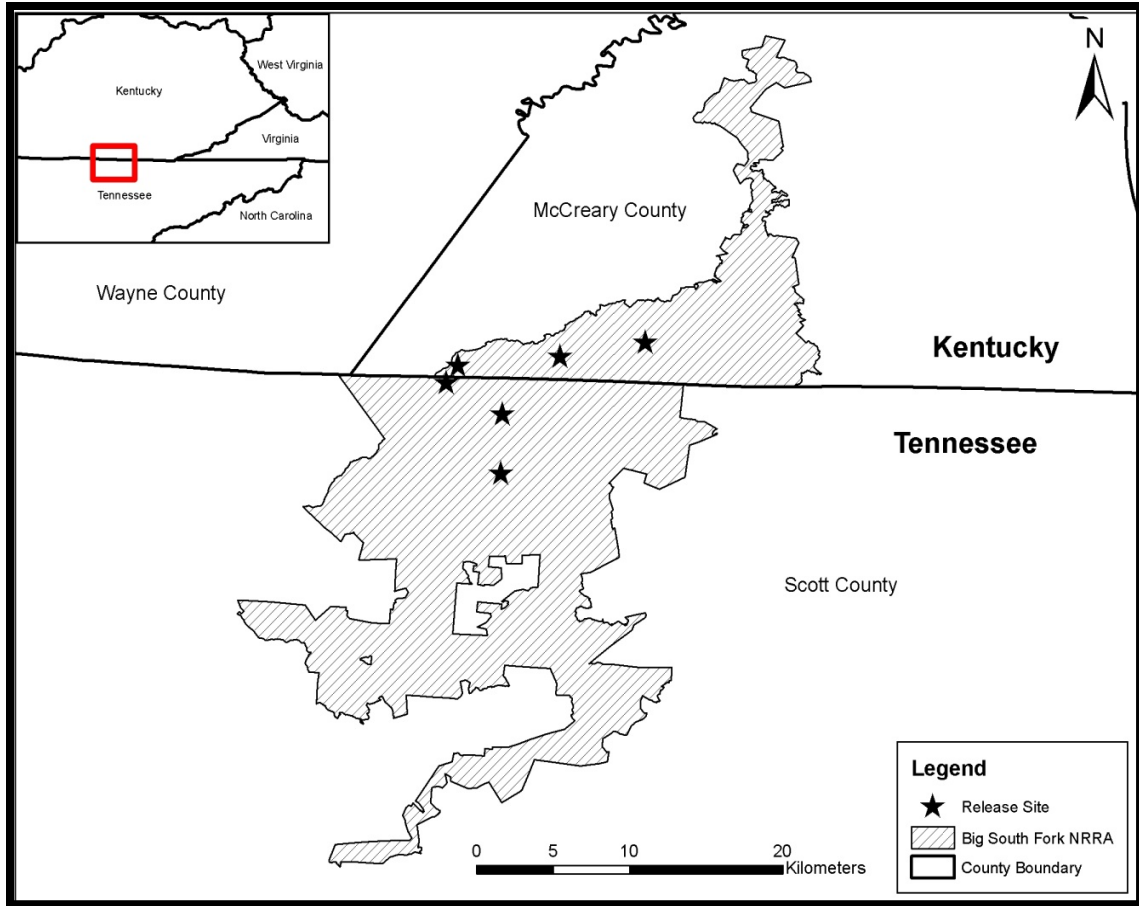


Figure 1.2: Approximate release site locations of 14 female black bears with 16 cubs translocated from the Great Smoky Mountains National Park to the Big South Fork National River and Recreation Area in 1996-1997. Three release sites were in McCreary County, Kentucky, and three release sites were in Scott County, Tennessee. Modified from Eastridge (2000).

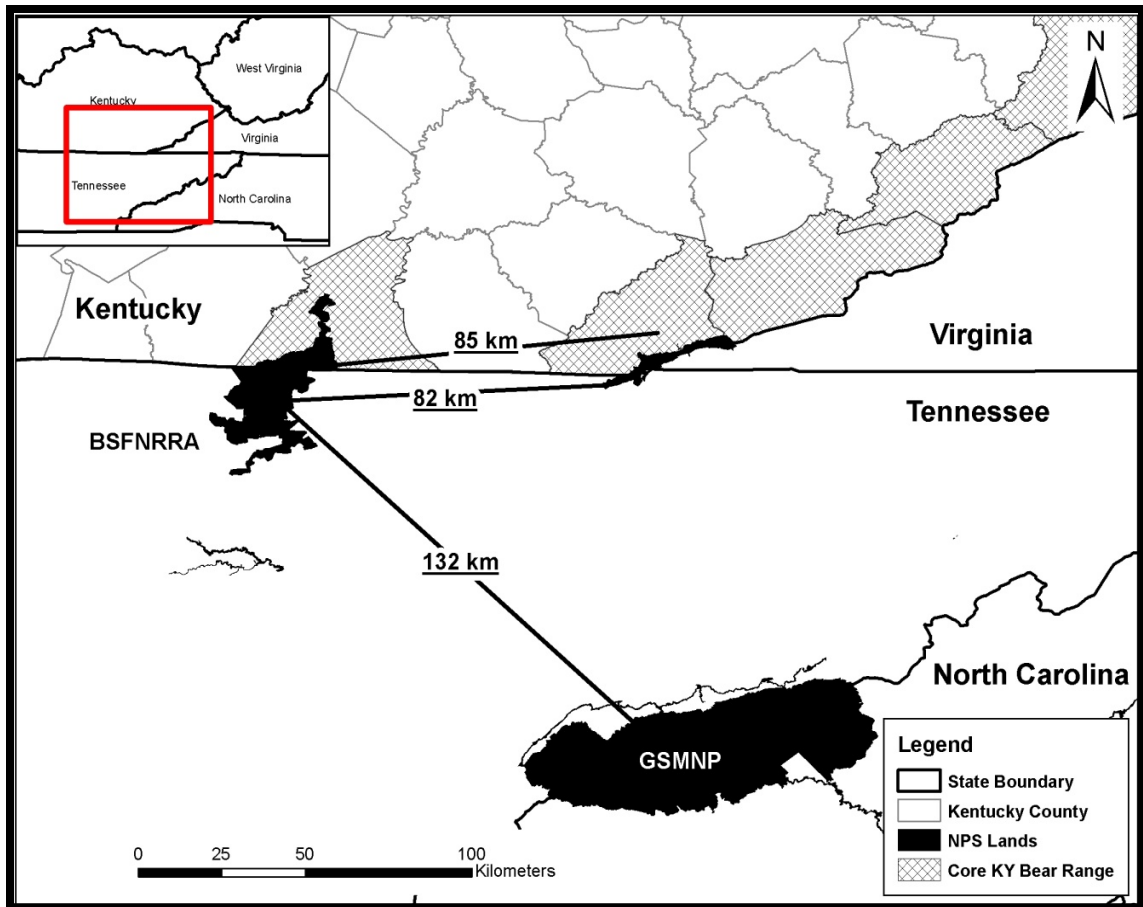


Figure 1.3: Locations of the nearest major black bear source populations relative to the Big South Fork population. Modified from Hast (2010).

## **CHAPTER 2: STUDY AREA AND METHODS**

### **Study Area**

The study area encompassed 1,270 km<sup>2</sup> of the western edge of the Cumberland Plateau physiographic region in south-central Kentucky at the Tennessee border (Figure 2.1). The Cumberland Plateau is characterized by forested, nearly horizontal ridge tops and deep, narrow valleys cut by multiple rivers and streams, such as the Cumberland River (Kleber 1992). Elevations ranged from 150-460 m. This region of the Cumberland Plateau typically has mild winters and hot, humid summers, with an average annual temperature of 13°C, and 133 cm of average annual precipitation (Shaw and Wofford 2003). The study area was bordered to the northeast by the Cumberland River, to the south by Scott County, Tennessee, and was bisected by highway U.S. 27 (Figure 2.1). Research was conducted in all 1,118 km<sup>2</sup> of McCreary County, and in neighboring portions of Laurel, Pulaski, Wayne, and Whitley counties (Figure 2.1).

Approximately 81% (906 km<sup>2</sup>) of McCreary County was owned by the federal government, the predominant cover type being second and third growth mixed-mesophytic forest. The Stearns Ranger District of the Daniel Boone National Forest (DBNF) and the National Park Service's Big South Fork National River and Recreation Area (BSF) managed approximately 63% (705 km<sup>2</sup>) and 18% (201 km<sup>2</sup>) of lands in the county, respectively. An additional 6% (69 km<sup>2</sup>) was state government land in the Beaver Creek Wildlife Management Area, managed by KDFWR (Figure 2.1). The remaining 12.8% (143 km<sup>2</sup>) of McCreary County was privately owned and consisted of a matrix of forest and agricultural lands, primarily located in the south-central portion of the county.

Forests in the study area are part of the mixed-mesophytic classification characterized as having up to 30 co-dominant canopy tree species (Braun 1950). At lower elevations, predominant tree species included maples (*Acer* spp.), oaks (*Quercus* spp.), hickories (*Carya* spp.), magnolias (*Magnolia* spp.), birches (*Betula* spp.), ashes (*Fraxinus* spp.), yellow poplar (*Liriodendron tulipifera*), blackgum (*Nyssa sylvatica*), American basswood (*Tilia americana*), beech (*Fagus grandifolia*), flowering dogwood (*Cornus florida*), eastern hemlock (*Tsuga canadensis*), and rhododendron (*Rhododendron* spp.) (Braun 1950, Wharton and Barbour 1973). At higher elevations, predominant tree species include maples (*Acer* spp.), oaks (*Quercus* spp.), hickories (*Carya* spp.), magnolias (*Magnolia* spp.), ashes (*Fraxinus* spp.), yellow poplar (*Liriodendron tulipifera*), pines (*Pinus* spp.), yellow buckeye (*Aesculus octandra*), sassafras (*Sassafras albidum*), eastern red cedar (*Juniperus virginiana*), and understory shrubs including mountain laurel (*Kalmia latifolia*), rhododendron (*Rhododendron* spp.), and highbush blueberry (*Vaccinium simulatum*) (Braun 1950, Wharton and Barbour 1973).

Forest management on federal government lands differed between the DBNF and BSF. Forests in the DBNF were managed for multiple uses, including timber, water, wildlife, fish, minerals, and recreation activities (DBNF 2009). Active management plans commonly implemented in the DBNF included prescribed burning and timber harvesting. Forests in the BSF were managed for multiple uses as well, primarily recreation and conservation, but did not include timber harvesting (NPS 2005). Removal of timber in the BSF was only permitted for development of public and administrative facilities (NPS 2005). Prescribed burning was permitted and used as a management tool in the BSF.

The human population in McCreary County, Kentucky, was estimated at approximately 18,300 individuals in 2010 (USCB 2010b). The largest community in McCreary County was Whitley City, Kentucky, which incorporated approximately 6 km<sup>2</sup> (0.5%) of land area. Other human-inhabited areas in McCreary County included the small communities of Stearns, Strunk, Pine Knot, and Parkers Lake.

## **Methods**

Because the size and distribution of the black bear population in the BSF has remained unknown since reintroduction, non-invasive genetic sampling was used in a systematic capture-mark-recapture framework to estimate population abundance, density, and range expansion. Many bear studies that investigate or monitor the status of reintroduced or small populations often seek additional information regarding population characteristics, such as genetic parameters, for effective management (De Barba et al. 2010); non-invasive genetic sampling affords researchers the opportunity to quantify this and other population parameters. Because the BSF black bear population originated from a small number of founders relatively recently (Eastridge 2000), non-invasive genetic sampling was used to quantify genetic diversity (*i.e.* expected heterozygosity), and to investigate relatedness and population structure of extant individuals.

### **Non-invasive hair sampling**

A non-invasive hair trap sampling grid composed of 127 contiguous sampling cells was created using ArcMap 9.3 Geographic Information Systems (ESRI, Redlands, CA) and superimposed across a map of the 1,270 km<sup>2</sup> study area (Figure 2.2). Each sampling cell encompassed 10 km<sup>2</sup>; an area equivalent to the average annual spring home

range (*i.e.* smallest annual home range) of adult female black bear in Kentucky based on ~3 years of Global Positioning Systems (GPS) radio-collar data (University of Kentucky, unpublished data). One baited, barbed-wire hair trap was constructed in each 10 km<sup>2</sup> sampling cell to collect black bear hair for DNA analysis (Woods et al. 1999). Settlage (2005) recommended  $\geq 4$  sampling sites/female home range for non-invasive black bear studies in the southeastern United States. Due to limited resources and personnel, however, such sampling intensity was not feasible in this study. One sampling cell within the grid was excluded because landowner permission for access was denied; therefore, 126 sampling sites, with 1 hair trap/site, were used (Figure 2.2). Hair trap placement was restricted to locations between 100-250 m from roads to enable efficient access; however, if campgrounds, picnic areas, or residential areas were present, a minimum buffer of 500 m was used to mitigate human-bear conflict.

A hair trap consisted of one, 4-point barbed-wire strand wrapped around 3-4 corner trees, ~35 cm above the ground to create a ~25-m enclosure (Woods et al. 1999). Hair traps were marked with fluorescent flagging as a human safety precaution. Traps were baited every 7 days with a combination of sardines and pastry, suspended between 2 trees ~3 m above the ground. Each barb on the barbed-wire was treated as a separate sample. Collected samples were placed in individually-labeled paper coin envelopes, categorized by trap session, trap number, and sample quality (*i.e.* approximate number of hairs). Following collection of samples, barbs were flame-sterilized to prevent future contamination. Hair samples were air-dried at room temperature for 24 hours and immediately frozen. All hair traps were checked and re-baited every 7 days for 7 consecutive, week-long sampling sessions from 23 May 2010 to 11 July 2010. Weekly



duration hair-trapping sessions were chosen to maintain equal trapping effort, and to reduce the risk of DNA degradation in the humid environment of the study area (Shaw and Wofford 2003). Traps were not moved between or during sampling sessions.

### **Genetic analyses**

All hair samples were shipped to Wildlife Genetics International (Nelson, British Columbia) for DNA extraction and amplification using the polymerase chain reaction (PCR). Eight black bear-specific microsatellite loci (G10B, G10H, G10J, G10P, G10L, G10M, MU23, and MU59) were used to identify individual black bear according to the methods described in Paetkau and Strobeck (1994) and Paetkau (2003). A gender marker, ZFX/ZFY, was used to delineate sex of identified individuals (Ennis and Gallagher 1994). These 9 markers were used to obtain capture histories of individual black bear for abundance and density estimates. An additional 14 microsatellite loci (G1A, G1D, G10C, G10X, G10U, MU50, MU51, Cxx20, Cxx110, 145P07, 144A06, CPH9, D1A, and MU26) were used to investigate relatedness of extant individuals and genetic diversity in the Big South Fork population (BSFP) of black bear. Methods described in Paetkau (2003) were used for data quality management.

Capture-mark-recapture (CMR) methods assume individuals are correctly identified and marked (Otis et al. 1978). Genotyping error can occur for a variety of reasons (Bonin et al. 2004), and the misidentification of individuals can impose bias on population parameter estimates derived from CMR methods (Paetkau 2004). For example, if separate samples from the same individual are assigned to different genotypes, too many individuals will be identified (Taberlet and Luikart 1999, Woods et al. 1999). In contrast, lack of variation in genetic markers can produce a low number of

unique genotypes, causing too few individuals to be identified (Woods et al. 1999, Waits and Paetkau 2005). Such errors can be mitigated by selecting highly variable markers, such as microsatellites, and by reanalyzing samples with similar genotypes (Woods et al. 1999). 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 microsatellite 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 investigate if differences existed at each locus (D. Paetkau, pers. comm., Paetkau 2003).

Because evidence suggested the BSF black bear population may be small and isolated (Hast 2010), multiple individuals could share the same genotype, and multiple samples could have come from closely-related individuals (Woods et al. 1999). Therefore, probability of identity (*PI*) was used to estimate the statistical power of individual identification (Mills et al. 2000). Probability of identity represents the probability that 2 individuals in a population have identical genotypes at multiple loci (Paetkau and Strobeck 1994). Additionally, the probability of collecting samples from closely-related individuals (*e.g.* siblings or mother-daughter) is not random in isolated populations. Therefore, the probability of identity between siblings ( $PI_{sibs}$ ) was used to estimate the probability that siblings had the same genotype (Waits et al. 2001). Probability of identity was calculated using Program GenALEX 6.1 (Peakall and Smouse 2006), and a *PI* of  $\leq 0.01$  was used to differentiate individuals (Taberlet and Luikart 1999).

Laboratory results provided by WGI for 22 microsatellite genotypes were used to test Hardy-Weinberg equilibrium (HWE) between genotypes and linkage disequilibrium between loci. Program Genepop 4.0 was used to complete these tests (Raymond and Rousset 1995). The HWE probability test in Genepop 4.0 (Raymond and Rousset 1995) was used to investigate departures from Hardy-Weinberg equilibrium by using the complete enumeration method (Louis and Dempster 1987) and a Markov Chain sampling regime (Guo and Thompson 1992) as per Hast (2010). A chi-square test was used to investigate if the difference between observed heterozygosity ( $H_O$ ) and expected heterozygosity ( $H_E$ ) was statistically significant. Linkage disequilibrium, the failure of alleles at two loci to be statistically independent, was investigated using the linkage disequilibrium test in Genepop 4.0 (Raymond and Rousset 1995) with P-values adjusted for multiple comparisons using a Bonferroni sequential correction (Rice 1989). These two tests were used to investigate the presence of non-amplifying alleles (Paetkau et al. 1997), heterozygote deficiency, and the presence of non-random mating. Data sets available from this study, Hast (2010), and live-captures (University of Kentucky, unpublished data) with  $\geq 20$  microsatellite markers were pooled to investigate overall genetic diversity in the BSFP using the allele identity method in Genepop 4.0 (Raymond and Rousset 1995). Finally, a paired t-test was used to evaluate whether a statistically significant change in genetic diversity had occurred since the 2002 KDFWR study.

### **Abundance**

To estimate abundance ( $N$ ), closed-population CMR models (Otis et al. 1978) were used in program MARK (White and Burnham 1999). Closed-population models assume: 1) demographic closure (*i.e.* no births, deaths, immigration, or emigration occur

during sampling) and geographic closure, 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, but variation often exists (Otis et al. 1978). To address sources of variation in equal capture probability, models that account for temporal variation, behavioral variation, and individual heterogeneity were constructed (Otis et al. 1978, Huggins 1989, Pledger 2000).

Individual heterogeneity (*i.e.* differences in the probability of capture of individuals), which can be influenced by age, sex, social status, and individual experience of study animals, can lead to biased estimates of abundance (Ebert et al. 2010).

Therefore, Boulanger et al. (2004) suggested attempts should be made to identify the sources of individual heterogeneity in non-invasive CMR studies. To investigate if gender was a potential source of individual heterogeneity, sex-specific models were constructed. Sex was used as a group variable, and 2-mixture models were constructed with 1 mixture for each sex (*i.e.* 1 mixture for males and 1 mixture for females), no mixtures, and different combinations of temporal and behavioral effects. Akaike's Information Criterion (AIC - Akaike 1973), corrected for small sample size (AICc - Burnham and Anderson 2002) was used to select models that best-fit the data within 7  $\Delta$ AICc values to provide a conservative abundance estimate (Burnham et al. 2011). Best-fit models were averaged according to the methods outlined in Burnham and Anderson (2002) to produce a final, model-averaged abundance estimate. Finally, sex-ratios were calculated using abundance estimates for each gender, and a chi-square test was used to investigate whether sex-ratios differed from 1:1 ( $P < 0.05$ ).

## Density

Spatially explicit capture-recapture (SECR) methods for estimating density ( $D$ ) have recently been developed (Efford et al. 2004). These methods incorporate the spatial distribution of captures and individual capture histories into maximum likelihood-based models (Borchers and Efford 2008). Spatially explicit methods generally provide more precise estimates of density than the traditional method, which divides estimated abundance by the effective sampling area ( $A$ ). The traditional method has a tendency to overestimate density if geographic closure is violated because the movement of individuals in and out of the study area results in low-biased capture probabilities and high-biased estimated abundance (Boulanger and McLellan 2001). Spatially explicit methods, however, are relatively new, and few black bear studies in the southeastern United States have used SECR to estimate density to-date.

A rather large data set currently exists for density estimates in the southeastern United States calculated by the traditional method. Therefore, to compare density in this study to previous studies in the southeastern United States, including small, isolated populations, the traditional method was used. Because radio-telemetry data was not available, the effective sampling area was estimated by extending the sampling grid by 5 km (radius of average annual spring home range of females in Kentucky; University of Kentucky, unpublished data) to create a buffer (Dice 1938). Density was estimated by dividing the model-averaged abundance estimate by the effective sampling area:  $D = N/A$ .

## **Relatedness**

Parentage analysis is a relatively new procedure with some caveats (Jones et al. 2010). The development of microsatellite markers, however, has vastly improved methods of parentage in recent years (Jones and Arden 2003, Jones et al. 2010). Additionally, new methods have been developed for assigning parentage, and incorporated into user-friendly computer programs (Jones et al. 2010). To investigate relatedness of extant individuals, a parentage analysis was performed with program PARENTE (Cercueil et al. 2002) using the categorical allocation method (Jones et al. 2010). Categorical allocation uses a likelihood-based approach to assign an entire offspring to a particular parent. For this reason, categorical allocation is advantageous over other methods of parentage, such as fractional likelihood, which splits an offspring among all compatible parents (Jones and Arden 2003). As such, categorical allocation is more likely to produce correct assignments that represent biological characteristics of a species (Jones and Arden 2003).

Data sets from this study, Hast (2010), and live-captures (University of Kentucky, unpublished data) were pooled to create a single data set for black bear in the BSFP (2009-2010). Genotype data at  $\geq 20$  microsatellite genotypes was used for parentage of individual bear identified in the BSFP. A minimum age difference of 3 years was assumed between parents and offspring when applicable (*e.g.* from live-captures) as black bear in Kentucky typically become sexually mature at the age of three (University of Kentucky, unpublished data). Because multiple genetic markers were used, genotyping errors must be accounted for in data sets used for parentage analysis (Morrissey and Wilson 2005). To mitigate the incorrect rejection of parentage, a

maximum of three incompatibilities between parent-offspring matches were accepted (Zeyl et al. 2009). Parentage was accepted if the probability of being the true parent was  $>0.5$  (Zeyl et al. 2009). Field and observational data were used to refine parentage. Pedigrees were drawn using GenoPro V software ([www.genopro.com](http://www.genopro.com)).

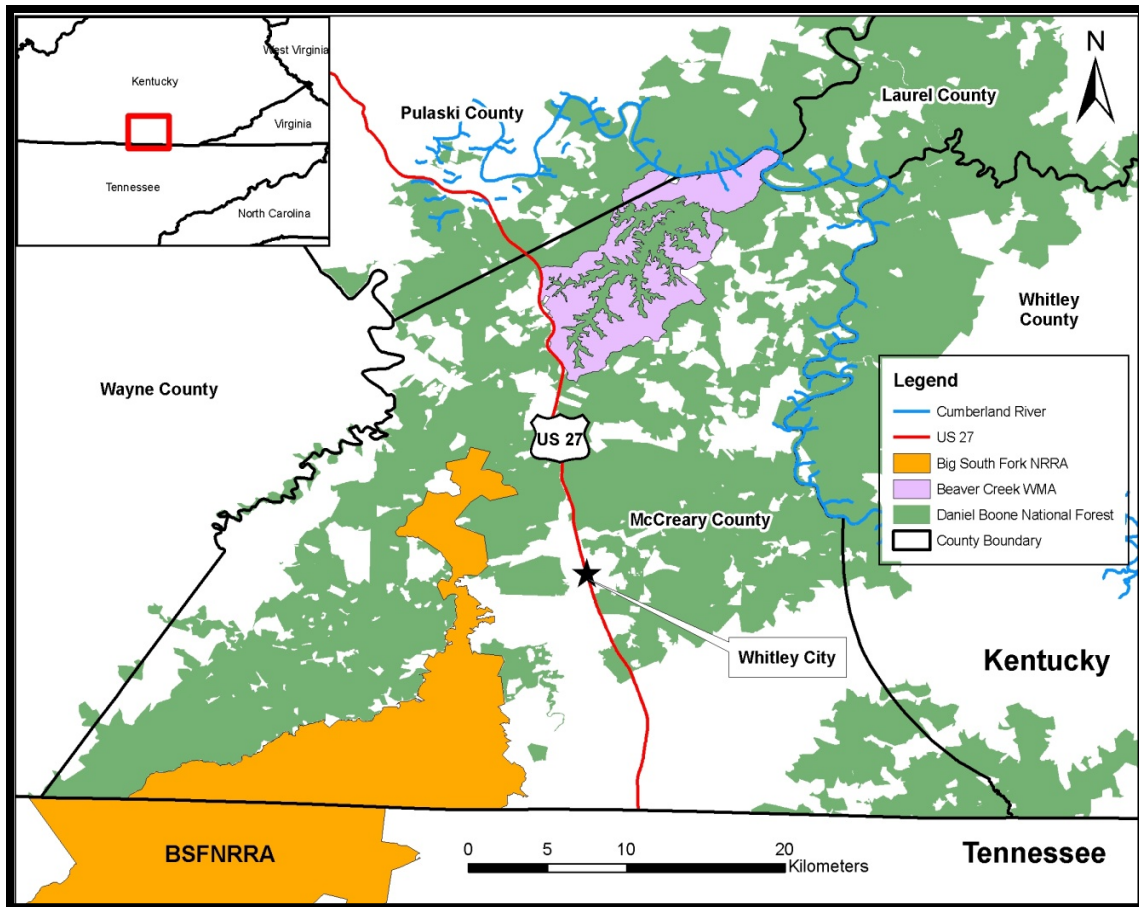


Figure 2.1: Black bear study area, McCreary County, Kentucky.



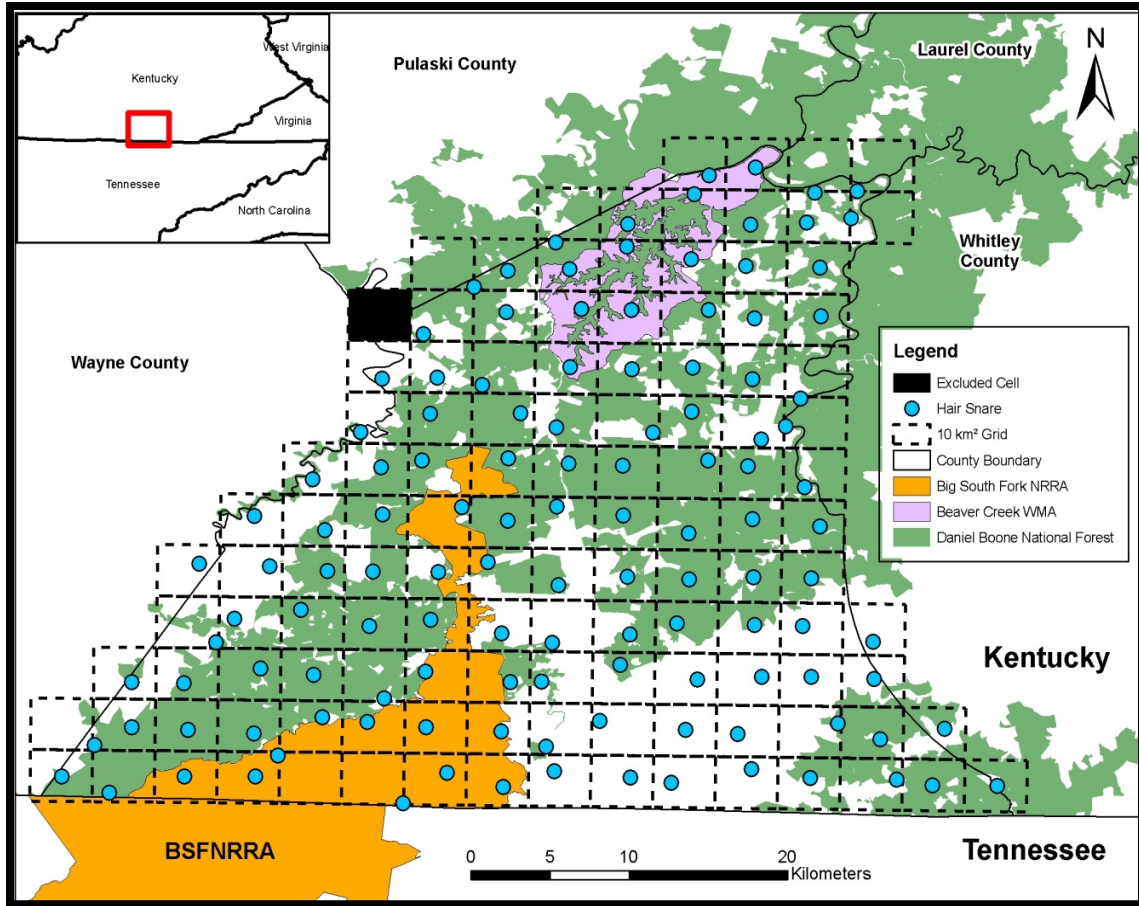


Figure 2.2: 2010 black bear study area, McCreary County, Kentucky, illustrating a hair trap sampling grid of 127 10 km<sup>2</sup> cells. One sampling cell was excluded due to accessibility, and one hair trap was constructed in each of the remaining 126 sampling cells.

## CHAPTER 3: RESULTS AND DISCUSSION

### Results

#### Non-invasive hair sampling

During the 7 sampling sessions, 156 black bear hair samples were collected. Bear visited a total of 23 sample sites (mean = 3.3 visited sites/sampling occasion) (Figure 3.1). All female hair captures occurred  $\leq 15$  km from original release sites in the BSF, whereas only male hair captures occurred outside of this range (Figure 3.1).

#### Genetic analyses

All 156 hair samples were selected for genotyping; however, 25 samples (16%) lacked sufficient DNA for analysis, and 44 samples (28%) failed during genetic analysis. DNA was extracted from the remaining 87 samples (56%), which produced successful individual identification. The mean number of guard hair roots/extracted sample was 1.7. A total of 29 individual bear (16M:13F) were uniquely identified from the 87 successful samples by genotyping with 22 microsatellite markers.

All individuals sampled during the 7 capture-mark-recapture sessions ( $n = 29$ ) were successfully genotyped for 22 microsatellites with no missing loci present. Samples were in Hardy-Weinberg equilibrium with no departures from equilibrium ( $\chi^2 = 32.65$ ,  $df = 42$ ,  $P = 0.85$ ). Of 230 loci pairings for 22 markers, however, 3 pairs ( $< 2\%$  of total) showed signs of linkage disequilibrium ( $P < 0.05$ ) following Bonferroni sequential correction. Genetic diversity indicated by expected heterozygosity ( $H_E$ ) was 0.709 (Table 3.1). Overall probability of identity (PI) was  $2.1 \times 10^{-17}$ , and overall probability of identity between siblings ( $PI_{sibs}$ ) was  $1.5 \times 10^{-7}$ . Therefore, the probability of encountering

identical genotypes was sufficiently low for capture-mark-recapture analysis (Taberlet and Luikart 1999, Mills et al. 2000).

The pooled data set from this study, Hast (2010), and live captures (University of Kentucky, unpublished data) totaled 48 individuals in the BSFP (2009-2010), which were successfully genotyped for  $\geq 20$  microsatellite markers with no missing loci present. Samples were in Hardy-Weinberg equilibrium with no departures from equilibrium ( $X^2 = 43.67$ ,  $df = 38$ ,  $P = 0.24$ ). Of 191 loci pairings, 5 pairs ( $< 3\%$  of total) showed signs of linkage disequilibrium ( $P < 0.05$ ) following Bonferroni sequential correction. Overall genetic diversity in the BSFP from pooled data (2009-2010), as indicated by expected heterozygosity ( $H_E$ ), was 0.698 (Table 3.1).

To enable the use of a paired t-test for evaluating the change in genetic diversity since 2002, I reduced the number of microsatellite markers in this study to 8 for all 48 individuals identified from 2009-2010 because the 2002 Kentucky Department of Fish and Wildlife Resources (KDFWR) study analyzed hair samples with only 8 markers (Hast 2010). Genetic diversity in the reduced 8-marker pooled data set ( $n = 48$ ) from this study was  $H_E = 0.758$  (Table 3.1). Although, genetic diversity exhibited a declining trend since 2002 ( $H_E = 0.819$ ), this decrease was not statistically significant ( $P = 0.13$ ) based on the 8-marker datasets.

### **Abundance**

There were 9 non-sex-specific closed-population models in the candidate set; 4 models had no support. The top 5 models were within 7  $\Delta AIC_c$  values, and considered plausible based on the data set (Table 3.2). Four of the top 5 models indicated capture heterogeneity. The top 5 models were model-averaged to produce an abundance estimate

of  $N = 40$  (95% CI = 30-113). Average capture probability throughout all 7 sessions was  $p = 0.22$ , and average probability of recapture was  $c = 0.23$ .

There were 12 sex-specific closed-population models in the candidate set; 2 models had no support. Ten models were within 7  $\Delta\text{AICc}$  values and considered competing (Table 3.3). Because multiple variations of similar model types (*i.e.* multiple null models, etc.) were constructed, models with the lowest AICc value of each type were selected, and considered plausible based on the data set (Table 3.4). This resulted in 5 competing models. Four of the top 5 models indicated gender variation (Table 3.4). The top 5 models were model-averaged to produce a male abundance estimate of  $N_{\text{male}} = 21$  (95% CI = 16-56), and a female abundance estimate of  $N_{\text{female}} = 17$  (95% CI = 13-36), totaling  $N = 38$  individuals. Sex ratio favored males, but was not statistically different from 1:1 (21M:17F,  $X^2 = 0.003$ ,  $P = 0.95$ ). Average capture probability of males was  $p_{\text{male}} = 0.30$ , and average capture probability of females was  $p_{\text{female}} = 0.27$ .

### **Density**

Because samples were acquired from hair traps in only a portion of the 1,270 km<sup>2</sup> sampling area (Figure 3.1), I reduced the effective sampling area to avoid underestimation of density. The reduced effective sampling area, which included a 5 km buffer, totaled 1,208 km<sup>2</sup> (Figure 3.2). Estimated density ( $D$ ) was 0.03 bear/km<sup>2</sup>.

### **Relatedness**

All 48 individuals identified in this study, Hast (2010), and live captures (University of Kentucky, unpublished data) from 2009-2010 with  $\geq 20$  microsatellite genotypes were analyzed in program PARENTE (Cercueil et al. 2002). Following initial analysis, multiple probabilities of parentage appeared incorrect based on known ages of

live-captured individuals. This was likely due to 13 individuals identified in Hast (2010), but not this study or live-captures (University of Kentucky, unpublished data), that were genotyped with only 20 markers, which created missing data at 2 markers. Therefore, only data from the 20 microsatellite markers that were complete for all 48 individuals were used for parentage.

Parentage analysis successfully assigned relationships among 45 individuals. Three individuals (M513, 024, 129) were not identified as relatives of any other bear in the Big South Fork population (BSFP). Eight mother-father-offspring triads with  $P \geq 95\%$ , and 6 mother-father-offspring triads with  $P > 50 < 95\%$  were discovered (Table 3.5), totaling 14 known reproductive pairs that produced 15 offspring (Figure 3.3). Six mother-offspring dyads were identified with  $P \geq 95\%$  (Table 3.6). Six father-offspring dyads were identified with  $P \geq 95\%$ , and 4 father-offspring dyads were identified with  $P > 50 < 95\%$  (Table 3.7). One full-sibling pair was discovered (Figure 3.3). Three matriline and 3 patriline were discovered (Figure 3.3). One patriline consisted of 3 individuals (M516, 101, M512) not identified as relatives of any other bear in the primary BSFP lineage (Figure 3.3). Additionally, parentage analysis suggested female bears 664 and 025 may be original founders, or direct offspring of original founders (Figure 3.3). Finally, 15 individuals (*i.e.* 30% of all sampled bear) were identified as descendents of male 622 (Figure 3.4).

## Discussion

Non-invasive genetic sampling (NGS) has become an effective, practical, and widely-used tool for researchers and managers studying bear populations. In recent years, non-invasive sampling methods have improved (Long et al. 2008), highly variable genetic markers have been developed (Woods et al. 1999), and genetic laboratories have refined protocols (Paetkau 2003), all of which have allowed NGS methods to be easily incorporated into capture-mark-recapture (CMR) studies of bear populations (Woods et al. 1999, Mowat and Strobeck 2000, Boulanger et al. 2008, Marucco et al. 2011). In this study, non-invasive hair traps were used in a CMR study design to collect hair samples for estimating demographic parameters, acquiring genetic and spatial information, and for investigating long-term population trends of a recently reintroduced, small black bear population.

When using hair samples as DNA sources from mammal species, root follicles must be present for successful analysis. Additionally, the number of high quality root follicles must be sufficient to successfully extract DNA and identify individuals (Taberlet et al. 1999). The success rate for analyses of collected hair samples in this study (56%) was lower than WGI's expectations (*i.e.* 70% - D. Paetkau, pers. comm.), and likely due to multiple factors, including sample quality (*i.e.* number of guard hair roots). This assumption was supported by the low mean number of guard hair roots/extracted sample (1.7 roots/sample), and reflected by the 25 samples (16%) that lacked sufficient material for analysis. A potential solution to improving sample quality would be constructing hair traps with >1 strand of barbed-wire. For example, Tredick et al. (2007) used 2 strands of barbed-wire, with the lowest strand ~ 25 cm above the ground, to improve sample quality

on the upper strand. Results from this study suggest future black bear studies that use non-invasive hair traps should consider using 2 strands of barbed-wire to improve hair sample quality.

In addition to sample quality, climatic and environmental factors can also reduce success rates of non-invasively collected hair samples (Kendall and McKelvey 2008). For instance, the combination of moisture and warm temperatures can increase the rate of hair sample degradation, thereby hindering DNA amplification (Kendall and McKelvey 2008). Shaw and Wofford (2003) described the BSF area as having high humidity and hot summers, and precipitation (*i.e.* rain) was confirmed within the boundary of the study area on 32 of 49 total trapping days (NWS 2010). Approximately 28% of hair samples in this study failed to amplify during genetic analysis, indicating those 44 samples were likely degraded. Taberlet et al. (1999) noted increased degradation of hair samples and greater difficulty amplifying DNA the longer hair samples remained in the field. Short sampling sessions (*i.e.* 7 days) were used in this study to combat the negative effects moisture and heat can have on hair samples. Other black bear studies in wet, humid regions of the United States (*i.e.* Louisiana) have used identical duration hair sampling sessions, yet had much higher success rates (Hooker 2010, Lowe 2011). Therefore, it is unlikely the combination of precipitation and humidity was the sole cause of sample degradation.

Taberlet et al. (1999) mentioned the importance of preservation methods to obtaining successful results from hair sample analysis. Perhaps compounding the effect of moisture and humidity on hair sample degradation was my choice of sample storage. All collected hair samples were placed in paper coin envelopes and frozen for

preservation, which likely trapped moisture in samples and increased degradation during the thawing process. As such, I suggest future studies that collect hair non-invasively should avoid freezing samples as thawing may degrade DNA. Taberlet and Luikart (1999) recommended storing hair samples in paper coin envelopes at room temperature to reduce degradation rates; however, this method may increase the rate of degradation if working in humid environments. Therefore, future studies in warm, humid, and/or wet environments that utilize non-invasive hair sampling should place hair samples in individual paper-coin envelopes inside a sealed container with non-chalky desiccant to mitigate sample degradation and improve success rates (L. Harris, WGI, pers. comm.).

Non-invasive genetic sampling was effective for detecting bear in the Big South Fork population, and sufficient for capture-mark-recapture (CMR) analysis. Otis et al. (1978) recommended capture probabilities between 0.2 and 0.4 to accurately estimate population parameters in CMR studies, and Boulanger et al. (2004) recommended capture probabilities  $\geq 0.2$  for NGS-based CMR studies. Although small populations (*i.e.*  $N < 100$ ) require higher capture probabilities for population parameter estimates to be precise and unbiased (White et al. 1982), estimated average capture probability for this study ( $p = 0.22$ ) was above the suggested minimum for CMR studies. If bias was present in this study, it was likely due to small sample size ( $n = 29$ ), reflected by the wide confidence interval (95% CI: 30-113) in the abundance estimate from non-sex-specific closed models, and capture heterogeneity.



Capture-mark-recapture studies that exhibit capture probabilities  $<0.4$  may have increased susceptibility to individual heterogeneity, which can cause biased estimates of abundance and density (Ebert et al. 2010). Four of the top 5 non-sex-specific closed models in this study indicated the presence of individual heterogeneity (Table 3.2). Individual heterogeneity can be attributed to multiple factors, such as an individual's size, age, gender, or capture experience (Boulanger et al. 2004, Chao and Huggins 2005). Gender, however, is one of the few sources of individual heterogeneity that can be evaluated in non-invasive genetic studies because animals are not physically observed or handled. To investigate if gender was a source of individual heterogeneity, sex-specific closed models were constructed. Four of the top 5 sex-specific closed models included gender variation, suggesting gender contributed to capture heterogeneity (Table 3.4).

Because the density of sampling sites was low compared to the recommended minimum for CMR studies in the southeastern United States (Settlage 2005; Settlage et al. 2008), it is likely that females, which have smaller home ranges, had a lower opportunity of encountering sample sites. Males, however, typically have larger home ranges and exhibit wide-ranging dispersal, thereby having a greater opportunity to encounter more sampling sites. This inference is further supported by the higher average probability of capture for males ( $p_{male} = 0.30$ ). Mitigating heterogeneity caused by gender differences would likely require a higher density of sample sites over a smaller area (Settlage et al. 2008). However, because the areas of McCreary County harboring resident bear were unknown prior to onset of this study, sampling a smaller area with higher sample site intensity was not feasible. As such, future bear studies in the southeastern United States that use NGS for estimating population parameters should

consider conducting pilot studies prior to implementing a CMR study design. This precautionary measure would likely allow researchers to identify optimal sampling areas, thereby potentially reducing heterogeneity in non-invasive CMR studies while maximizing results.

Reliable estimates of demographic parameters are necessary for effective management and timely conservation actions. Frary (2008) produced the only abundance estimate for black bear in Kentucky ( $N = 130$ ), and suggested this estimate was representative of all black bear in the state. This statement, however, was not valid because the Big South Fork area, including McCreary County, was not included in the study area (Frary 2008). Therefore, this study provided the first ever abundance estimate for black bear in the Big South Fork area.

The abundance estimator models I used were based on multiple assumptions, including population closure (Otis et al. 1978). Because sampling occurred for a relatively short duration (49 days), and during summer months in which black bear do not reproduce and survival is typically high, demographic closure was likely satisfied. Geographic closure, however, is often much more difficult to attain, and has been violated in numerous bear studies (Boulanger et al. 2004). It is unlikely geographic closure was satisfied in this study because forested lands, and presumably quality bear habitat existed outside of the sampling grid (van Manen 1990, van Manen and Pelton 1997). Furthermore, bear were released in the Tennessee portion of the BSF as part of the reintroduction project (Eastridge 2000). Resident bears likely inhabited the BSF in Tennessee during the duration of this study; although, empirical evidence did not exist for confirmation. In any case, violation of geographic closure typically results in

overestimation of abundance and density because animals that may not reside within the sampling grid can be captured (Woods et al. 1999).

The abundance estimate reported by this study ( $N = 40$ ) indicated the BSF population is much smaller than the neighboring Pine Mountain population ( $N = 130$ ; Frary 2008), and comparable in size to threatened black bear populations in Florida (Maehr et al. 2001, Brown 2004, Dobey et al. 2005) and Louisiana (Triant et al. 2004, Lowe 2011) that currently have conservation protection. Additionally, the density estimate for black bear in the BSFP ( $0.03 \text{ bear/km}^2$ ) is in the lower-range of reported densities for black bear populations in the southeastern United States (Table 3.8). Assuming geographic closure was violated, actual population size and density of the BSFP may be lower than estimated by this study.

While population size in the BSFP has increased since reintroduction, range expansion appears to have been minimal (Figure 3.1). All female bear hair samples were collected from hair traps  $\leq 15 \text{ km}$  from original release sites, whereas, all hair samples collected  $> 15 \text{ km}$  from release sites were from male bear (Figure 3.1). This pattern of range expansion is typical of black bear populations still in the early stages of colonization, as females are highly philopatric and establish home ranges adjacent to or near mothers, whereas, males are typically dispersers that exhibit long-ranging movements (Clark 2009). Furthermore, the estimated sex ratio in the BSF population (21M:17F), although not significantly different from 1:1, appears to favor males. Most bear populations that have moved beyond the initial stages of colonization typically exhibit female biased sex ratios and higher population densities (Unger 2007, Frary 2008).

The BSF black bear population remains small ( $N = 40$ ) and at low density ( $0.03$  bear/km<sup>2</sup>), and has colonized very little new range within the last decade (Figure 3.1), suggesting a slow population growth rate. Clark et al. (2002) characterized black bear as poor colonizers, and results from this study may support this description. Observations of bear poaching in this area since reintroduction (J. Plaxico and M. Strunk, KDFWR, pers. comm.), however, suggest that poaching may have been an important factor in retarding population growth and expansion. Buchalczyk (1980) identified illegal poaching of brown bears in Poland as the primary cause of population loss 25 years post-reintroduction. Therefore, it is certainly possible that continued illegal poaching, coupled with other sources of mortality, such as bear-vehicle collisions, could have substantial deleterious consequences on the already small BSF population.

Relatedness is a useful biological characteristic to evaluate, especially in small populations that may be isolated. Since the BSF population originated only 14 years prior to onset of this study, a unique opportunity was presented to investigate relatedness, family lineages, and breeding structure of this small, recently reintroduced black bear population. Parentage analysis was used to investigate genetic relationships of extant individuals in the BSFP, and multiple multigenerational lineages were discovered (Figure 3.3). The identification of two females, 664 and 025, as original founders is plausible based on available known ages of select descendents. For example, M504, which was the first-order offspring of female 664 and male 622 (Table 3.5), was 9 years of age when live-captured in 2010 (Figure 3.3). Based on the average earliest breeding age of female bear in Kentucky (*i.e.* 3 years of age), female 664, whose exact age remains unknown as she has not been live-captured to-date, was likely born no later than 1998, one year

following reintroduction. A similar inference can be made for female 025, whose second-order offspring was M509 (Figure 3.3), a 5 year-old individual in 2010.

Interestingly, 3 individuals (M516, 101, M512) formed one patriline that constituted a family separate from all other bear in the BSFP (Figure 3.3). Therefore, it is possible that not all individuals in the BSFP were sampled in 2009-2010; however, those individuals may not have been alive during sampling, or could have moved off of the sampling grid.

Perhaps the most revealing discovery from parentage analysis was male 622's lineage (Figure 3.4). Approximately 30% (15 individuals) of bears sampled from 2009-2010 shared male 622 as a common ancestor. The age of this individual remains unknown as he has not been live-captured to-date. Male 622, however, was not identified as a migrant by Hast (2010), and was comprised of a genotype similar to other individuals in the BSFP and the Great Smoky Mountains (Hast 2010). Furthermore, because one of male 622's first-order offspring was M504 (Table 3.5), male 622 was likely either a founder cub or was present in the Big South Fork area prior to reintroduction. Cumulatively, results from parentage analysis suggest many individuals in the BSFP are closely related.

Hast (2010) alluded the BSF population may be isolated from nearby subpopulations, including the neighboring Pine Mountain population. Isolated populations are vulnerable to deleterious genetic effects such as genetic drift, genetic bottleneck, and inbreeding depression (Hartl 2000, Boersen et al. 2003). Additionally, reintroduced populations that remain isolated typically exhibit reduced levels of genetic diversity over time (Maudet et al. 2002). The paired t-test for the 8 microsatellite marker data sets did not reveal a statistically significant decline in genetic diversity since 2002

( $P = 0.13$ ). However, genetic diversity in the BSFP based on pooled data at  $\geq 20$  microsatellites was  $H_E = 0.698$ , which suggests a declining trend (Table 3.1). Linkage disequilibrium was detected among 5 allele pairs, which could indicate the presence of genetic drift (Boersen et al. 2003). Furthermore, 14 years is extremely short compared to evolutionary timescales, and based on the average generation time of black bear (*i.e.* 6 years – Onorato et al. 2004), only 2-3 generations have likely occurred in the BSFP. Therefore, substantial decreases in genetic diversity may occur in future generations if the BSF population remains isolated (Dixon et al. 2007). The proportion of closely related individuals identified by parentage analysis, a decline in genetic diversity, and minimal gene flow into the BSFP as suggested by Hast (2010) support the possibility of genetic drift and founder effects caused by isolation.

If the BSFP is in fact isolated from nearby populations to the east, the Interstate 75 barrier posited by Hast (2010) may be impeding movement between the BSF and Pine Mountain populations. Although a few radio-collared bear from the PMP have been documented successfully crossing this roadway (B. Augustine, pers. comm.), multiple individuals have been killed by vehicles while attempting to cross (J. Plaxico, KDFWR, pers. comm.). To date, no radio-collared bear originating in the BSFP are known to have crossed Interstate 75 (University of Kentucky, unpublished data). Additionally, range expansion in the BSFP displays a movement westward and northward, away from Interstate 75 and the PMP (Figure 3.1). For example, in recent years, confirmed sightings of females with cubs, and nuisance complaints have increased in southeastern Wayne County (M. Strunk, KDFWR, pers. comm.). Therefore, establishing connectivity between the BSFP and populations to the east may be delayed until resident female bear

in the BSFP establish home ranges east of highway U.S. 27 or female bear in the PMP establish home ranges west of Interstate 75. Such expansion rates, however, will likely require many years.

The BSF black bear population has persisted for more than a decade, and population size has increased approximately 4-fold since 1997, indicating the reintroduction successfully established a breeding population without management intervention. Therefore, this study represents the first evidence of successful reintroduction and associated expansion of black bear in Kentucky. Seddon (1999), however, warned that reintroduction success may only be representative of the time at which assessments are made, and that momentary self-sustainability is not synonymous with long-term population persistence. Seddon's (1999) admonition is further supported by the multiple reintroduction projects that have been initially declared successful, only to have declining populations years or decades later (Buchalczyk 1980, Wolf et al. 1996, Seddon 1999, Clark 2002, Clark 2009, Gusset 2009, Hayward and Somers 2009). As such, declaring the reintroduction of black bear in the BSF area a success does not imply the BSF population will persist in the future, especially since this small population, comprised of numerous closely-related individuals, appears to have declining genetic diversity, and may be substantially influenced by human-induced mortality.

Table 3.1: Estimated genetic diversities (expected heterozygosity) of black bears in the Big South Fork area of Kentucky since reintroduction, 2002-2010.

Year	# Individuals	# Markers	$H_E$	Study
2002	16	8	0.819	KDFWR
2009	19	20	0.770	Hast (2010)
2010	29	8	0.758	This study
2010	29	22	0.709	This study
2009-2010	48	20	0.698	This study



Table 3.2: Closed-population non-sex-specific models and model selection based on AICc and  $\Delta\text{AICc}$  to estimate population parameters of the Big South Fork black bear population in McCreary County, Kentucky, 2010. I modeled abundance ( $N$ ), proportion of the population belonging to 1 of 2 unknown mixtures (*mixture*), capture probability ( $p$ ), and recapture probability ( $c$ ). I also modeled variations due to behavioral response, no behavioral response, time (*time*), no time, heterogeneity ( $\pi$ ), and no heterogeneity.

Model	$K^a$	AICc	$\Delta\text{AICc}^b$	$w_i^c$	Dev
$\pi(\sim 1)p(\sim \text{mixture} * c)c()N(\sim 1)$	6	85.47	0.00	0.46	63.93
$\pi(\sim 1)p(\sim \text{mixture})c()N(\sim 1)$	4	86.76	1.29	0.24	69.45
$\pi(\sim 1)p(\sim \text{time} + \text{mixture})c()N(\sim 1)$	10	87.16	1.69	0.20	56.91
$\pi(\sim 1)p(\sim \text{mixture} + c + \text{time})c()N(\sim 1)$	11	88.85	3.38	0.08	56.36
$p(\sim 1)c()N(\sim 1)$	2	91.35	5.88	0.02	78.19
$p(\sim \text{time})c()N(\sim 1)$	8	92.72	7.25	0.02	66.87
$p(\sim 1)c(\sim 1)N(\sim 1)$	3	92.72	7.25	0.02	77.49
$p(\sim \text{time})c(\sim 1)N(\sim 1)$	9	92.85	7.38	0.01	64.81
$\pi(\sim 1)p(\sim \text{mixture})c(\sim 1)N(\sim 1)$	5	96.90	11.43	0.00	77.49

<sup>a</sup>: Number of model parameters

<sup>b</sup>: Relative difference between AICc of model and AICc of model with lowest AICc.

<sup>c</sup>: Model weight.

Table 3.3: Closed-population sex-specific models and model selection based on AICc and  $\Delta\text{AICc}$  to estimate population parameters of the Big South Fork black bear population in McCreary County, Kentucky, 2010. I modeled abundance ( $N$ ) of each gender (*group*), proportion of each gender belonging to 1 of 2 sex mixtures (*mixture*), capture probability ( $p$ ), and recapture probability ( $c$ ). I also modeled variations due to sex (*Sex*), no sex, behavioral response, no behavioral response, time (*time*), no time, heterogeneity ( $\pi$ ), and no heterogeneity.

Model	$K^a$	AICc	$\Delta\text{AICc}^b$	$w_i^c$	Dev
$\pi(\sim 1)p(\sim \text{mixture})c()N(\sim \text{group})$	5	127.49	0.00	0.44	86.95
$\pi(\sim \text{Sex})p(\sim \text{mixture})c()N(\sim \text{group})$	6	128.31	0.82	0.29	85.65
$p(\sim 1)c()N(\sim \text{group})$	3	131.46	3.98	0.06	95.11
$p(\sim \text{Sex})c()N(\sim \text{group})$	4	131.89	4.40	0.05	93.50
$p(\sim \text{time})c()N(\sim \text{group})$	9	132.87	4.38	0.03	83.70
$\pi(\sim \text{Sex})p(\sim \text{mixture}*\text{Sex}*c)c()N(\sim \text{group})$	12	133.20	5.71	0.03	77.32
$p(\sim 1)c(\sim 1)N(\sim \text{group})$	4	133.28	5.80	0.02	94.84
$p(\sim c + \text{Sex})c()N(\sim \text{group})$	5	133.30	5.81	0.02	92.76
$p(\sim \text{time} + \text{Sex})c()N(\sim \text{group})$	10	133.37	5.88	0.02	81.99
$p(\sim c*\text{Sex})c()N(\sim \text{group})$	6	133.47	5.98	0.02	90.08
$p(\sim \text{time} + c + \text{Sex})c()N(\sim \text{group})$	11	134.93	7.44	0.01	81.32
$p(\sim \text{time}*\text{Sex})c()N(\sim \text{group})$	16	142.69	15.20	0.00	77.53

<sup>a</sup>: Number of model parameters

<sup>b</sup>: Relative difference between AICc of model and AICc of model with lowest AICc.

<sup>c</sup>: Model weight.

Table 3.4: Top closed-population sex-specific models that were model-averaged to estimate population parameters of the Big South Fork black bear population in McCreary County, Kentucky, 2010.

Model	$K^a$	AICc	$\Delta\text{AICc}^b$	$w_i^c$	Dev
$\pi(\sim 1)p(\sim \text{mixture})c()N(\sim \text{group})$	5	127.49	0.00	0.79	86.95
$p(\sim \text{Sex})c()N(\sim \text{group})$	4	131.89	4.40	0.09	93.50
$\pi(\sim \text{Sex})p(\sim \text{mixture}*\text{Sex}*c)c()N(\sim \text{group})$	12	133.20	5.71	0.05	77.32
$p(\sim \text{time} + \text{Sex})c()N(\sim \text{group})$	10	133.37	5.88	0.04	81.99
$p(\sim c*\text{Sex})c()N(\sim \text{group})$	6	133.47	5.98	0.04	90.08

<sup>a</sup>: Number of model parameters

<sup>b</sup>: Relative difference between AICc of model and AICc of model with lowest AICc.

<sup>c</sup>: Model weight.

Table 3.5: Mother-father-offspring triads in the Big South Fork black bear population identified by parentage analysis of pooled data (2009-2010).

Offspring ID	Mother ID	MM <sup>a</sup> Mother	Father ID	MM <sup>a</sup> Father	Probability
649	031	3	610	0	1
F508	658	0	632	0	1
F511	F503	1	613	0	0.9983
005	025	0	014	0	0.9836
618	669	0	M504	0	0.9821
M504	664	1	622	0	0.9809
M514	044	3	624	1	0.9747
107	034	0	619	0	0.9711
F505	669	2	005	1	0.9197
F506	607	0	604	3	0.8751
M510	607	3	668	1	0.8300
034	601	0	622	1	0.7021
F503	077	0	M504	2	0.6499
011	034	1	619	0	0.5366

<sup>a</sup>: Mismatch between parent-offspring. A maximum of 3 incompatibilities were accepted.

Table 3.6: Mother-offspring dyads in the Big South Fork black bear population identified by parentage analysis of pooled data (2009-2010).

Offspring ID	Mother ID	MM <sup>a</sup> Mother	Probability
031	034	0	1
M509	044	1	1
668	044	0	1
044	025	2	0.99
064	669	1	0.99
M515	658	1	0.98

<sup>a</sup>: Mismatch between mother-offspring. A maximum of 3 incompatibilities were accepted.

Table 3.7: Father-offspring dyads in the Big South Fork black bear population identified by parentage analysis of pooled data (2009-2010).

Offspring ID	Father ID	MM <sup>a</sup> Father	Probability
602	622	0	1
632	065	1	1
M512	101	1	1
140	M504	0	0.99
043	632	2	0.99
104	M504	0	0.95
607	M504	0	0.95
613	065	2	0.90
101	M516	2	0.88
M507	604	2	0.81

<sup>a</sup>: Mismatch between father-offspring. A maximum of 3 incompatibilities were accepted.

Table 3.8: Reported population densities (bear/km<sup>2</sup>) of select black bear populations in the southeastern United States.

Location	Bear/km <sup>2</sup>	Reference
Camp LeJeune, NC	0.02	Brandenberg (1996)
McCreary County, KY	0.03	This study
Carvers Bay, SC	0.04	Drewry (2010)
Osceola National Forest, FL	0.14	Dobey et al. (2005)
White River National Wildlife Refuge, AR	0.22-0.25	Clark et al. (2010)
Upper Atchafalaya River Basin, LA	0.15-0.18	Lowe (2011)
Lewis Ocean Bay, SC	0.31	Drewry (2010)
Tensas River National Wildlife Refuge, LA	0.36	Boersen et al. (2003)
Tensas River Basin, LA	0.66	Hooker (2010)

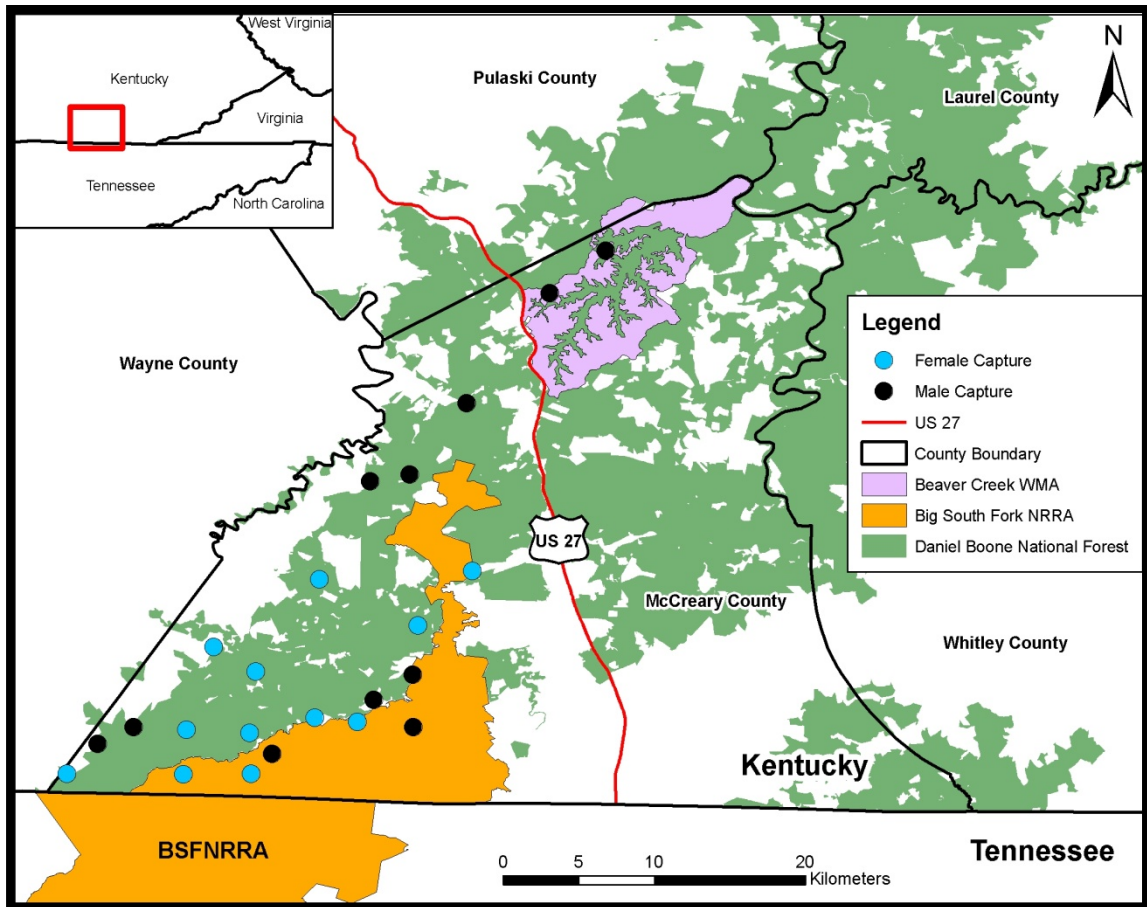


Figure 3.1: Locations of 23 hair traps visited by black bears in McCreary County, Kentucky, 2010. All female hair samples were captured at hair traps  $\leq 15$  km from reintroduction release sites in the Big South Fork National River and Recreation Area.



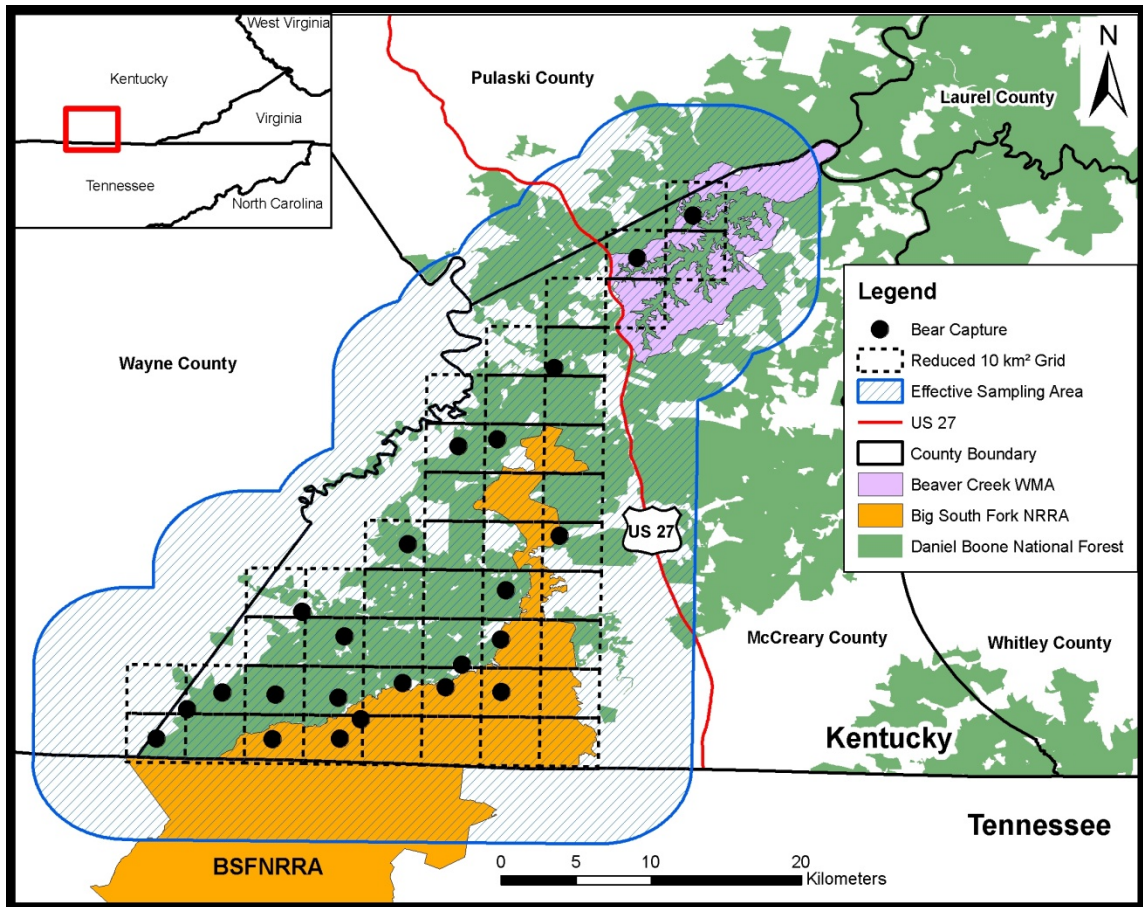


Figure 3.2: Reduced grid and effective sampling area with 5 km buffer used for density 2010 estimation of the Big South Fork black bear population in McCreary County, Kentucky.

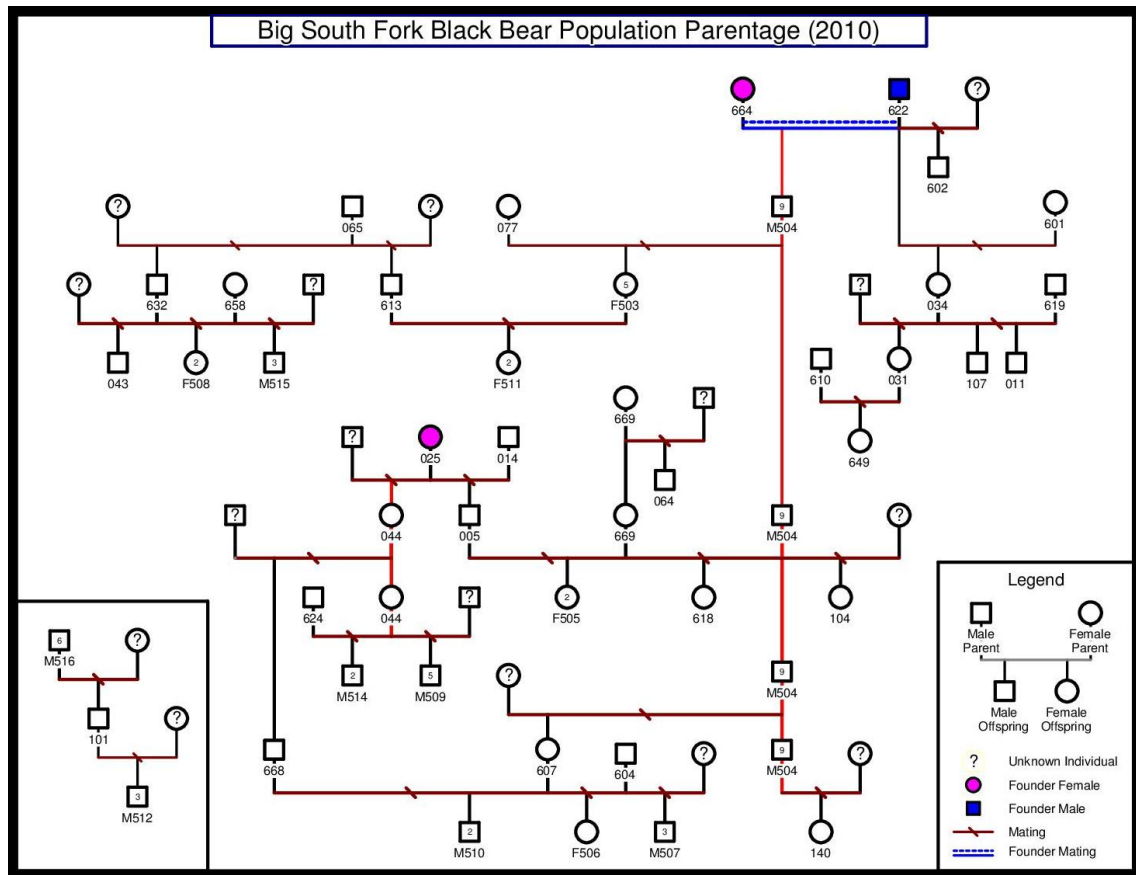


Figure 3.3: Lineage results from parentage analysis of the Big South Fork black bear population using data from this study, Hast (2010), and live-captures. Females 664 and 025 were likely founders in the Big South Fork population.



## **CHAPTER 4: MANAGEMENT AND RESEARCH IMPLICATIONS**

This study provided an important post-reintroduction status assessment of black bear in the BSF area, including the first population abundance and density estimates. While the population currently appears to be small, but stable, the future of the BSF black bear remains unpredictable based on current information and known problems with reintroduced populations. Empirical data from this study and Hast (2010), however, indicate the population may be vulnerable. The BSF black bear population exhibits numerous characteristics of small, isolated populations that are susceptible to deleterious genetic effects and overexploitation. Many individuals in the population appear to be closely related, genetic diversity demonstrates a declining trend, and the population may be experiencing isolation-induced genetic drift. As such, I recommend that black bear in the BSF population should not be considered for inclusion in the Kentucky bear hunt given the potential detrimental effects on the population, including a further reduction of a presumed slow population growth rate, risk of overharvest, and possibly extinction.

Results from this study demonstrate the critical need for continued monitoring and immediate research of this small black bear population. Because the status and number of bears in the BSF population in neighboring Tennessee remains unknown, I suggest investigating it to further characterize bears in this area. Despite the difficulties of monitoring small populations, future research should begin to examine population growth rate, a parameter that is often more useful for black bear population management than abundance estimates (Clark et al. 2010). In addition, I strongly recommend repetition of this study within 5-10 years to evaluate changes in abundance, density, and range expansion. These studies should also allow continued monitoring of the genetic

health of this small population to assess changes in genetic diversity, and provide a landscape genetics perspective into whether connectivity becomes established between the BSF and Pine Mountain populations.

I also recommend that survival rates of bears in the BSF population be investigated. Although limited data exists, unexplained cub mortality has been documented in the BSF population (University of Kentucky, unpublished data), and cub survival and population recruitment may be low. Additionally, cases of illegal poaching of all age classes of black bear have been confirmed in McCreary County, Kentucky (KDFWR, unpublished data), but the true extent of such occurrences remains unknown. Therefore, future research should also investigate causes of mortality. I suggest development of a comprehensive black bear monitoring plan for the BSF population that includes said research recommendations, along with pre-defined time intervals for implementation. This measure will be important for natural resource agencies challenged with ensuring the long-term persistence of the black bear in the Big South Fork area.

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