To the Graduate Council:

I am submitting herewith a thesis written by Laura M. Thompson titled "Abundance and Genetic Structure of Two Black Bear Populations Prior to Highway Construction in Eastern North Carolina". I have examined the final paper copy of this thesis for form, content, and quality and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

Frank T. van Manen, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Vice Provost and Dean of Graduate Studies

AG-VET-MED.

ABUNDANCE AND GENETIC STRUCTURE OF TWO BLACK BEAR POPULATIONS PRIOR TO HIGHWAY CONSTRUCTION IN EASTERN NORTH CAROLINA

A Thesis

Presented for the

Master of Science Degree

The University of Tennessee, Knoxville

Laura Marie Thompson

May 2003

ACKNOWLEDGMENTS

I would like to acknowledge all the people who made my study possible. First, my major professor, Dr. Frank T. van Manen, provided me with the unique opportunity to pursue graduate studies by researching black bears. I am especially grateful to Dr. van Manen for his willingness to offer assistance or advice when needed, regardless of his schedule. I would like to thank my committee members, Dr. Joseph Clark, Dr. Arnold Saxton, and Dr. Lisa Muller. They, too, were always willing to help and provided me with invaluable information on numerous occasions.

Dr. Tim King, at the Leetown Science Center, contributed greatly to my knowledge of genetics and was almost like a committee member. I am grateful to him for being so patient when answering my many questions. I would like to thank Shannon Julian, also at the Leetown Science Center, for her contribution to the study by graciously taking the time to analyze the black bear hair samples.

The North Carolina Department of Transportation provided funding for this project through the North Carolina Wildlife Resources Commission. Mark Jones, David Cox, Dale Evans, William Wescott, and T. Lindsey Riddick provided valuable logistical assistance during the study. I am especially grateful to all of the landowners, including Weyerhaeuser Forests Products Company, Bluestone Farms, Mike Noles, Robert Saunders, Harry Chesson, the Vernon G. James Research and Extension Center, Pocosin Lakes National Wildlife Refuge, and Lake Phelps Hunting Club, for providing access to their land.

I owe a special thanks to my fellow graduate student on the project, Jason Kindall, who made enormous contributions to this portion of the study through his long hours of collecting data in the field. Also, I am appreciative to his wife, Heather Kindall, who was gracious enough to serve as a technician on the project. The other technicians, Beth Vincent, Ryan Myers, Matt Conners, and Byron Weckworth also made large contributions to the study by collecting data in, sometimes, not so ideal conditions. I am grateful to David and Rebecca Telesco, who were almost like partners in the field with their frequent visits to Country Club Drive. They were always eager to help and offer advice and have been great friends to me. I would like to thank Steve Dobey for sharing his bear trapping expertise with me. I am also grateful to Mark Boersen for advising me on closed population models. Of course, I owe a debt of gratitude to the remainder of "the gang", Kim Asmus, Kent Hersey, Jaime McNutt, Jennifer Dobey, Katie Settlage, Benny and Cindy Thatcher, and Brandon and Meaghan Wear, for the camaraderie and friendship they shared. I will never forget those late office afternoons and Fridays at five.

Finally, I would like to thank my family, Dad, Mom, Beth and David, Rebekah and Steve, and Ricky and Carmen for their love and support throughout my work on this study. My niece, Megan, and nephews David, Eli, Matthew, and Logan always know how to make me smile and I am grateful to them for that.

ABSTRACT

A 19.3-km segment of U.S. Highway 64 in Washington County, North Carolina is being re-routed and upgraded to a 4-lane highway in what is considered to be crucial black bear (*Ursus americanus*) habitat. To lessen the impacts on bears and other wildlife, 3 passageways are currently under construction in this segment of highway. In 2000, I began research on a project to determine the effects of the 4-lane highway on black bear ecology and to determine whether wildlife passageways can mitigate impacts of such highways. Research was divided into 2 phases: before highway construction (phase I; June 2000–June 2001) and after the anticipated completion of the highway (phase II; 2005). In addition to the treatment area (area of the proposed highway) a control area was established. I collected baseline information during phase I by estimating population abundance and density on the 2 study areas with DNA from hair samples collected from barbed-wire enclosures. Microsatellite analysis of the DNA was used to identify individual bears for mark-recapture estimates. I examined 3 different groups of closed population estimation models (modified Lincoln-Petersen, Bailey's triple catch, and multiple mark-recapture models). A multiple mark-recapture model with varying capture probabilities provided the best fit to the data and produced the most precise population estimates. Population estimates for the treatment and control areas were 85 (1.20 bears/km^2) and 165 (1.78 bears/km^2), respectively. Differences in density may be related to differences in hunting pressure; however, both densities were high because of large tracts of forests interspersed with agricultural food crops. Additionally, I used the microsatellite data to compare genetic relatedness, population structure, and gene flow

between the treatment and control areas, between areas north and south of the proposed highway within the treatment area, and between areas north and south of a fictitious highway within the control area. The genetic distance between the north and south populations within the treatment area was greater than the genetic distance between the treatment and control areas and between areas north and south of the fictitious highway within the control area. Unrooted neighbor-joining trees constructed from the genetic distance analyses suggested some subpopulation structure. The F_{ST} values (0.029, 0.040, and 0.006 for the 3 scales, respectively), which measure the reduction in heterozygosity of subpopulations relative to the entire population, indicated that the amount of population structure was low, but slightly greater within the treatment area than between the 2 study areas or within the control area. Gene flow rates also supported those results, with fewer individuals traveling north and south of the proposed highway (3.3 migrants per generation) than between the 2 study areas (4.6 migrants per generation) or between the north and south populations within the control area (7.6 migrants per generation). I examined isolation-by-distance with Mantel tests to determine relationships between genetic distance and geographic distance based on sample locations. Male bears showed less isolation-by-distance ($r = 0.06$) than female bears ($r = 0.26$), probably because of larger home range sizes, which may also explain why the overall population structure was low. Thus, because of the relatively small scale of the study, the unrooted trees likely depict family structure rather than population structure. Finally, I used a geographic information system (GIS) to map allelic diversity throughout the 2 study areas. Allelic diversity was positively correlated with forest density ($r = 0.29$), indicating that the size and, possibly, the connectivity of habitat patches plays a role in gene exchange, even on a

small scale. The density and genetic structure patterns I observed for black bears on the 2 study areas before highway construction will be crucial to understanding the potential impacts of the highway on black bear ecology. However, it may be some time before potential genetic impacts can be detected once highway construction is completed.

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CHAPTER I

INTRODUCTION

GENERAL PROBLEM STATEMENT

The impacts of habitat fragmentation on wildlife can be substantial and can be a serious threat to population stability and persistence (Wilcox 1980, Wolff et al. 1997). Habitat fragmentation separates blocks of contiguous habitat into ≥1 disconnected pieces and has the potential to disrupt a variety of ecological processes (Forman 1995). Although the source of habitat fragmentation can be natural or anthropogenic, highway networks represent one of the primary concerns for wildlife managers. There has been increasing emphasis in recent years to study the impacts of highways on wildlife and how to mitigate such impacts by incorporating wildlife passageways into highway construction projects. Rare and wide-ranging carnivores have received particular attention because they are dependent on regional landscapes (Ruediger 1998). Black bears (*Ursus americanus*) occur in relatively low densities and use their habitat on a landscape scale (Schoen 1990). Thus, this species can provide valuable ecological insights regarding the effects of highways on wildlife.

Highways have the potential to impact black bear populations in many ways. Direct mortalities caused by collisions with vehicles may impact black bear densities in areas surrounding a highway. Bear-vehicle collisions have become commonplace in areas where high-speed roads traverse high-quality habitat (Pelton et al. 1999). The frequency of collisions likely will increase as the network of highways throughout the United States is upgraded and expanded to accommodate the growing number of

motorists (Lehnert and Bissonette 1997). Also, disturbances associated with highways, such as noise and artificial lighting (Spellerberg 1998), may cause avoidance among wildlife populations (Noss et al. 1996), resulting in displacement of animals. Human development often follows highway construction, potentially causing further displacement of wildlife as a secondary effect. Finally, highways often are barriers to ecological processes, such as dispersal (Clevenger et al. 2001, Wilcox 1980).

Many wildlife species, including bears, exist as part of a metapopulation, or a group of several subpopulations that are linked by immigration and emigration (Gotelli 1998). Metapopulation theory suggests that regional populations may persist, even when local extinctions occur, because of movements by individual animals among subpopulations. Such movements of animals can supplement declining populations, maintain gene exchange, and recolonize habitats after local population extinctions. However, fragmentation due to highways can potentially disrupt such movements, thereby undermining those processes (Jackson 1999).

Besides the potential impacts of fragmentation on demographic processes, the resulting isolation of populations may, over time, reduce genetic diversity (Fleischer et al. 1995). In the absence of evolutionary forces such as migration, mutation, and selection, small populations are more susceptible to random genetic drift and inbreeding depression; when populations are sampled from one generation to the next, allele frequencies are likely to fluctuate more in small populations than in larger populations (Lacy 1987). Although large carnivores typically exhibit low levels of polymorphism and genetic variation (Merola 1994), they often are distributed at low densities and, thus, more susceptible to loss of genetic diversity caused by fragmentation (Paetkau et al.

1998*b*). Large areas of relatively contiguous habitat are important for bears to encompass large home ranges (Schoen 1990, Noss et al. 1996). However, as smaller subpopulations become isolated from larger populations, the potential for genetic drift and inbreeding depression increases.

Because of increasing interest in the ecological effects of highways and traffic, transportation planners have spent substantial resources in recent years to construct wildlife passageways (Clevenger et al. 2002). However, few studies have been conducted to assess the efficacy of wildlife passageways in reducing road kills, facilitating animal movements, or promoting genetic exchange (Clevenger et al. 2002).

BACKGROUND AND JUSTIFICATION

The North Carolina Department of Transportation (NCDOT) is in the process of expanding U.S. Highway 64 between Raleigh and The Outer Banks. This expansion is expected to improve the local economy and increase the efficiency of hurricane evacuations. The NCDOT plans to expand the highway in segments within the next 10–15 years, upgrading it from a 2-lane road to a 4-lane highway. A 19.3-km segment of U.S. Highway 64 in Washington County is presently under construction and is the focus of my research project. A new route was identified south of the current U.S. Highway 64 between the towns of Roper and Creswell, bisecting important black bear habitat. The new highway route borders large forested tracts of land, potentially restricting bear movements to adjacent agricultural fields, which are important for foraging. The NCDOT and North Carolina Wildlife Resources Commission (NCWRC) incorporated plans for 3 wildlife underpasses into the highway construction design to reduce vehicle

collisions and to mitigate the potential effects of the highway on bear movements. The locations of the wildlife underpasses were determined based on surveys of bear sign conducted by the NCWRC in 1999 (Scheick and Jones 1999).

My study is 1 component of a project designed to determine the impacts of 4-lane highway with wildlife underpasses on black bear ecology (van Manen et al. 2001). Few studies have been able to document ecological responses of wildlife to highways because data are not collected before construction. This project was designed to collect data before highway construction (phase I) to be compared with data gathered after the anticipated completion of the highway in 2005 (phase II). Establishment of a control area was necessary to ensure that observed effects on the treatment area are not due to factors other than the presence of the highway, particularly because it is unlikely that study animals during phase I will still be alive during the second phase of the project. The final analysis of the project will be based on 4 sets of data: (1) treatment area, pre construction; (2) treatment area, post construction; (3) control area, pre construction; and (4) control area, post construction (Figure 1). Treatment effects occur only if differences exist between data from groups 1 and 2, after adjusting for differences between groups 3 and 4.

A pre-construction population estimate will be crucial to document demographic changes once construction is complete. Any alteration in population abundance that is not accounted for by the control area may indicate a highway impact (e.g., increased vehicle mortalities). Furthermore, deriving estimates of genetic relatedness, gene flow, and population structure from phase I data will be important to determine if the highway is a barrier to genetic exchange. Finally, determining the associations between genetic

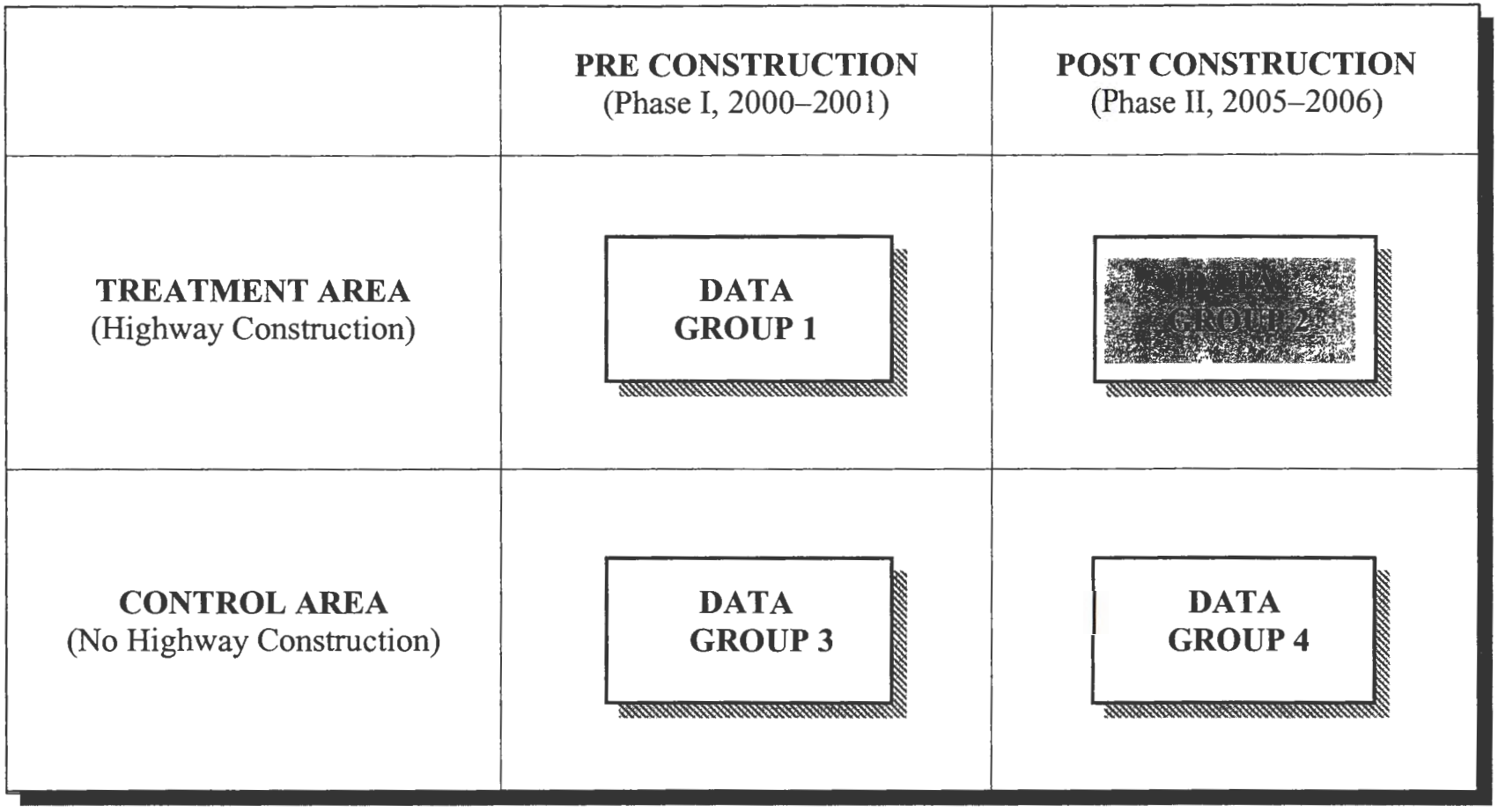

Figure 1. Experimental design to determine the effects of a 4-lane highway with wildlife underpasses on black bear ecology in Washington County, North Carolina. Treatment effects occur only if differences exist between data groups 1 and 2, after adjusting for differences between data groups 3 and 4.

diversity and landscape features (e.g., habitat connectivity) may provide further insights into the ecological effects of the highway.

The main goal of my study was to provide baseline data on black bear population abundance and genetic structure during the pre-construction phase of the new U.S. Highway 64. The specific objectives of my study were to:

(1) estimate the abundance and density of black bears on the treatment and control areas,

(2) determine genetic relatedness, population structure, and gene flow of black bears between the treatment and control area populations and between the north and south populations within the 2 study areas, and

(3) map genetic diversity of black bears within both study areas and determine its association with landscape features.

CHAPTER II

STUDY AREA

LOCATION

The 2 study areas were located in Washington County in the coastal plain region of eastern North Carolina (Figure 2). The center of the overall study area was located at approximately 35° 87′ north latitude, 76° 65′ west longitude. The treatment area was approximately 10,750 ha in size; this area was bordered by the current U.S. Highway 64 to the north, Newland Road to the south, the town of Roper to the west, and the town of Creswell to the east. The control area was approximately 12,270 ha in size and was bordered by U.S. Highway 64 to the north, State Highway 99 to the south, State Highway 32 to the west, and State Road 1127 to the east. The new route of U.S. Highway 64 will pass through the center of the treatment area, separating the forested tracts from the agricultural tracts (Figure 2). The 3 wildlife underpasses will be evenly spaced along the 19.3-km segment of highway (i.e., eastern, central, and western site) with the goal to maintain existing movement corridors (Figure 2). The control area was chosen to closely resemble the landscape of the treatment area, particularly the juxtaposition and area of agricultural (38%) and forested lands (62%).

GEOLOGY AND SOILS

Washington County lies within 2 major river basins. The northwestern portion of the county drains into the Roanoke River basin, the southern portion drains into the Pungo River basin, and the remainder of the county drains into the Albemarle Sound.

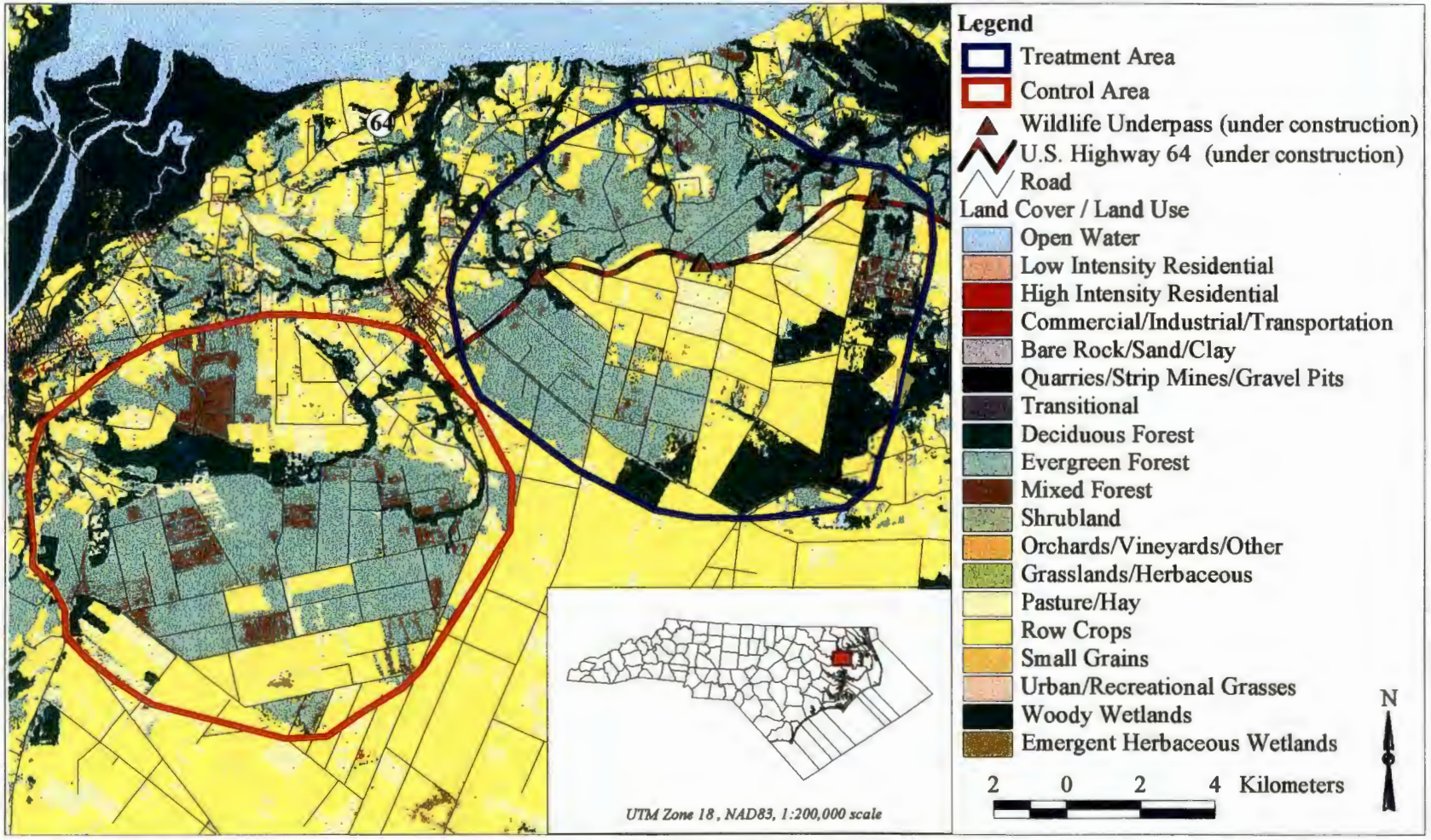

Figure 2. Study area locations to determine the impacts of a 4-lane highway on black bear ecology, Washington County, North Carolina.

The elevation ranges from 4.6–15.2 m above sea level in the western portion of the county to <1.5 m on the flood plains of the Scuppernong River and Bull's Bay Swamp in the northeastern portion (Soil Conservation Service 1979). Washington County has many coastal plain characteristics, including shallow valleys and meandering stream channels. Most of these valleys terminate in estuaries along the coast, where local relief is <1 m.

Seven soil types occur in Washington County. The Augusta-Altavista-Wahee, Conetoe-Wickham-Tarboro, and Dragston-Conetoe-Altavista series occur on uplands and range from well-drained to somewhat poorly-drained soils. Those soils contain loamy and sandy surface layers and loamy, sandy, or clayey subsoil layers. The Cape Fear-Portsmouth-Roanoke, Dorovan, Belhaven-Wasda-Roper, and Pungo series occur on stream terraces, flood plains, and level flats and all are very poorly drained. Those associations contain loamy and mucky surface soil and subsoil layers (Soil Conservation Service 1979).

LAND USE

Human development was limited to a few large farms within the study areas and several small towns near the study area boundaries. Weyerhaeuser Forest Products Company was the largest landowner within the study area, with private farms and woodlots accounting for the remaining portion. Agriculture was the primary land use in the study area, with approximately 38% of the lands in crop production. Major agricultural crops included corn, wheat, soybeans, peanuts, and cotton. Additionally, small acreages of pasture and turf existed. Irrigation of farmlands and aerial application of fertilizers and pesticides was common.

Managed pine forests provided the primary source of income, with pulpwood as the leading product, followed by lumber and naval products. During my study, several managed pine stands in both study areas were either thinned or cleared. Weyerhaeuser Forest Products Company leased most of their lands to hunting clubs. Hardwood drainages existed mostly on private lands and were commonly leased to hunting clubs as well.

CLIMATE

Washington County is hot and humid in summer, but is frequently cooled by Atlantic sea breezes. Winters are typically cool, with occasional, brief cold spells. The average minimum and maximum summer temperatures are 25° C and 31° C, respectively, compared with the average minimum and maximum temperatures of 0° C and 6.7° C, respectively, during winter (Soil Conservation Service 1979). Average annual precipitation ranges from 115 to 127.5 cm. Rains occur throughout the year and can be heavy at times. Maximum precipitation occurs in the summer between April and September. Fall typically is the driest season in Washington County. The average annual snowfall accumulation is 10.2 cm. The average freeze-free period ranges from 200 to 300 days.

FLORA

Managed pine forests comprised the majority of the forested lands, whereas hardwoods were restricted to natural drainages. Managed pine forests contained age classes ranging from seedlings to 60-year-old stands. Common overstory species were

loblolly pine (*Pinus taeda*), yellow-poplar (*Liriodendron tulipifera*), sugar maple (*Acer saccharum*), red maple (*Acer rubrum*), and sweetgum (*Liquidambar styraciflua*). Common understory species included switch cane (*Arundinaria gigantea*), red bay (*Persea borbonia*), muscadine grapes (*Vitis rotundifolia*), greenbriar (*Smilax* spp.), and poison ivy (*Toxicodendron radicans*). Most hardwood drainages were composed of trees in the 40- to 120-year age classes and were dominated by oaks (*Quercus* spp.), bald cypress (*Taxodium distichum*), and blackgum (*Nyssa sylvatica*).

FAUNA

All of Washington County is classified as occupied black bear habitat by the NCWRC, with 17% classified as core habitat. The juxtaposition of managed and unmanaged forests with agricultural areas provided food, cover, and protection for many other wildlife species. Common upland game species were white-tailed deer (*Odocoileus virginianus*), gray fox (*Urocyon cinereoargenteus*), eastern gray squirrel (*Sciurus carolinensis*), eastern cottontail (*Sylvilagus floridanus*), eastern wild turkey (*Meleagris gallopavo*), mourning dove (*Zenaida macroura*), northern bobwhite (*Colinus virginianus*), several species of waterfowl, and hundreds of songbird species. Furbearers also were abundant, including river otter (*Lutra canadensis*), mink (*Mustela vison*), raccoon (*Procyon lotor*), muskrat (*Ondatra zibethica*), and Virginia opossum (*Didelphis virginiana*). Other species of mammals include red wolf (*Canis rufus*), coyote (*Canis latrans*), and bobcat (*Lynx rufus*).

CHAPTER III

METHODS

APPROACH

Conventional mark-recapture techniques based on live-trapping often provide population estimates with relatively low precision and poor accuracy because of low sample sizes and sampling biases. Advances in DNA technology offer alternative methods for population estimation that may help overcome problems with conventional mark-recapture techniques (Woods et al. 1999). A relatively new technique is to "mark" animals based on DNA collected from hair samples; this technique has many advantages over live-trapping, including greater capture probabilities, no tag loss, lower sampling bias, and decreased intrusiveness (Woods et al. 1999, Mills et al. 2000). Researchers have effectively used hair sampling to determine abundance of brown bears (*Ursus arctos*) in British Columbia (Mowat and Strobeck 2000) and Glacier National Park (Kendall et al. 2002) and of black bears on Tensas River National Wildlife Refuge in Louisiana (Boersen et al. 2003) and the Okefenokee National Wildlife Refuge in Georgia and Osceola National Forest in Florida (Dobey 2002). I used DNA from hair samples to estimate population abundance and density for the treatment and control study areas.

DNA from hair and tissue samples also can be used to assess genetic structure within animal populations (Paetkau et al. 1995, Warrillow et al. 2001) and, thus, to identify potential barriers to genetic exchange (Paetkau et al. 1998*a*). I used DNA from hair and tissue samples to determine genetic relatedness, population structure, gene flow rates, and genetic diversity of black bears on the 2 study areas.

DATA COLLECTION

Capture and Handling

Livetrapping was included as a part of this study with the primary objective to deploy radio collars (J. L. Kindall, University of Tennessee, Knoxville, unpublished data). Trap sites were established based on recent bear activity, such as scats, tracks, or visual observations (Figures 3 and 4). Bears were captured with Aldrich, spring-activated, foot snares (Aldrich Animal Trap Co., Clallam Bay, Washington, USA) modified with an automobile hood spring to reduce injuries (Johnson and Pelton 1980).

Trap sites were set within close proximity to logging and farm roads to facilitate access. All trap site locations were recorded in Universal Transverse Mercator (UTM; Zone 18) coordinates with global positioning system (GPS) receivers (Garmin GPS 12 XL, Olathe, Kansas, USA). From 9 June 2000 to 8 September 2000, 15–30 trap sites were checked daily for captures.

Captured bears were immobilized with an intra-muscular injection of ketamine hydrochloride (Bristol Laboratories, Syracuse, New York, USA) and xylazine hydrochloride (Haver-Lockhart, Inc., Shawnee, Kansas, USA) at 8.8 mg/kg and 4.4 mg/kg estimated body mass, respectively. Once bears were sedated, a wetting agent (Akwa Tears, Akorn Incorporated, Abita Springs, Louisiana, USA) was immediately applied to the eyes to prevent desiccation. A blindfold was used to cover the eyes for protection from debris, insects, and to reduce visual stimuli. Bears were carefully inspected for capture injuries or signs of previous injury. Vital signs, including body temperature, respiration, and pulse, were monitored throughout the handling period.

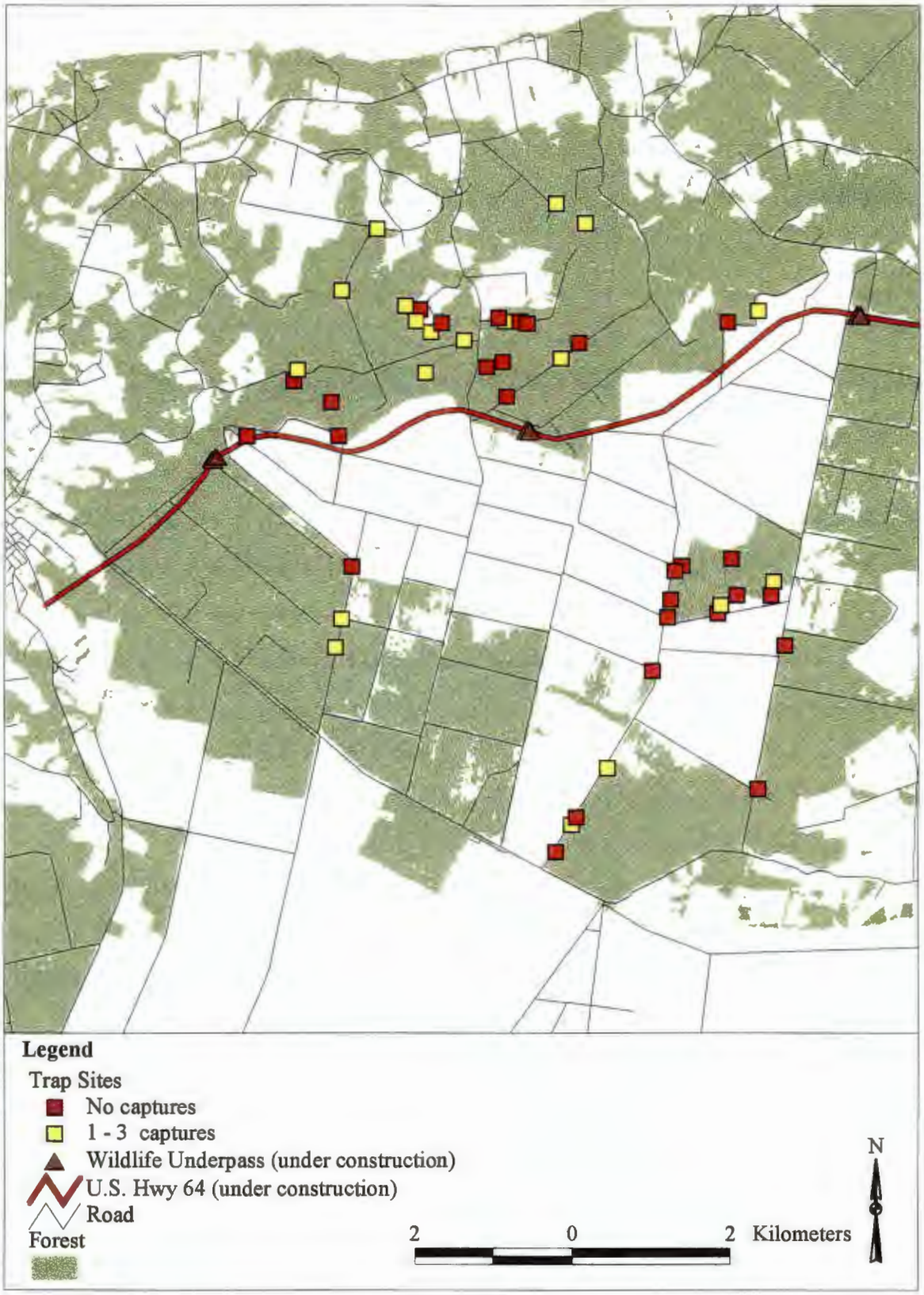

Figure 3. Locations of live-trapping sites of black bears on the treatment area, Washington County, North Carolina, 2000.

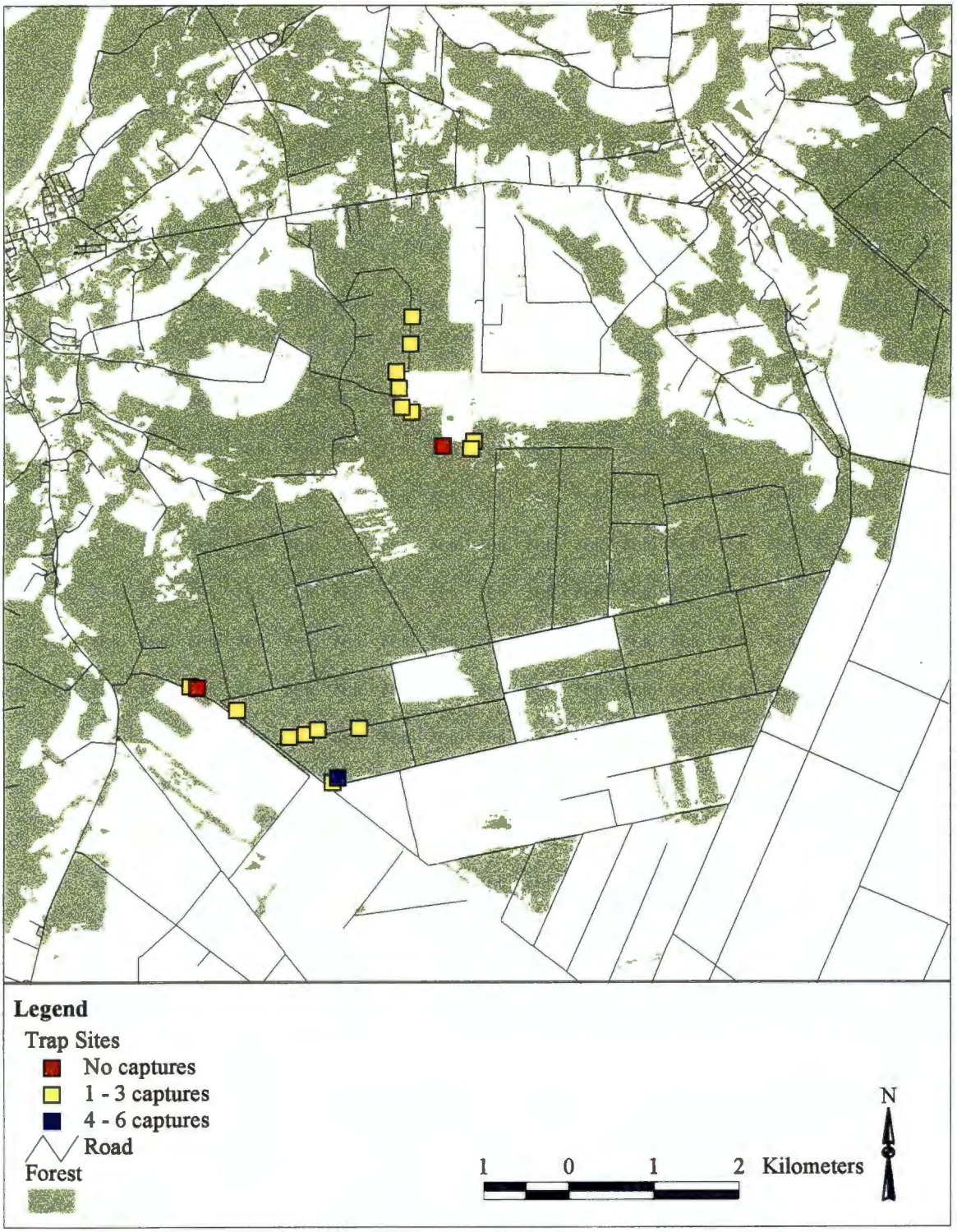

Figure 4. Locations of live-trapping sites of black bears on the control area, Washington County, North Carolina, 2000.

Every bear received 2 metal eartags with a unique identification number and a lip and inner thigh tattoo with a corresponding number. An upper premolar tooth was extracted from each bear for cementum annuli aging by Mattson Laboratories (Milltown, Montana, USA). A tissue sample (2–4 mm wedge) was taken from the tip of the ear for DNA microsatellite analysis.

Standard body measurements were collected on all captured bears (Eason et al. 1996. All adult females and large males (>100 kg) were fitted with a radio collar (Telonics, Inc., Mesa, Arizona, USA and Advanced Telemetry Systems, Isanti, Minnesota, USA). Leather spacers served as a breakaway function on each radio collar, which enabled the collars to drop off after 1–2 years. Spacers were soaked in vegetable oil for 24 hours and were approximately 0.75 cm thick, 5 cm wide, and ranged from 10.2 to 15.2 cm in length. After completion of data collection, and at least 1 hour after the initial immobilization, an intravenous injection of yohimbine hydrochloride (Spectrum Laboratory Products, New Brunswick, New Jersey, USA) was administered at a dosage of 1 ml per 22.7 kg as an antagonist to xylazine hydrochloride. All captured bears were handled according to animal handling protocols approved by the University of Tennessee Office of Laboratory Animal Care (IACUC #1020).

Hair Sampling

I used ArcView® (ESRI, Redlands, California, USA) geographic information system (GIS) software to generate random location coordinates for 70 hair-sampling sites on both the treatment and control areas. Coordinates for hair-sampling sites were only generated within forested habitats. The number of sites was chosen so that all bears had

access to at least 1 sampling site based on minimum home range estimates. Field personnel used GPS receivers to establish the actual sampling sites within a 250-m radius from the designated point location to facilitate access by roads and trails (Figures 5 and 6). Some sites (5 treatment area sites, 7 control area sites) were relocated to a new random location to avoid recently cut or inaccessible areas.

Each hair-sample site consisted of a barbed-wire enclosure that was baited with bakery products and scented with raspberry extract. A single strand of 15.5-gauge barbed wire was stretched around 4 corner trees at 40–50 cm above the ground. The barbed wire was positioned high enough for bears to travel underneath but still low enough to snag hair. Bait was hung in the center of the trap from a metal wire stretched diagonally between 2 corner trees approximately 1.8 m above the ground. The dimensions of the enclosure were approximately 5 × 5 m, or large enough to prevent bears from reaching the bait without entering the trap. Each site was marked with surveyor flagging and identified with a sign explaining its purpose (Figure 7).

Starting on 1 October 2000, sites were checked for hair samples once each week for 7 weeks. On each study area the 70 sampling sites were divided into 3 groups of 23–24 traps that were checked on the same day each week. Hair samples with ≥5 hairs were collected from the barbs and placed in labeled envelopes. Remaining hair on the barbs was burned off to prevent mixing of DNA with future samples. After hair collection, each station was re-baited and scented. All collected hair samples were frozen at the field site and later prepared at the University of Tennessee for microsatellite analysis. Preparation consisted of thawing frozen samples and inspecting for the presence of hair roots. Approximately 0.6 cm of the root ends was clipped from all hairs of each sample.

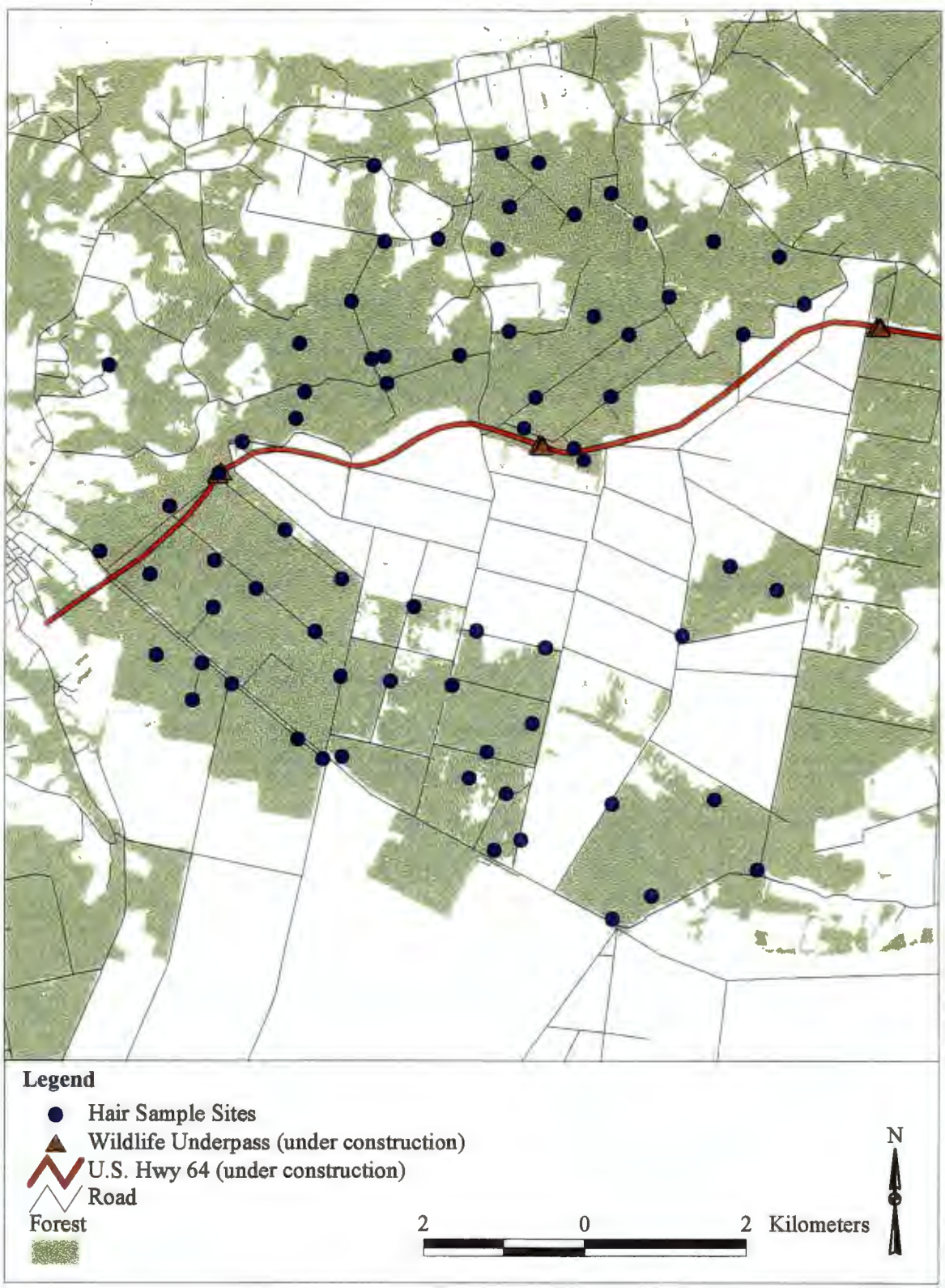

Figure 5. Locations of black bear hair-sampling sites on the treatment area, Washington County, North Carolina, 2000.

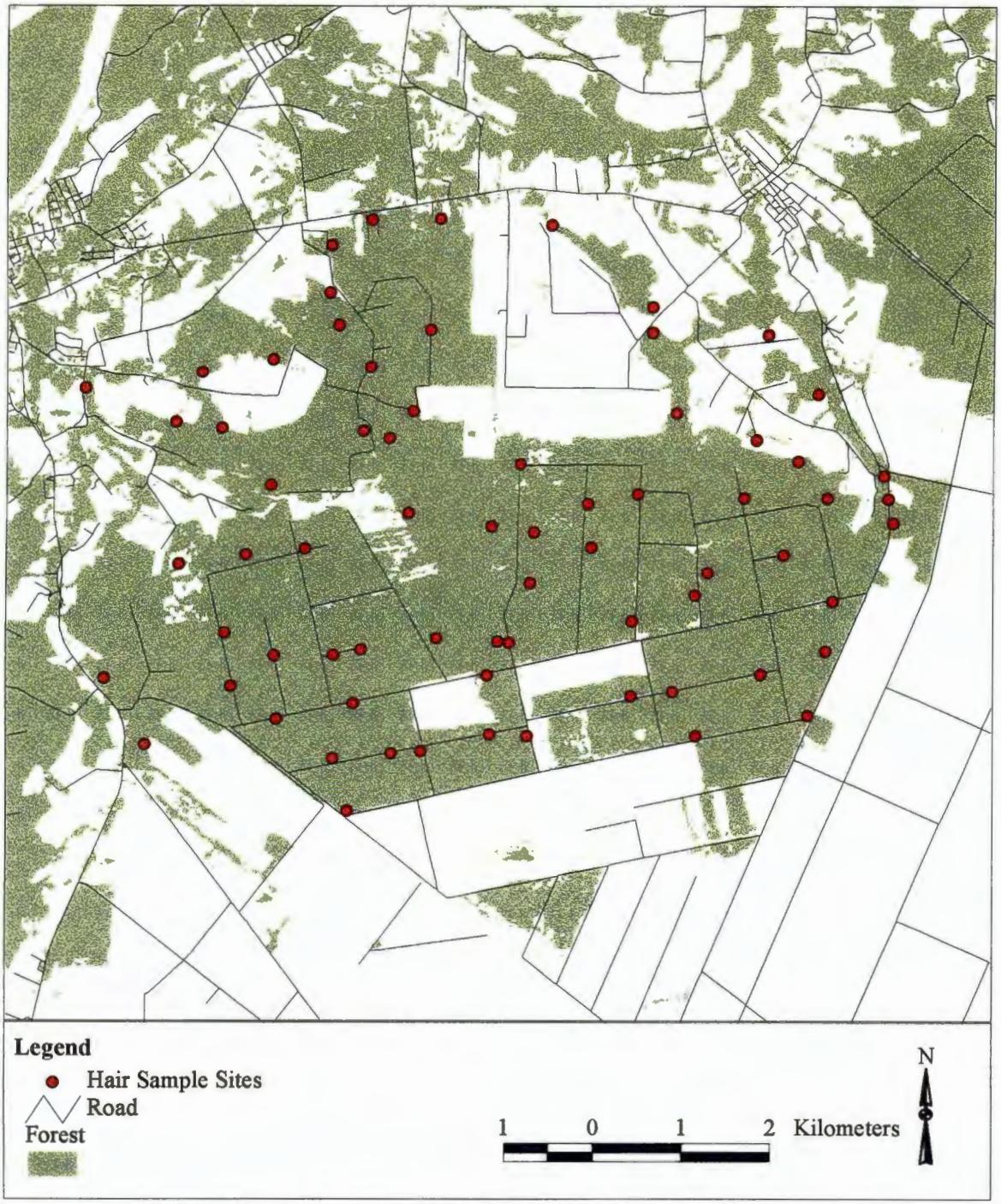

Figure 6. Locations of black bear hair-sampling sites on the control area, Washington County, North Carolina, 2000.

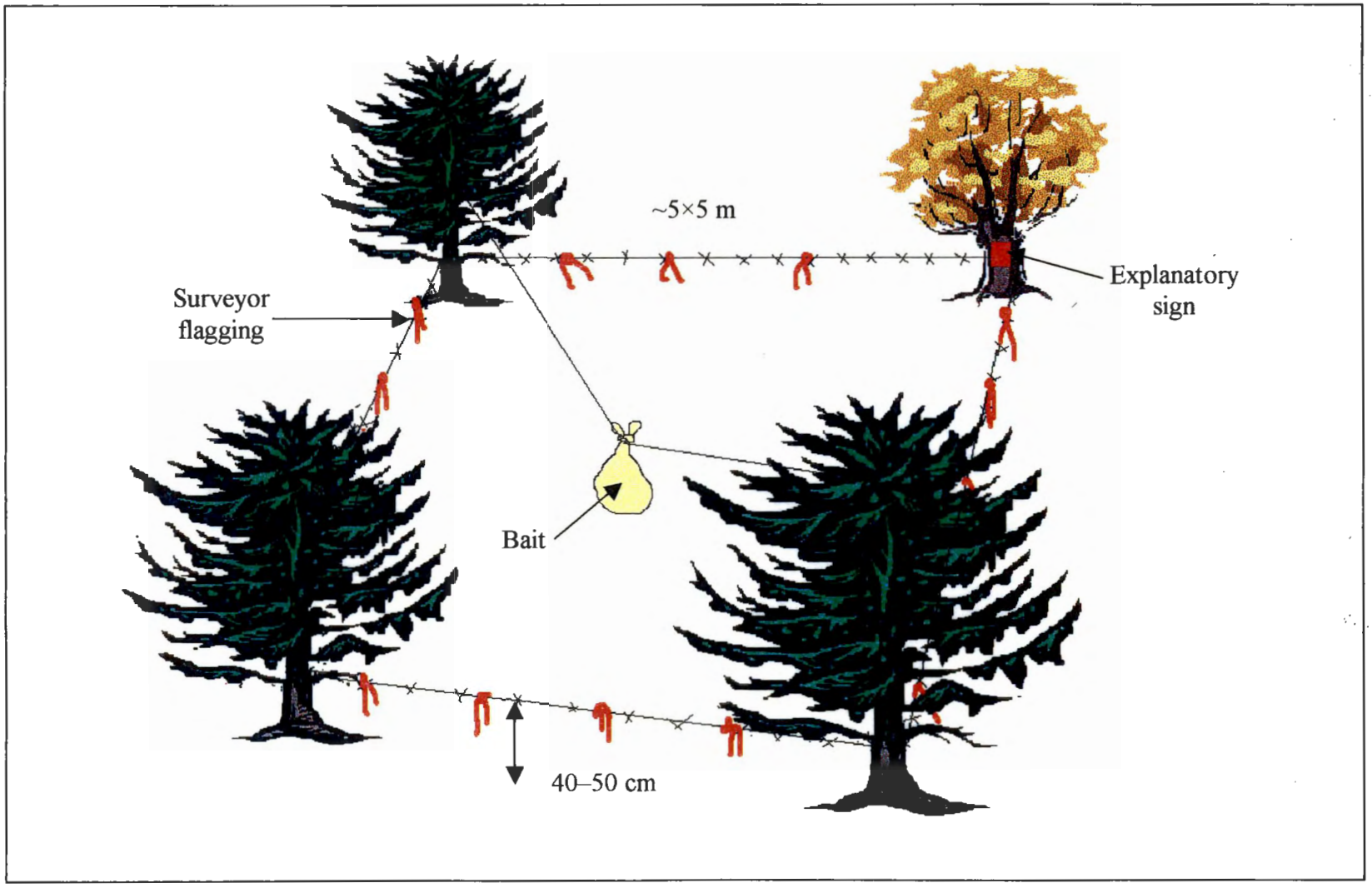

Figure 7. Design of black bear hair-sampling sites on treatment and control areas, Washington County, North Carolina, 2000.

Each sample was then placed into a 1.5-ml centrifuge tube and shipped to the U.S. Geological Survey Aquatic Ecology Branch at the Leetown Science Center in Kearneysville, West Virginia for microsatellite analysis.

Microsatellite Analysis

Molecular markers, such as allozymes and mitochondrial DNA, have been used on a variety of species to identify unique individuals, but these techniques only allow detection of relatively low genetic variation (McConnell et al. 1995). Microsatellites have become increasingly popular to study population genetics because they are highly variable and allele frequencies are easily interpreted. Microsatellite analysis uses polymerase chain reactions (PCR) to amplify DNA; therefore, data collection is rapid and small or degraded DNA samples can be used, making PCR suitable for analysis of hair samples (Paetkau et al. 1995, Mills et al. 2000).

I randomly selected 25 hair-sampling sites for each sampling period and randomly chose 1 hair sample from each of those sites for microsatellite analysis. That number of samples was a conservative estimate of samples needed to achieve a coefficient of variation (CV) of $\leq 20\%$ with a projected population size of 50–150 bears, using the general Lincoln-Petersen model. All tissue samples collected during the live-trapping season also were sent for microsatellite analysis. Ten microsatellite loci were analyzed for each selected hair sample at the following markers (Appendix B): G1A, G1D, G10B, G10L, G10C, G10M, G10P, G10X, MU23, and MU50 (Paetkau and Strobeck 1994, Paetkau et al. 1995).

POPULATION ABUNDANCE

Sampling

For the population abundance analysis, I only included individuals identified from the hair-sampling season. A random sample is required for population estimation and the hair-sampling sites were randomly distributed throughout both study areas. However, no specific sampling method was used for individuals captured during the live-trapping season because the main objective was to deploy radio-collars.

Allele Frequencies

Gene or allele frequency is the fundamental parameter in population genetic studies (Nei 1987). The frequency of a particular allele is the proportion of all alleles of the gene that are of the specified type (Hartl and Clark 1997). For example, a single locus with 2 alleles, i and j, potentially can have 3 genotypes: A_iA_i, A_iA_j, and A_jA_j. Out of a sample of n individuals selected from a population and a locus with m codominant alleles, the allele frequencies may be calculated, where n_{ij} is the number of individuals (n) for genotype A_iA_j. The allele frequency of A_i can then be estimated by:

$$\hat{x}_i = \left(2n_{ii} + \sum_{j \neq i} n_{ij} \right) \Big/ 2n \text{ (Nei 1987).}$$

In population studies, genetic change can be described by the change in allele frequencies (Nei 1987). Changes can be caused by a number of factors, such as migration (movement from 1 area to another, often to reproduce, with a subsequent return), selection, and non-random mating. Using program BIOSYS-1 (Swofford and

Selander 1989), I calculated the allele frequency of each allele sampled during the hair-sampling season at all 10 loci for the treatment and control area populations.

Hardy-Weinberg Equilibrium

The frequencies of homozygotes and heterozygotes in a population will stay constant for generations if specific criteria are met (Nei 1987); this condition is known as the Hardy-Weinberg law and is an important assumption for population abundance analyses that are based on genetic samples (i.e., probability of identity). This law states that an equilibrium of allelic and genotypic frequencies will arise and be maintained in any diploid population if it is (1) large in size, (2) undergoes random mating, (3) has no mutation or migration of individuals, and (4) is not subject to selection (Krebs 1994). These frequencies conform to Hardy-Weinberg proportions if:

$$p^2 + 2pq + q^2 = 1,$$

where p is the frequency of allele A_i and q is the frequency of allele A_j. Deviations from Hardy-Weinberg proportions can be the result of a number of factors, including inbreeding, assortative mating (mating pairs are more or less similar in phenotype than expected by chance), the existence of null alleles, and natural selection (Nei 1987, Hartl and Clark 1997).

I used the probability test in program GENEPOP (version 3.1; Raymond and Rousset 1995), which tests for the random union of gametes, to determine if the frequencies of sampled alleles were inconsistent with Hardy-Weinberg expectations. The program uses Guo and Thompson's (1992) Markov chain randomization test to determine

the *P*-values for each locus. I used sequential Bonferroni adjustments (Rice 1989) to determine statistical significance of multiple tests.

Linkage Disequilibrium

When mating is random, alleles at each locus are in random association with one another (i.e., Hardy-Weinberg equilibrium); however, an allele at one locus may not be in random association with an allele at another locus (Hartl and Clark 1997). This phenomenon is known as linkage disequilibrium and occurs when the rate of gametic recombination during meiosis is so low that certain alleles are associated in the same manner as the previous generation (Hartl and Clark 1997). Thus, alleles at different loci appear as if they are "linked" because they consistently occur together. Unlike Hardy-Weinberg equilibrium, which can be attained after 1 generation, linkage equilibrium may require several generations to be attained, and the rate at which it is attained is dependent on the rate of recombination (Hartl and Clark 1997).

Because the 10 microsatellite loci used in my study have been found to be independent (Paetkau and Strobeck 1994, Paetkau et al. 1995), detected associations may indicate sampling bias, sampling of siblings, the presence of immigrants, or stochastic processes (T. L. King, U.S. Geological Survey, personal communication). Therefore, I used the genotypic linkage disequilibrium test in program GENEPOP to test whether the genotypes at one locus were independent from genotypes at another locus. Sequential Bonferroni adjustments were used to determine statistical significance.

Probability of Identity

Unique marking of animals is essential for all mark-recapture studies. However, obtaining DNA samples that uniquely identify an individual can be problematic; depending on the number of loci considered, different animals can share the same genetic profile (Mills et al. 2000). Moreover, extracting DNA from hair can lead to genotyping errors because of problems with 'allelic dropout', when only one allele of a heterozygous individual is detected thereby producing false homozygotes. Common causes of allelic dropout are low DNA content in hair samples and degradation of DNA (Taberlet and Luikart 1999).

When obtaining a population estimate based on a recapture technique with DNA markers, close relatives can be mistaken for recaptures, causing the population estimate to be biased low (Taberlet and Luikart 1999, Mills et al. 2000). Therefore, it is important to quantify the power of the molecular markers (microsatellites) to resolve between different individuals. The probability of identity (PI) is the statistic most commonly used to quantify that power and is defined as the probability of obtaining identical genotypes given certain allele frequency distributions (Taberlet and Luikart 1999). This frequency can be calculated for a single locus with multiple alleles by:

$$\mathrm{PI}_{\text{single locus}} = \sum_i p_i^4 + \sum_i \sum_{j>i} \left(2 p_i p_j\right)^2 ,$$

where p_i and p_j are the frequencies of the ith and jth alleles, assuming the allele genotypes are in Hardy-Weinberg proportions (Taberlet and Luikart 1999). The overall PI for a given population can be estimated as:

$$\mathrm{PI}_{\text{overall}} = \prod (\mathrm{PI}_{\text{single locus}}).$$

This formula is calculated across all sampled loci and is based on the assumption that loci are independent (linkage equilibrium). If loci are not independent, the overall PI will be biased low (Mills et al 2000). Substantial bias can occur in populations containing many siblings (Donnelly 1995). One way to determine how many loci are sufficient to obtain a low PI is to calculate the PI for siblings:

$$\text{PI}sibs = 0.25 + (0.5\sum p_i^2) + [0.5(\sum p_i^2)^2] - (0.25\sum p_i^4),$$

where p_i is the frequency of the ith allele (Taberlet and Luikart 1999). This formula represents the upper limit on the possible range of PIs in a population and it gives an upper limit for the number of loci needed to likely resolve all individuals, assuming independence among all loci. Taberlet and Luikart (1999) found that approximately 14 loci are required to achieve a low probability (e.g., 0.0001) of finding 2 siblings with identical genotypes, in contrast to approximately 7 loci to achieve the same probability for 2 random individuals with identical genotypes. I used 10 loci to determine genotypes in my study, which is considered to be conservative for populations that do not contain many siblings (Taberlet and Luikart 1999).

Woods et al. (1999) described 3 match tests that provide PI estimates for each individual genotype, unlike $\text{PI}_{\text{overall}}$ and PI*sibs*, which estimate PI over all observed genotypes (Boersen 2001). The match tests are based on the assumption that samples taken from individuals are not necessarily random and may come from individuals that are related (Woods et al. 1999). For example, sibling bears may visit hair traps together or a female may visit a hair trap with her cubs (Woods et al. 1999). The tests described by Woods et al. (1999) determine the probability that a parent, offspring, or sibling would have the same observed genotype. Because the probability for siblings produces the most

conservative estimate, I did not consider estimators for parents and offspring. The sibling match test can be calculated for homozygotes as,

$$P_{sib} = (1 + 2p_i + p_i^2)/4;$$

and for heterozygotes as,

$$P_{sib} = (1 + p_i + p_j + 2p_ip_j)/4 \text{ (Woods et al. 1999).}$$

I computed the sibling match test (P_{sib}) for the 10-locus genotypes of all individuals on the treatment and control areas. Genotypes that produced a $P_{sib} > 0.05$ were not considered to be unique individuals and were excluded from population estimation (Woods et al. 1999, Mowat and Strobeck 2000, Boersen 2001, Dobey 2002).

Population Estimation

Population closure is defined as a population size that remains constant over the period of investigation; that is, where no recruitment (births or immigration) and no losses (death or emigration) occur. Thus, closed population estimators are appropriate for studies conducted over relatively short time periods (Pollock et al. 1990). The population closure definition is divided into 2 components: geographic and demographic closure. A study area is geographically closed when it has a distinct boundary that limits the population. A population closed to birth, immigration, death, and emigration is demographically closed (White et al. 1982).

Because of the short duration of the hair-sampling season, I only considered closed models for population estimation, including the modified Lincoln-Petersen estimator (Seber 1982), Bailey's triple catch estimator (Caughley 1977), and multiple mark-recapture models (Otis et al. 1978). Based on guidelines given by Otis et al. (1978)

and White et al. (1982), estimates for all models were produced with the objective to obtain a CV of $\leq 20\%$ and capture probabilities $\geq 20\%$. Capture histories were pooled into different configurations as appropriate to increase capture probabilities (Menkens and Anderson 1988).

Modified Lincoln-Petersen Model.--The "Petersen estimator" is a simple method for estimating the population size N based on the ratio of marked and unmarked individuals as given by Seber (1982):

$$\hat{N} = \frac{M_2 n_2}{m_2},$$

where M_2 = the number of animals marked in capture period i, n_2 = the number of animals captured in period $i+1$, and m_2 = the number of marked animals captured in period $i+1$. A modified version of the Lincoln-Petersen model was given by Chapman (1951) to decrease bias with small sample sizes:

$$\hat{N} = \frac{(M_2 + 1)(n_2 + 1)}{(m_2 + 1)} - 1,$$

with an approximately unbiased estimate of variance:

$$\text{var}\,\hat{N} = \frac{(M_2 + 1)(n_2 + 1)(M_2 - m_2)(n_2 - m_2)}{(m_2 + 1)^2 (m_2 + 2)}.$$

The modified Lincoln-Petersen model is based on the following assumptions (Pollock et al. 1990):

(1) the population is closed to additions (births or immigrants) and deletions (deaths or emigrants),

(2) all animals are equally likely to be captured in each sample, and

(3) marks are not lost and are not overlooked by the observer.

The modified Lincoln-Petersen model requires 2 sampling periods; therefore, I pooled the 7 sampling periods based on 2 different configurations for each study area (i.e., 4 and 3 weeks; 3 and 4 weeks; Figure 8). The most appropriate pooling configuration was selected by minimizing both the number of recaptures lost due to pooling and the CV.

Bailey's Triple Catch.--Bailey (1951) described a population estimator that allows for the closure assumption to be relaxed when individuals are either born or migrate into the study area between the marking and recapture periods (Caughley 1977). The model requires 3 sampling occasions (0, 1, and 2), or 2 marking occasions and 2 recapturing occasions, where the middle occasion (occasion 1) is the second marking occasion and the first recapture occasion. Population size can be calculated as:

$$N_1 = \frac{M_1(n_1 + 1)m_{02}}{(m_{01} + 1)(m_{12} + 1)},$$

where N_1 is the size of the population at occasion 1, M_1 is the number of animals marked during occasion 1, n_1 is the number of individuals examined for marks at occasion 1, m_{01} and m_{02} are the recaptures from occasion 0 at occasions 1 and 2, and m_{12} is the recapture frequency of occasion 1 at occasion 2. The standard error can be calculated as (Caughley 1977):

$$S.E. = \sqrt{\frac{M_1^2(n_1 + 1)(n_1 + 2)(m_{02} - 1)m_{02}}{(m_{01} + 1)(m_{01} + 2)(m_{12} + 1)(m_{12} + 2)}}.$$

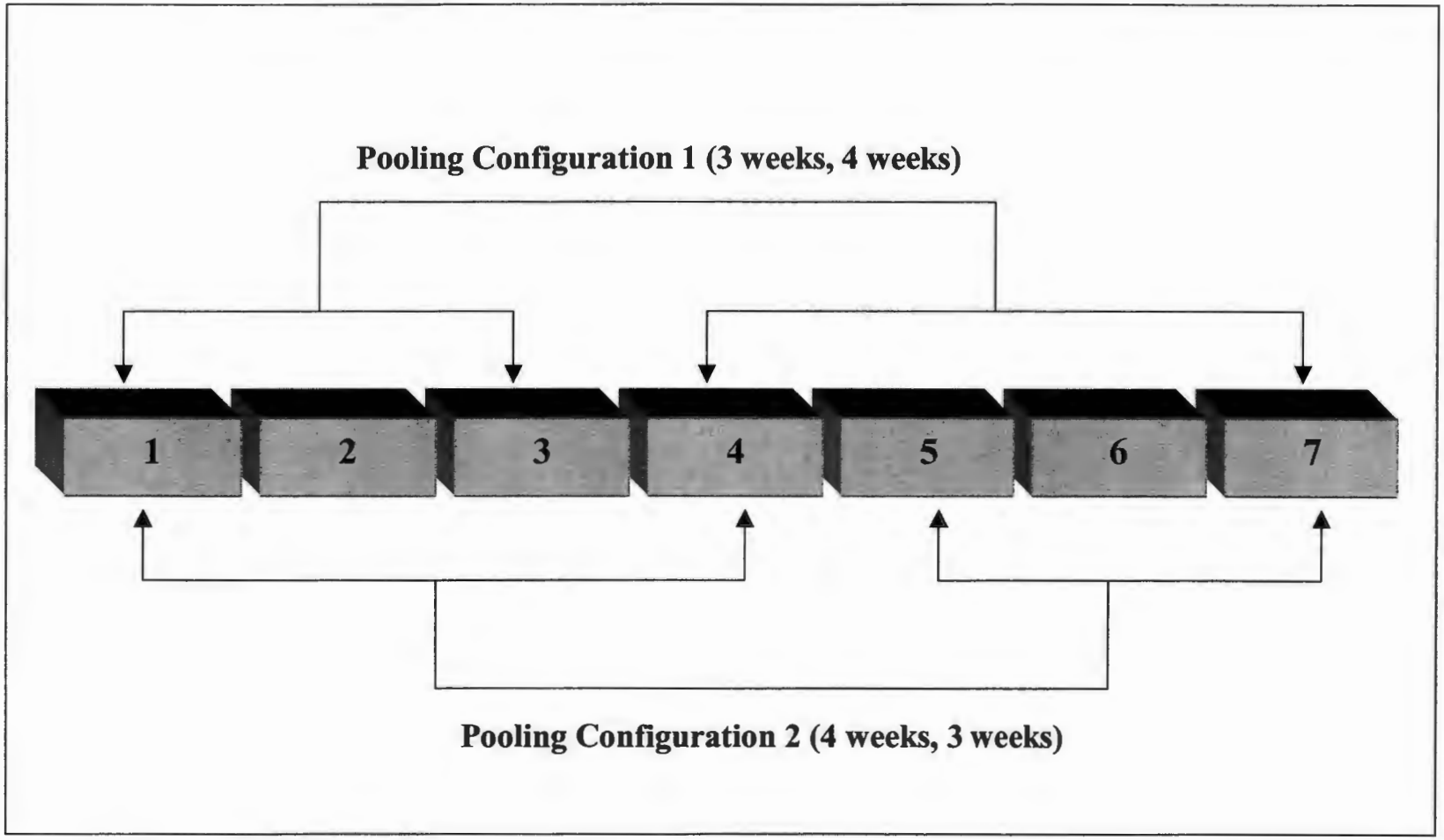

Figure 8. Pooling configurations of sampling periods used to estimate black bear population abundance based on the modified Lincoln-Petersen model for the treatment and control areas, Washington County, North Carolina, 2000.

I pooled data from the 7 sampling periods into 3 sampling occasions using 3 different pooling configurations (Figure 9) for the treatment and control areas.

Multiple Mark-Recapture Models.--Eight mark-recapture models described by Otis et al. (1978) can be used for study designs with >2 sampling periods. The equal catchability model (M_o or null model) is based on the following assumptions:

(1) the population is closed,

(2) animals do not lose their marks during the experiment,

(3) all marks are correctly noted and recorded at each capture occasion, and

(4) each animal has a constant and equal probability of capture during each trapping period (Otis et al. 1978).

The null model is the most restrictive of the 8 models and is somewhat unrealistic. It is widely recognized that the fourth assumption is not met in most capture-recapture studies (White et al. 1982). Thus, variations of the model were developed to allow the fourth assumption to be relaxed (Otis et al. 1978).

Model M_t allows capture probabilities to vary by time. This model is useful in situations where, for example, weather conditions or different trapping methods may reduce trap activity. Model M_b allows capture probabilities to vary by behavioral response. This model is useful for situations where animals become "trap happy" or "trap shy" based on previous trapping experiences (White et al. 1982). Model M_h, the heterogeneity model, allows capture probabilities to vary by individual animal so that differences in capture probabilities because of age, sex, social dominance, or accessibility to traps can be accounted for (Otis et al. 1978). Combinations of these 3 unequal capture probability models also exist and were considered: models M_{tb}, M_{th}, M_{bh}, and M_{tbh}.

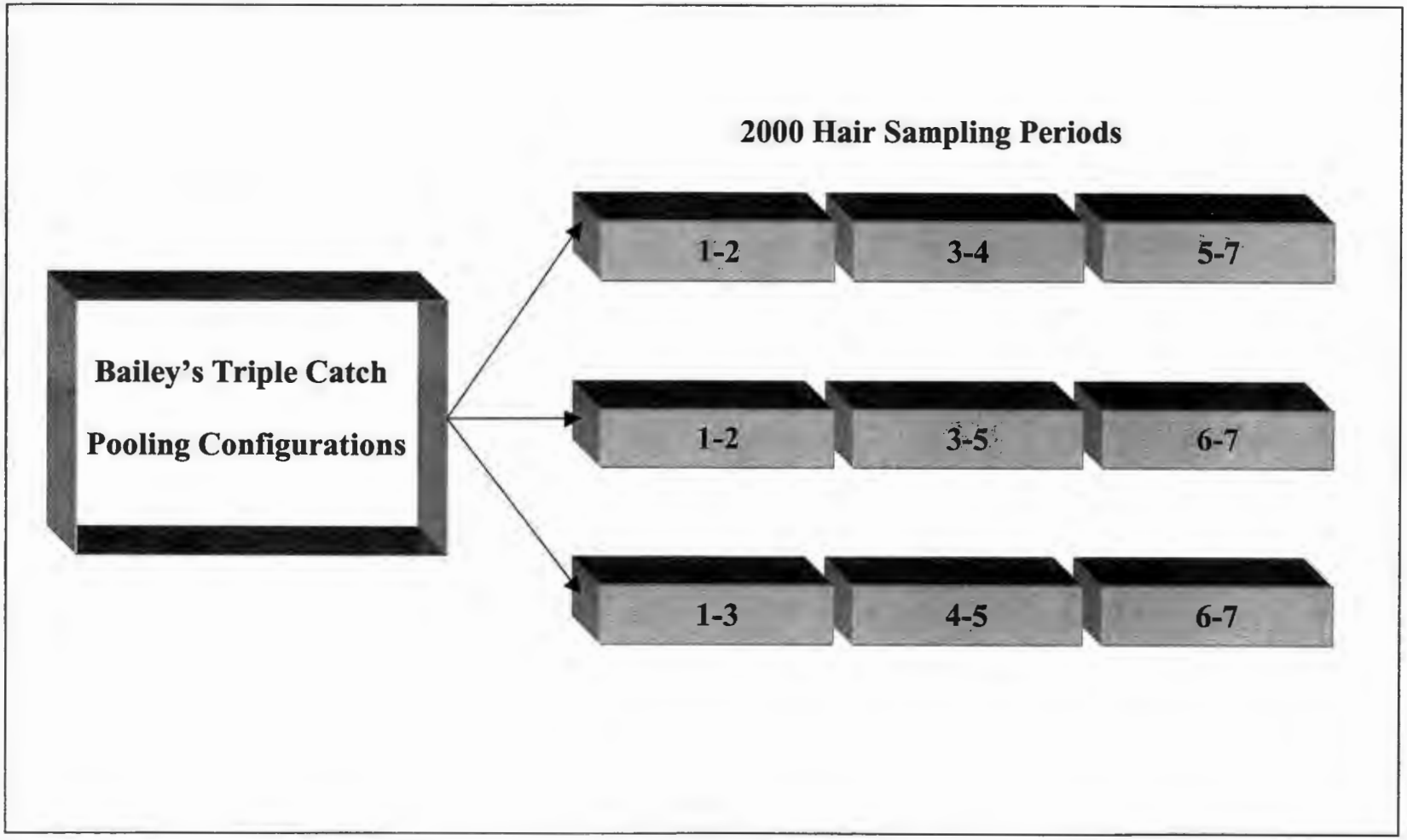

Figure 9. Pooling configurations of 7 sampling periods used to estimate black bear population abundance based on the Bailey's Triple Catch estimator for the treatment and control areas, Washington County, North Carolina, 2000.

Model M_{tbh} only exists conceptually, and no statistical analyses have been developed to facilitate its use (Otis et al. 1978). Models M_o, M_t, M_{bh}, and M_h, are based on maximum likelihood algorithms and do not have closed form estimators for population abundance. Therefore, population estimates cannot be produced from a simple formula that equates to N (Otis et al. 1978).

I considered 3 additional multiple mark-recapture models described by Chao (1987, 1988, 1989) and Chao et al. (1992). These additional models are based on modified calculations of M_h and M_t as described by Otis et al. (1978) and are specifically designed to provide reliable population estimates when capture probabilities are relatively low. Assumptions for the Chao M_h and Chao M_t estimators are the same as the heterogeneity and time models described by Otis et al. (1978). In the presence of both time and heterogeneity, Chao et al. (1992) developed model M_{th}, which only existed conceptually before.

Otis et al. (1978) claimed that M_h generally produces adequate population estimates if many individuals are caught a large number of times. However, M_h has a tendency to underestimate population size when many individuals have very small capture probabilities (Chao 1987). Therefore, Chao (1987, 1988) developed Chao M_h, which is a closed form estimator and is appropriate when many individuals are captured only a few times (Chao 1987). Population abundance can be estimated with this model as:

$$\text{Chao } \hat{N}_h = S + \frac{f_1^2}{2f_2},$$

or,

$$\text{Chao } \tilde{N}_h = S + \left[\frac{f_1^2}{2f_2}\right]\left\{\left[1 - \frac{2f_2}{tf_1}\right] \Big/ \left[\frac{1-3f_3}{tf_2}\right]\right\} \text{ if } tf_1 > 2f_2, tf_2 > 3f_3, \text{ and } 3f_1f_3 > 2f_2^2,$$

where S is the number of individuals captured in the experiment, f_k is the number of animals captured exactly k times, and t is the number of sampling occasions (Chao 1988). Estimates of variance are calculated as:

$$\text{var(Chao } \hat{N}_h) = f_2\left[0.25\left(\frac{f_1}{f_2}\right)^4 + \left(\frac{f_1}{f_2}\right)^3 + 0.5\left(\frac{f_1}{f_2}\right)^2\right],$$

and,

$$\text{var(Chao } \tilde{N}_h) = f_2\left[0.25A^2\left(\frac{f_1}{f_2}\right)^4 + A^2\left(\frac{f_1}{f_2}\right)^3 + 0.5A\left(\frac{f_1}{f_2}\right)^2\right],$$

where,

$$A = \left[\frac{(1-2f_2)}{tf_1} \Big/ \frac{(1-3f_3)}{tf_2}\right] \text{(Chao 1988).}$$

Closed form estimators for models Chao M_t and M_{th} are described by Chao (1989) and Chao et al. (1992).

I used program CAPTURE (Rextad and Burnham 1992) to compute population estimates and associated standard errors for all multiple mark-recapture models. I calculated population estimates for the 7 individual sampling periods and for 7 different pooling configurations (Figure 10). In addition to evaluating the most consistent source of bias and using biological judgment, I also considered a model selection technique available within program CAPTURE to determine the most appropriate model. The model selection criteria are based on a series of chi-square goodness-of-fit tests, which detect the various sources of unequal catchability and determine which model best fits the

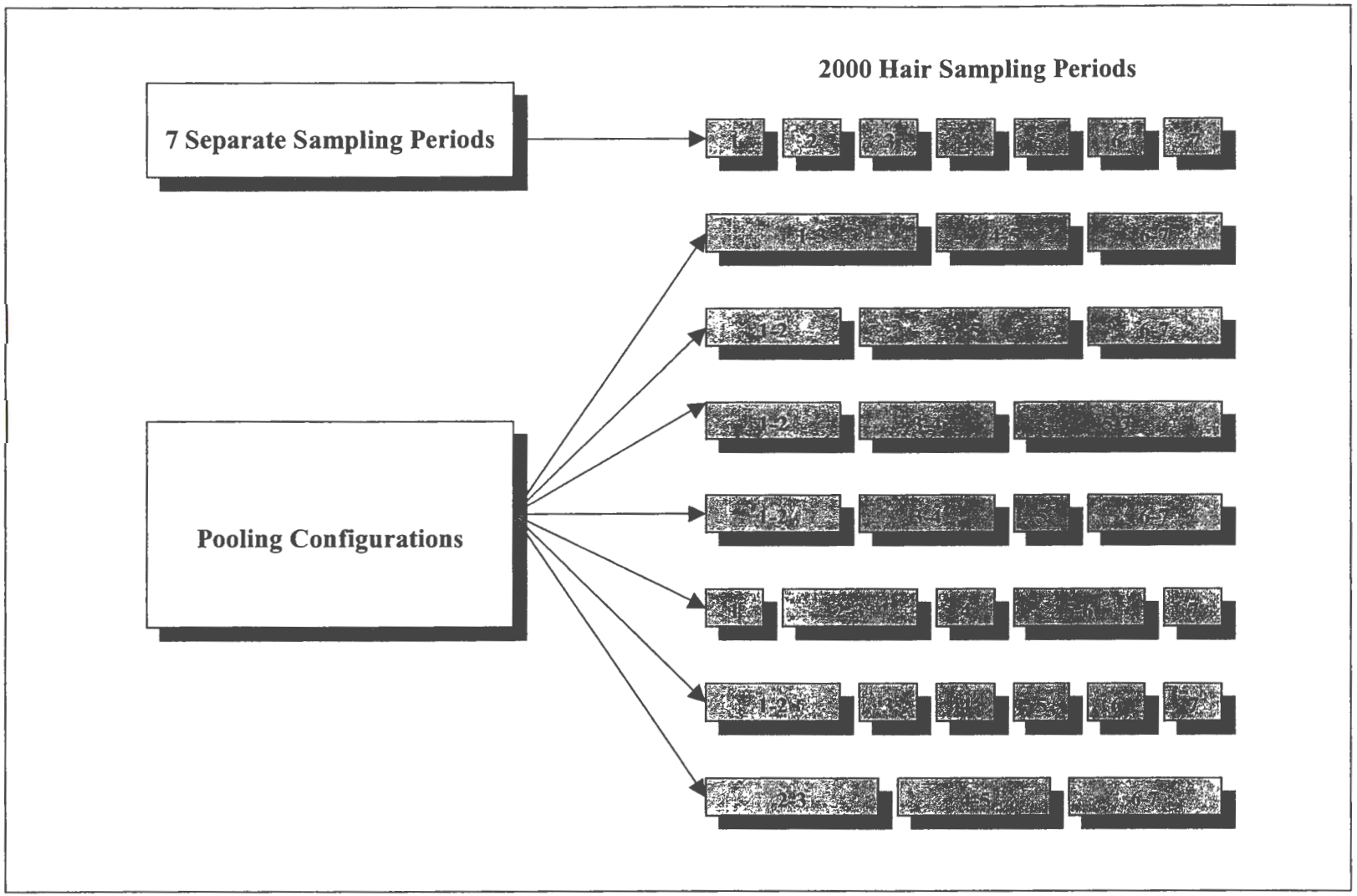

Figure 10. Pooling configurations of 7 sampling periods used to estimate black bear population abundance based on multiple mark-recapture models for the treatment and control areas in Washington County, North Carolina, 2000.

data. Using a multivariate discriminant function analysis, the most appropriate model was chosen based on the observed significance levels of the chi-square tests (White et al. 1982).

When using closed models, the assumption that the population is closed is important; hence, program CAPTURE provides a test for the closure assumption (Otis et al. 1978). Results of the test, however, can be confounded when either behavioral or time variation is present in the capture probabilities because failure of closure cannot be distinguished from either one. Thus, the population closure test only is reliable when M_h or M_o is assumed to be the underlying model (White et al. 1982). I computed the test of closure for the 7 separate sampling periods and all pooling configurations.

Study Area Delineation and Population Density

Some animals have home ranges that overlap the boundaries of a designated study area and this 'edge effect' can lead to overestimation of density (Otis et al. 1978). Animals sampled at the edge of a study area are incorporated in the population estimate but may only spend a small portion of their time in the study area (White et al. 1982). Therefore, it is important to use biological criteria to determine the "effective" study area size. First, I delineated all forested tracts, including a 250-m buffer beyond the forest edge (approximately the maximum observed distance of black bears outside of forested areas). Second, I circumscribed the 70 hair sampling sites on each area with a distance equivalent to the radius of the average female home range for each study area (Boersen 2001, Dobey 2002). Home ranges (95% fixed kernel method) were based on data collected from July 2000–June 2001, comprising ≥ 30 locations for each bear. Finally, I

used ArcView® GIS to delineate the forested areas and corresponding 250-m buffer within the home range buffer of hair-sampling sites to determine the size of the effective study area. I estimated population density for both study areas by dividing the population estimate (N) by the area of the effective study area.

GENETIC STRUCTURE

Sampling

For the genetic structure analysis, I included individuals identified during both the hair-sampling and the live-trapping seasons. There were 2 main advantages for combining live-trapping data with the hair-sampling data. First, data from the 2 sampling occasions provided additional opportunities to sample a greater proportion of genotypes occurring in the population (Weir 1996). Secondly, the sex and home range characteristics of individuals sampled during the live-trapping season were known, which enabled comparisons with individuals for which those characteristics were not known.

Allele Frequencies

Allele frequencies, which initially included individuals from the hair-sampling season only, were re-calculated to incorporate the genotypes of individuals captured during the live-trapping season. I used program BIOSYS-1 to compute allele frequencies for all hair and tissue samples.

Hardy-Weinberg Equilibrium and Linkage Disequilibrium

Before analyzing genetic data, it is important to test for the violation of underlying assumptions to ensure that calculated parameters are not biased. Thus, I re-calculated the test for Hardy-Weinberg equilibrium for the combined hair-sampling and live-trapping data with the probability test in program GENEPOP. Most genetic analyses also require the independent assortment of loci. I re-calculated the test for linkage disequilibrium to also include genotypes sampled during the live-trapping season using the genotype disequilibrium test in program GENEPOP. Sequential Bonferroni adjustments were used to determine statistical significance for both tests.

Heterozygosity

Heterozygosity is the proportion of genotypes in a population composed of chemically dissimilar alleles and represents the existence of variation in diploid populations (Weir 1996, Hartl and Clark 1997). Low levels of heterozygosity may be an indicator of inbreeding and genetic isolation, whereas an excess of heterozygotes may indicate a recent genetic bottleneck (Luikart and Cornuet 1998). Observed heterozygosity is a simple measure of genetic variation in a population and can be calculated for each locus as (Weir 1996):

$$\tilde{H}_l = \sum_i \sum_{i \neq j} \frac{n_{lij}}{n},$$

where n_{lij} is the observed count of A_iA_j heterozygotes at locus l in a sample of size n and $i \neq j$. Average heterozygosity is the observed heterozygosity averaged over all loci and can be calculated as:

$$\tilde{H} = \frac{1}{m}\sum_{l=1}^{m}\tilde{H}_l,$$

where m is the number of loci scored (Weir 1996). I used program BIOSYS-1 to compute average heterozygosity for samples collected during the hair-sampling and live-trapping seasons on the 2 study areas.

Genetic Relatedness

Genetic Distance.--Microsatellite analysis may be used to determine the relatedness of individuals, which is a useful parameter to describe genetic relationships (King et al. 2001). I used pair-wise distance measures to describe the genetic distance among individuals within each population, between the 2 populations, and among subpopulation clusters. Within the treatment area population, I identified individuals based on whether they were captured north or south of the proposed highway to determine the degree of relatedness before highway construction. To allow similar comparisons within the control area, I created a fictitious highway, comparable to the proposed U.S. Highway 64, to separate individuals captured in the north and south portions of the control area (Figure 11).

The proportion of shared alleles provides one of the simplest estimates of genetic similarity and can be calculated for 2 individuals as (Bowcock et al. 1994):

$$P_{SA_I} = \frac{\sum_u S}{2u},$$

where the number of shared alleles, S, is summed over all loci u. The distance between a pair of individuals (D_{SA}) is estimated by:

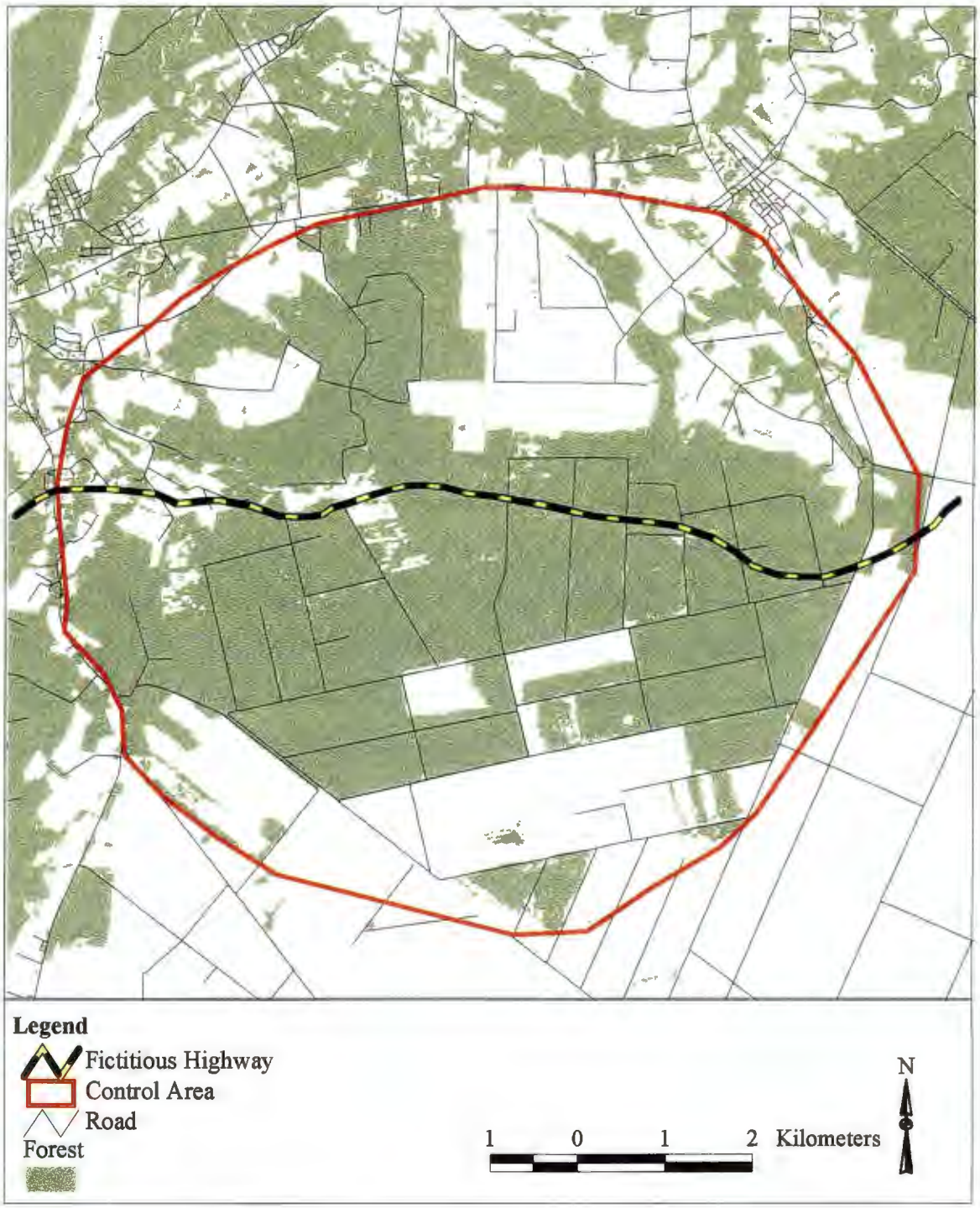

Figure 11. Location of the fictitious highway to separate north and south populations within the control area, Washington County, North Carolina

$$D_{SA_I} = 1 - P_{SA_I}.$$

I used the Chord distance of Cavalli-Sforza and Edwards (1967) to determine the genetic distance between populations and among subpopulation clusters. This measure transforms data to produce an angular distance, θ, between 2 populations that exist conceptually as points in an m-dimensional Euclidean space and are specified by m allele frequencies, where m is the number of alleles in the 2 populations. The distance, θ, is the angle between the 2 points where,

$$\cos(\theta) = \sum_i \sqrt{x_i y_i},$$

and x_i and y_i are the frequencies of the ith allele in populations x and y, respectively. A chord distance (D_{CH}) can be calculated as the distance between the 2 points measured along a straight line (Cavalli-Sforza and Edwards 1967):

$$D_{CH} = \left(\frac{2\sqrt{2}}{\pi}\right)\sqrt{1-\cos\theta}.$$

I calculated individual pair-wise distance matrices based on D_{SA} for both the treatment and control areas combined and for each area separately with program MICROSAT (version 1.5d; E. Minch, Stanford University, Stanford, California, USA). I used program BIOSYS-1 to compute D_{CH} between the treatment and control area populations and between the north and south populations within the treatment and control areas. Finally, I used ArcView® GIS to define subpopulations by clustering individuals that were sampled in close proximity relative to other clusters. I calculated D_{CH} distance matrices with program BIOSYS-1 for these subpopulation clusters within the treatment and control areas separately and for both areas combined.

Unrooted Neighbor-Joining Trees.--The evolutionary relationships of a group of operational taxonomic units, or OTUs, often are represented by a tree. Several methods exist to construct trees. However, the neighbor-joining method of Saitou and Nei (1987) is most appropriate for distance matrices (Weir 1996). The neighbor-joining method is designed to track the nodes of a tree and starts with the formation of a star, in which all OTUs are assumed to be equally related to each other. Once a distance matrix is applied, the nodes shift and join the least distant pair of nodes and continues to pair nodes until no more joining can be done. Unlike other methods, the neighbor-joining method is not based on the assumption that all lineages evolve at an equal rate. Thus, constructed trees are "unrooted", only reflecting distances among OTUs and not which unit is ancestral to which (Weir 1996).

I constructed unrooted neighbor-joining trees at 6 different scales. The first tree was based on pair-wise distances of all individuals (D_{SA}) from both the treatment and control areas combined. Next, I constructed 2 trees based on the pair-wise distances of individuals (D_{SA}) sampled in the north and south populations within the treatment and control areas. The fourth tree was constructed from pair-wise distances of all subpopulation clusters (D_{CH}) from both the treatment and control areas combined. Finally, I constructed 2 trees based on pair-wise distances of subpopulation clusters (D_{CH}) sampled in the north and south populations within the treatment and control areas. I constructed trees by fitting an unrooted tree to the D_{SA} and D_{CH} matrices with the neighbor-joining algorithm in program PHYLIP (Felsenstein 1993). I used program TREEVIEW (Page 1996) to display the trees.

Population Structure and Gene Flow

Population structure can be thought of as a hierarchy in which subpopulations are aggregated into progressively more inclusive groups (Hartl and Clark 1997). When populations are subdivided, genetic differentiation (i.e., differences in allele frequencies) almost always occurs (Hartl and Clark 1997). However, the degree to which these subpopulations differentiate genetically is of primary interest. I used several methods to determine population structure at 3 different scales: (1) between the treatment and control area populations, (2) between animals captured north and south of the proposed U.S. Highway 64 within the treatment area, and (3) between animals captured north and south of the fictitious highway within the control area.

Assignment Test.--The assignment test is a relatively new method, which can be used to assign individuals of unknown origin to populations based on how representative the genotypes are of a sampled population (Paetkau et al. 1995, Cornuet et al. 1999, King et al. 2001). I used the distance method described by Cornuet et al. (1999), which assigns an individual to the "closest" population based on the probability of observing that given genotype in a group of reference populations. Assignments tests may be used for a wide range of applications, including determining population differentiation (Paetkau 1995, Cornuet et al. 1999). I used the Chord distance (D_{CH}) option in program GENECLASS (Cornuet et al. 1999) to calculate assignment probabilities for all individuals in the treatment and control area populations and for individuals captured in the north and south populations within the treatment and control areas.

Heterogeneity Test.--Determining differences in allele frequencies between populations is useful to understand the degree of population structure (T. L. King, U.S.

Geological Survey, personal communication). I used the population differentiation randomization test (or heterogeneity test) in program GENEPOP to determine whether the treatment and control area populations and the north and south populations within the treatment and control areas were homogeneous or heterogeneous with respect to their allele frequencies at each locus. The test is based on a series of contingency tables for each locus, which contain the frequencies of every allele for both populations and tests the null hypothesis that the allelic distribution is identical across populations. I used sequential bonferroni adjustments to determine statistical significance.

F_{ST}.--Reduction of overall heterozygosity may occur when a population is subdivided. Wright (1921) presented a method (fixation index) to measure that effect at various hierarchical levels. A useful property of the fixation index is that it provides an objective method to assess population substructure, but is not complicated by actual allele frequencies or observed levels of heterozygosity (Hartl and Clark 1997). The fixation index is composed of a series of hierarchical *F*-statistics, which are denoted by the genetic symbol *F*, with subscripts indicating the levels of hierarchy being compared (Hartl and Clark 1997). In population studies, F_{ST} is the *F*-statistic of primary interest because it measures the lowest level in the hierarchy (subpopulation [S]) and the highest level (total population [T]) (J. B. Wolf, University of Tennessee, Knoxville, personal communication). That is, F_{ST} measures the reduction in average heterozygosity of a subpopulation ($\overline{H}_S$) relative to the total expected for the population (H_T) based on allele frequencies, or

$$F_{ST} = \frac{H_T - \overline{H}_S}{H_T}$$ (Hartl and Clark 1997).

F_{ST} values tend to be greater in fragmented habitats (i.e., barriers to gene exchange) than in continuous habitats (Hastings and Harrison 1994). Wright (1978) proposed general guidelines for interpreting F_{ST} values. For example, an F_{ST} value of <0.05 indicates very little population differentiation, whereas values ≥0.25 suggest no gene exchange (Wright 1978). I computed F_{ST} values using the uniform method of Weir and Cockerham (1984) in program GENEPOP to determine the degree of population substructure between the treatment and control area populations and between the north and south populations within the treatment and control areas.

Gene Flow Rates (Nm).--The extent of gene exchange among subpopulations determines the potential for genetic differentiation (Slatkin 1985). Gene flow can be defined as the average number of animals (migrants) exchanged between local populations per generation (*Nm*), where *m* is the fraction of individuals replaced by immigrants out of *N* individuals (Slatkin 1985). Mills and Allendorf (1996) evaluated the "1-migrant-per-generation" rule, which states that for many natural populations 1 migrant per generation is sufficient to prevent the loss of polymorphism and heterozygosity, but still allows for the divergence in allele frequencies among subpopulations. I calculated gene flow rates between the treatment and control area populations and between the north and south populations within the treatment and control areas. I used the private allele method of Barton and Slatkin (1986) in program GENEPOP to determine gene flow at the 3 different scales.

Isolation-by-Distance

Isolation-by-distance refers to the process by which geographically restricted gene flow generates a genetic structure (Rohlf and Schnell 1971). That is, in a continuous population, limits to dispersal cause allele frequencies to vary across a region, forming 'neighborhoods', which make the population appear as if it were discontinuous (Chambers 1995, Falconer and Mackay 1996). This offers an alternative approach for determining genetic differentiation and how rapidly the process of differentiation proceeds (Rohlf and Schnell 1971, Campbell and Dooley 1992).

I examined isolation-by-distance with the Mantel test (Mantel 1967), which is a simple correlation method (standardized Mantel statistic or r) that determines the association between 2 distance matrices; this test often is used to compare genetic and geographic distances within or among populations. To measure the degree of a relationship, the Mantel test statistic, Z, is calculated as (Mantel 1967):

$$Z = \sum_{i<j}^{n} X_{ij} Y_{ij} ,$$

where X_{ij} and Y_{ij} are the off-diagonal elements of matrices X and Y. If the matrices exhibit similar relationships, then Z will be large in comparison with what is expected by chance (Rohlf 1993). I used the Mantel test to determine correlations between genetic and geographic distances among black bears sampled individually or as subpopulations. I used 3 sets of matrix configurations to compute the test statistic at different scales based on the 2 genetic distance measures, D_{SA} and D_{CH}. The first set of configurations was based on the genetic distance among individuals (D_{SA}) for each study area separately and

both areas combined (scales 1, 2, and 3). Pair-wise geographic distances for the first 3 scales were based on the Euclidean distance between each hair-sampling or live-trapping location for each individual. Average UTM coordinates of sampling locations were generated in Microsoft Excel® (Microsoft Corporation, Redmond, Washington, USA) for animals that were sampled at multiple sites. The second set of configurations was based on the genetic distance among subpopulation clusters (D_{CH}) for each study area separately and both areas combined (scales 4, 5, and 6). Geographic distances between clusters were calculated using the average sampling location of all animals within a specified cluster. The third set of configurations was designed to determine whether sex and home-range size contribute to differences in associations between genetic and geographic distance. Thus, the last 4 scales were only calculated for live-captured bears and were based on D_{SA}. I used the activity centers of home ranges (J. L. Kindall, University of Tennessee, Knoxville, unpublished data) instead of capture locations to calculate geographic distances. The Mantel test was computed for live-captured females on the treatment and control areas separately and for both areas combined (scales 7, 8, and 9). Finally, I computed a Mantel statistic only for live-captured males on both study areas combined (scale 10) because the sample of radio-collared males on the control area was small ($n = 3$).

I used the Distance by ID extension in ArcView® GIS (J. Jenness, Jenness Enterprises, Flagstaff, Arizona, USA) to compute all pair-wise geographic distances. I created the final geographic distance matrices with Microsoft Excel®. I used program NTSYS-PC (version 1.8; Rohlf 1993) to compute the Mantel test for the different

configurations and scales. Significance tests were performed by comparing observed *Z*-values with permutational distributions (999 permutations; Rohlf 1993).

Allelic Diversity

Genetic or allelic diversity can be described as the number of different alleles that occur in a population (T. L. King, U.S. Geological Survey, personal communication). I used allele frequencies from the microsatellite analysis to spatially map allelic diversity throughout the treatment and control areas. For each allele sampled ($n = 72$), I first mapped the frequency of occurrence at each hair-sampling site. Individual genotypes only were included once for each hair-sampling site; however, recaptures at other sampling locations were included in the analysis. Because local bear density may affect allelic diversity, I standardized all allele frequencies at each site by the number of individuals captured at that site. I then used inverse distance weighted (IDW) interpolation in ArcInfo® GRID (ESRI, Redlands, California, USA) to predict the frequency of alleles in areas that were not sampled (i.e., areas between hair-sample sites). The IDW interpolator is a simple method, where each sample point has a local influence that diminishes with distance. Specifically, weights of sample locations closer to the processing cell are greater than those farther away (Burrough and McDonnell 1998). I created a separate GIS grid for each allele for an area encompassing both the treatment and control areas. Because some alleles were more frequently observed than others, I divided the interpolated frequencies by the maximum frequency of each allele to produce a standardized 0–1 scale (index of allele presence). I then summed the standardized

index values of all 72 allele grids to map allelic diversity for the treatment and control areas.

I calculated 3 landscape variables with program FRAGSTATS (version 3.3; McGarigal et al. 2002) to determine whether allelic diversity was associated with landscape features. Because gene exchange may be limited by the size and fragmentation of habitat patches, I chose 1 measure of habitat area and 2 measures of habitat connectivity. I calculated all 3 variables in ArcInfo GRID® GIS using a circular moving window with a radius of 1,300 m (average female home range size). The first variable I considered was forest density, which is the proportion of the moving window comprised of forest (McGarigal and Marks 1995). The second variable was connectance; this variable was measured based on joinings between each pair of patches as a percentage of the maximum possible connectance, given the number of patches (McGarigal and Marks 1995). I chose 210 m (average hourly movement distance) as the threshold distance for a functional joining between 2 habitat patches. Finally, I considered cohesion, which also is a measure of connectivity. Values of this variable increase monotonically as habitat patches become more aggregated (Schumaker 1996). I used the CORR procedure in SAS® (version 8.2; SAS Institute, Cary, North Carolina, USA) to determine whether relationships existed between allelic diversity and the 3 landscape variables, based on measurements at the 140 hair-sampling sites.

CHAPTER IV

RESULTS

DATA COLLECTION

Capture and Handling

Forty-eight trap sites were established on the treatment area (up to 27 active sites at one time), totaling 1,700 trap nights. Twenty-three bears (12 males, 11 females) were captured 27 times (Appendix A) with a capture success rate of 1.6%. Bears were captured at 19 of 48 trap sites (39.6%), and activity was confirmed at approximately 50% of the trap sites. Tissue samples were collected from 23 individual bears.

Eighteen trap sites were established on the control area (up to 17 active sites at one time), totaling 383 trap nights. Thirty-two bears (8 males, 24 females) were captured 33 times (Appendix A) with a capture success rate of 8.6%. Bears were captured at 16 of the 18 trap sites (88.9%), with confirmed bear activity at all sites. Tissue samples were collected from all 32 bears.

Hair Sampling

On the treatment area, the 7 hair-sampling periods resulted in 686 hair samples (range = 0–26 per site per period). Fifty-five sampling sites were visited. All 70 traps were baited weekly for 490 trap events and a trap success rate of 41.8%. Of the 686 hair samples, 162 were randomly selected for DNA analysis (Table 1).

On the control area, 7 sampling periods resulted in 1,240 hair samples (range = 0–25 per site per period). Sixty-four different sample sites were visited. All 70 traps were

Table 1. Number of black bear hair-sampling sites visited, samples collected, and samples analyzed on the treatment area, Washington County, North Carolina, 2000.

Period	Number of samplings sites visited	Number of samples collected	Number of samples selected for DNA analysis
1	20	61	17
2	26	105	21
3	30	78	25
4	33	94	21
5	31	108	25
6	34	123	28
7	31	117	26
Average	29.3	98.0	23.3
Total	55[a]	686	163
Samples analyzed (%)	23.6		

[a] Total number of hair-sampling sites out of 70 that were visited by black bears.

baited once a week, resulting in a trap success rate of 63.7%. Of the 1,240 samples, 175 were randomly selected for DNA analysis (Table 2).

Microsatellite Analysis

Because of insufficient amounts of DNA, microsatellite analysis was not possible on 30 hair samples from the treatment area and 22 hair samples and 1 tissue sample from the control area. Therefore, 132 and 153 hair samples and 23 and 31 tissue samples were analyzed for the treatment and control areas, respectively. From the hair samples and live captures combined, 66 multilocus genotypes were identified as unique individuals on the treatment area. Fifty-three bears were identified from the hair samples alone, 10 of which were recaptures from the live-trapping season. Therefore, 43 new individuals were identified from the hair sampling. On the control area, 115 multilocus genotypes were identified as unique individuals based on the hair sampling and live trapping combined. Ninety-two bears were identified from the hair samples, of which 8 were recaptures from the live-trapping season. Hence, 84 new individuals were identified during the hair-sampling efforts.

POPULATION ABUNDANCE

Allele Frequencies

Allele frequencies were calculated for all individuals identified from the hair samples on the 2 study areas. On the treatment area, 4–9 alleles were observed at each locus, whereas on the control area 4–10 alleles were observed. Allele frequencies ranged

Table 2. Number of black bear hair-sampling sites visited, samples collected, and samples analyzed on the control area, Washington County, North Carolina, 2000.

Period	Number of samplings sites visited	Number of samples collected	Number of samples selected for DNA analysis
1	39	150	25
2	45	171	25
3	43	147	25
4	47	164	25
5	44	209	25
6	47	198	25
7	47	201	25
Average	44.6	177.1	25.0
Total	64[a]	1240	175
Samples analyzed (%)	14.1		

[a] Total number of hair-sampling sites out of 70 that were visited by black bears.

from 0.009 to 0.877 (n = 53) on the treatment area and from 0.005 to 0.908 (n = 92) on the control area (Table 3).

Hardy-Weinberg Equilibrium and Linkage Disequilibrium

For the treatment area hair samples, I detected evidence of non-random mating for 2 loci (G10B, P = 0.006; MU50, P = 0.005). However, only 1 test was significant after applying the sequential Bonferroni adjustment (MU50, P = 0.005) at α = 0.0056. On the control area, only 1 test was significant (G10L, P = 0.002) and remained so after the sequential Bonferroni adjustment.

I observed associations among genotypes at different loci between 23 pairs of loci out of 45 comparisons for the treatment area (α = 0.05). However, only 10 tests were significant after sequential Bonferroni adjustment (α = 0.0014). On the control area, 19 of 45 comparisons were significant at α = 0.05, but only 5 tests were significant after sequential Bonferroni adjustment (α = 0.0013).

Probability of Identity

On the treatment area, the overall probability of identity was 1.07×10^{-9} for the 53 individuals captured during the hair-sampling period (Table 4). This probability corresponds to a 1 in 934 million chance that 2 individuals had the same genotype at the loci examined. $PI_{singlelocus}$ ranged from 0.040 to 0.606. On the control area, the $PI_{overall}$ was 1.28×10^{-7} for the 92 individuals captured during the hair-sampling period, corresponding to a 1 in 7.8 million chance that an individual could be misclassified as another individual (Table 5). The PI estimates for single loci ranged from 0.086 to 0.700.

Table 3. Observed allele frequencies of black bears identified from hair samples on the treatment and control areas, Washington County, North Carolina, 2000.

Locus	Treatment Area	Control Area
G10C		
(N)	**53**	**92**
1	0.038	0.000
2	0.038	0.022
3	0.038	0.049
4	0.877	0.908
5	0.009	0.022
G1A		
(N)	**53**	**92**
1	0.009	0.005
2	0.000	0.082
3	0.179	0.103
4	0.330	0.266
5	0.330	0.277
6	0.151	0.266
G10B		
(N)	**53**	**92**
1	0.189	0.076
2	0.000	0.005
3	0.387	0.620
4	0.283	0.190
5	0.019	0.060
6	0.000	0.000
7	0.104	0.033
8	0.019	0.016
G10M		
(N)	**50**	**92**
1	0.090	0.049
2	0.110	0.201
3	0.150	0.038
4	0.410	0.451
5	0.010	0.065
6	0.000	0.005
7	0.230	0.190

Table 3. (Continued)

Locus	Treatment Area	Control Area
G10X		
(N)	**53**	**92**
1	0.000	0.022
2	0.057	0.217
3	0.594	0.511
4	0.000	0.005
5	0.038	0.065
6	0.311	0.147
7	0.000	0.033
G10L		
(N)	**53**	**92**
1	0.038	0.043
2	0.028	0.038
3	0.057	0.082
4	0.000	0.005
5	0.104	0.087
6	0.255	0.098
7	0.094	0.098
8	0.160	0.299
9	0.142	0.207
10	0.123	0.043
G1D		
(N)	**52**	**92**
1	0.144	0.228
2	0.519	0.500
3	0.029	0.103
4	0.269	0.114
5	0.038	0.054
MU23		
(N)	**53**	**92**
1	0.217	0.109
2	0.009	0.027
3	0.019	0.000
4	0.217	0.277
5	0.038	0.103
6	0.226	0.147
7	0.208	0.125
8	0.066	0.212

Table 3. (Continued)

Locus	Treatment Area	Control Area
MU50		
(N)	**52**	**92**
1	0.029	0.043
2	0.038	0.120
3	0.192	0.185
4	0.010	0.060
5	0.221	0.043
6	0.000	0.022
7	0.010	0.022
8	0.125	0.071
9	0.375	0.435
G10P		
(N)	**51**	**92**
1	0.000	0.005
2	0.010	0.005
3	0.196	0.397
4	0.000	0.022
5	0.225	0.299
6	0.147	0.060
7	0.422	0.212

Table 4. Probability of identity estimates of black bears identified from hair samples on the treatment area, Washington County, North Carolina, 2000 (n = 53).

Locus	Number of alleles	Probability of identity	Probability of identity (siblings)
G10C	4	0.606	0.789
G1A	5	0.124	0.417
G10B	6	0.123	0.419
G10M	6	0.088	0.391
G10X	4	0.279	0.547
G10L	9	0.040	0.335
G1D	5	0.175	0.470
MU23	8	0.067	0.364
MU50	8	0.089	0.390
G10P	5	0.114	0.413
Overall	6.0[a]	1.07×10^{-9}[b]	2.73×10^{-4}[b]

[a] Average number of alleles
[b] Product of individual values

Table 5. Probability of identity estimates of black bears identified from hair samples on the control area, Washington County, North Carolina, 2000 (n = 92).

Locus	Number of alleles	Probability of identity	Probability of identity (siblings)
G10C	4	0.701	0.836
G1A	6	0.174	0.392
MU23	7	0.106	0.355
G10B	7	0.296	0.521
G10M	7	0.200	0.426
G10X	7	0.238	0.456
MU50	9	0.140	0.398
G10L	10	0.086	0.347
G1D	5	0.236	0.452
G10P	7	0.246	0.433
Overall	6.9[a]	1.28×10^{-7}[b]	3.19×10^{-4}[b]

[a] Average number of alleles
[b] Product of individual values

On the treatment area, the overall probability of identity for siblings (PI_{sibs}) was 2.73×10^{-4}, corresponding to a 1 in 3,663 chance of encountering identical genotypes. PI_{sibs} for single loci ranged from 0.335 to 0.789. On the control area, the overall PI_{sibs} was 3.19×10^{-4}, corresponding to a 1 in 3,135 chance that an individual could be identical to another individual at the loci examined. Single loci estimates of PI_{sibs} ranged from 0.347 to 0.836.

Genotypes with $P_{sib} > 0.05$ cannot be considered unique individuals. The sibling match test for each 10-locus genotype was $P_{sib} < 0.001$ on both study areas. Consequently, I included all multilocus genotypes for population estimation.

Study Area Delineation

Average female home ranges (95% fixed kernel method) for the treatment and control areas were 5.4 km^2 and 3.2 km^2, respectively (J. L. Kindall, University of Tennessee, Knoxville, unpublished data). Therefore, I circumscribed the treatment area sites with a radius of 1,312 m and the control area sites with a radius of 1,006 m to delineate effective sampling areas. After overlaying forested areas, including the 250-m buffer, the effective study area size was 70.5 km^2 for the treatment area and 92.7 km^2 for the control area (Figures 12 and 13).

Population Abundance and Density

Modified Lincoln-Petersen Model.--The 7 weeks of hair sampling were split into 2 sampling occasions for both the treatment and the control areas. For the treatment area, I pooled data for the first 3 weeks to form the first sampling occasion and the last 4 weeks

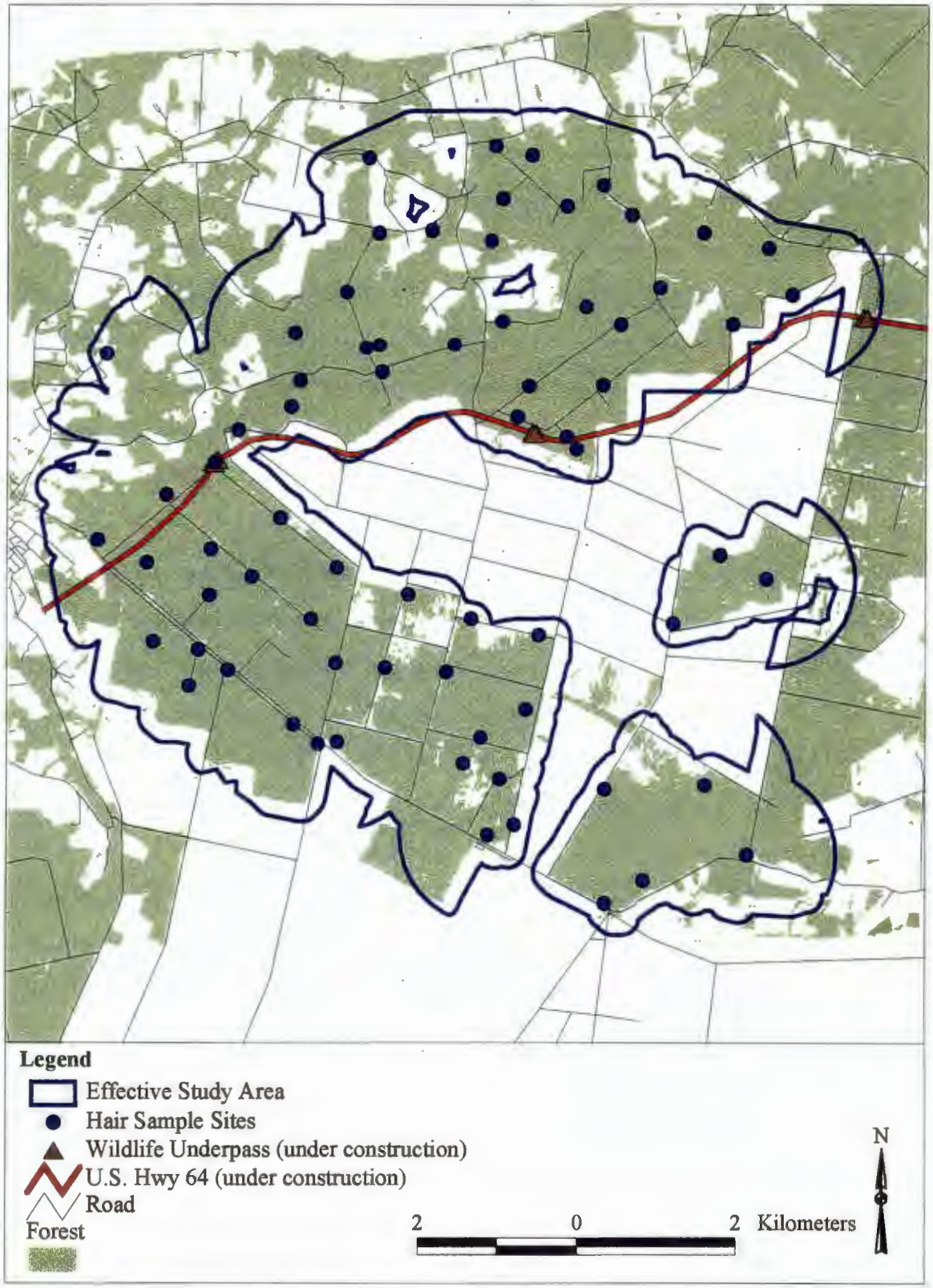

Figure 12. Effective study area for estimation of black bear population abundance, treatment area, Washington County, North Carolina, 2000.

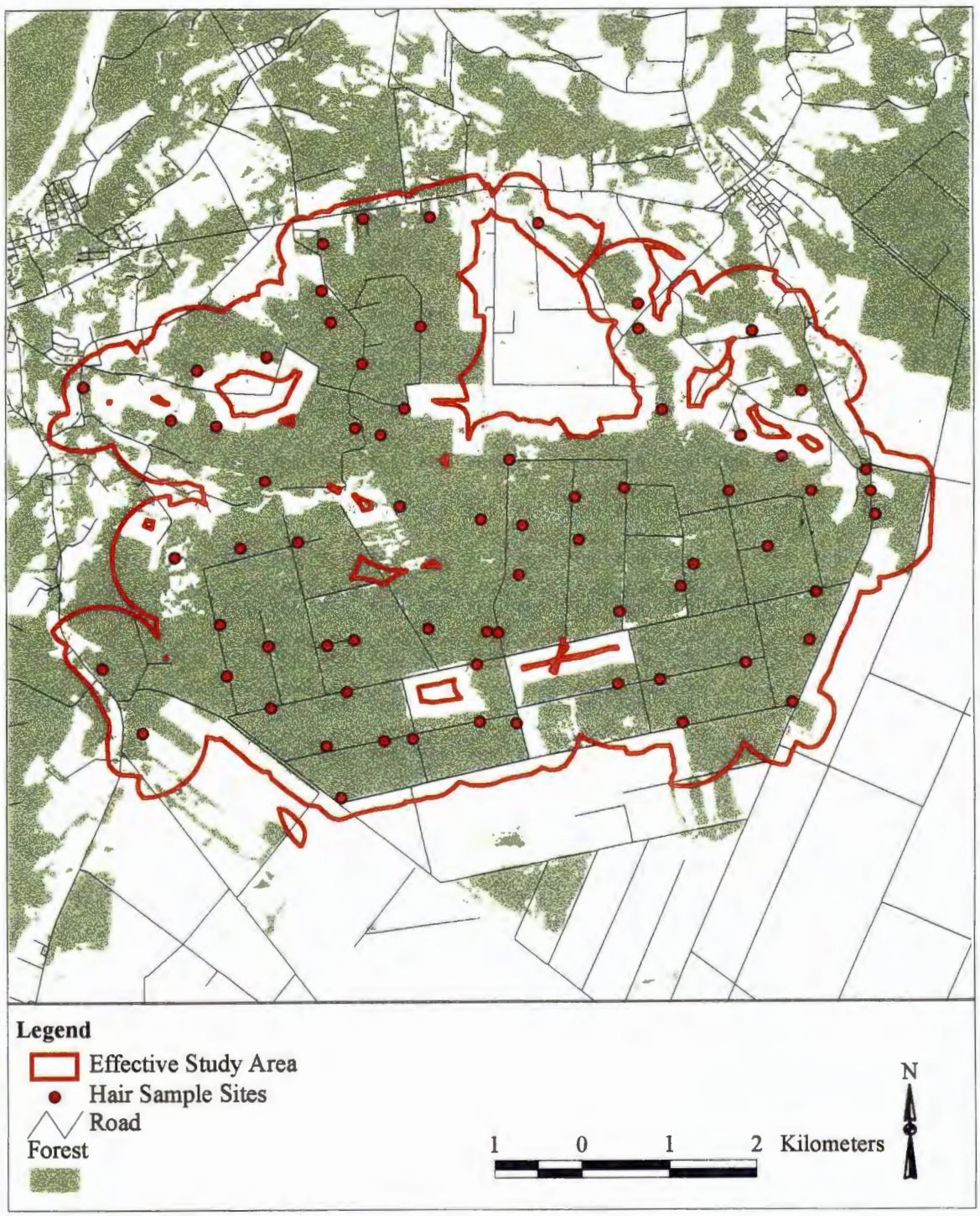

Figure 13. Effective study area for estimation of black bear population abundance, control area, Washington County, North Carolina, 2000.

to form the second sampling occasion. This configuration had the lowest CV (10%) and maximized the number of recaptures, producing a population estimate of 65 bears or 0.92 bears/km^2 (Table 6). On the control area, I pooled data for the first 4 weeks and the last 3 weeks to form the first and second sampling occasions, respectively. This configuration had the lowest CV (14%) and maximized the number of recaptures, producing a population estimate of 143 bears or 1.54 bears/km^2 (Table 7).

Bailey's Triple Catch.--I combined the 7 weeks of sampling for both the treatment and the control areas into 3 sampling occasions. On the treatment area, the most precise estimate (N = 29 bears; 0.41 bears/km^2; CV = 44%) was produced by pooling weeks 1–3, weeks 4–5, and weeks 6–7 (Table 6). On the control area, the best pooling configuration was weeks 1–2, weeks 3–4, and weeks 5–7, producing the most precise estimate (CV = 46%) of 79 bears or 0.85 bears/km^2 (Table 7).

Multiple Mark-Recapture Models.--Program CAPTURE consistently detected heterogeneity for the different sampling period configurations on the treatment area. Consequently, the jackknife estimator (M_h) consistently scored high for the different sampling period configurations based on the model selection results. The test for heterogeneity, based on 7 separate weeks of sampling, was highly significant ($\chi^2_{0.05}$ = 10.94, P = 0.004), but behavioral variation ($\chi^2_{0.05}$ = 0.37, P = 0.550) and time variation ($\chi^2_{0.05}$ = 1.48, P = 0.960) were not significant sources of unequal catchability. When I pooled the data into the 7 different pooling configurations (Figure 10), I detected heterogeneity 5 times; significance was marginal in a sixth configuration ($\chi^2_{0.05}$ = 3.28, P = 0.070). No behavioral variation was detected, but a time effect was detected in 2 of the

Table 6. Estimates of black bear population abundance, treatment area, Washington County, North Carolina, 2000.

Model	Sampling period configuration (weeks)	Population estimate (*N*)	95% confidence interval	Coefficient of variation (%)	Density (bears/km^2)	95% confidence interval
Modified Lincoln-Petersen	3,4	65	53–78	10.0	0.92	0.75–1.11
Bailey's Triple Catch	3,2,2	29	4–53	44.0	0.41	0.06–0.75
M_h[a]	2,3,2	85	73–105	9.7	1.20	1.04–1.49
M_h	1,1,1,1,1,1,1	122	93–173	16.4	1.73	1.32–2.45
Chao M_h	1,1,1,1,1,1,1	110	76–197	26.0	1.56	1.08–2.79

[a] Indicates chosen model

Table 7. Estimates of black bear population abundance, control area, Washington County, North Carolina, 2000.

Model	Sampling period configuration (weeks)	Population estimate (*N*)	95% confidence interval	Coefficient of variation (%)	Density (bears/km^2)	95% confidence interval
Modified Lincoln-Petersen	4,3	143	105–182	14.0	1.54	1.13–1.96
Bailey's Triple Catch	2,2,3	79	8–151	46.0	0.85	0.09–1.69
M_h[a]	2,2,3	165	145–192	7.4	1.78	1.56–2.07
M_h	1,1,1,1,1,1,1	255	207–322	11.4	2.75	2.23–3.47
Chao M_h	1,1,1,1,1,1,1	290	189–501	26.2	3.13	2.04–5.40

[a] Indicates chosen model

7 configurations. Because time was not consistently detected, I narrowed the model selection to models M_h and Chao M_h.

On the control area, I detected heterogeneity in the majority of the sampling period configurations, and model M_h consistently scored high using the model selection technique. When estimating population size based on the 7 separate weeks of sampling, the heterogeneity test was strongly significant ($\chi^2_{0.05} = 11.42$, $P = < 0.001$), and neither behavioral variation ($\chi^2_{0.05} = 0.27$, $P = 0.610$) or time variation ($\chi^2_{0.05} = 1.06$, $P = 0.980$) were a factor. When capture data were pooled into the 7 different configurations (Figure 10), heterogeneity was detected 4 times; however, 3 of the tests were not calculated because expected values were too small. Behavioral variation was detected once, and time variation was detected twice. Because time and behavior were not a consistent source of unequal catchability throughout the pooling configurations, I again narrowed the model selection down to models M_h and Chao M_h.

I selected the most appropriate sampling period configuration based on a number of factors, including the closure test, CV, capture probabilities, goodness-of-fit tests, and the number of recaptures lost because of pooling. The closure assumption was upheld for all sampling period configurations on both the treatment and control areas and was, therefore, not a factor in selecting the best model. The 7 separate weeks of sampling was the only configuration in which maximum recaptures could be attained because there was no pooling of data. Estimates based on M_h for the 7 separate weeks were 122 (CV = 16.4%; 1.73 bears/km^2; Table 6) and 255 (CV = 11.4%; 2.75 bears/km^2; Table 7) for the treatment and control areas, respectively; however, capture probabilities were low (11% and 7%, respectively). The Chao M_h model, which is more appropriate when capture

probabilities are low, produced estimates of 110 bears (CV = 26.0%; 1.56 bears/km^2) on the treatment area and 290 bears (CV = 26.2%; 3.13 bears/km^2) on the control area (Tables 6 and 7).

The 3 different configurations in which weeks were pooled into 3 sampling periods all had similar numbers of recaptures with relatively high capture probabilities (>30% on the treatment area and >20% on the control area). Therefore, I considered the CV and goodness-of-fit tests to determine the most appropriate pooling configuration with model M_h. On the treatment area, pooling weeks 1–2, weeks 3–5, and weeks 6–7 provided an estimate of 85 bears (1.20 bears/km^2; Table 6) with a low CV (9.7%) and good M_h model fit ($P = 0.550$). On the control area, the configuration of weeks 1–2, weeks 3–4, and weeks 5–7 resulted in a CV of 7.4% and a M_h goodness-of-fit of $P = 0.090$; this configuration provided an estimate of 165 bears or 1.78 bears/km^2 (Table 7).

GENETIC STRUCTURE

Allele Frequencies

Allele frequencies were calculated for all individuals identified from the hair samples and tissue samples on the 2 study areas. At each locus, 5–9 alleles were observed on the treatment area and 4–10 alleles were observed on the control area. Allele frequencies ranged from 0.008 to 0.864 ($n = 66$) on the treatment area and from 0.004 to 0.904 ($n = 115$) on the control area (Table 8).

Table 8. Observed allele frequencies of black bears identified from hair samples and tissue samples on the treatment and control areas, Washington County, North Carolina, 2000.

Locus	Treatment Area	Control Area
G10C		
(N)	**66**	**115**
1	0.038	0.000
2	0.045	0.022
3	0.045	0.057
4	0.864	0.904
5	0.008	0.017
G1A		
(N)	**66**	**115**
1	0.008	0.004
2	0.015	0.091
3	0.182	0.091
4	0.326	0.243
5	0.326	0.300
6	0.144	0.270
G10B		
(N)	**66**	**115**
1	0.174	0.070
2	0.000	0.004
3	0.424	0.643
4	0.265	0.183
5	0.015	0.048
6	0.008	0.000
7	0.098	0.039
8	0.015	0.013
G10M		
(N)	**62**	**115**
1	0.081	0.043
2	0.113	0.200
3	0.153	0.035
4	0.435	0.439
5	0.016	0.065
6	0.016	0.009
7	0.185	0.209

Table 8. (Continued)

Locus	Treatment Area	Control Area
G10X		
(N)	**66**	**115**
1	0.000	0.043
2	0.053	0.209
3	0.598	0.500
4	0.000	0.004
5	0.045	0.065
6	0.295	0.148
7	0.008	0.030
G10L		
(N)	**66**	**115**
1	0.038	0.048
2	0.030	0.039
3	0.068	0.074
4	0.000	0.004
5	0.098	0.083
6	0.265	0.100
7	0.091	0.096
8	0.159	0.304
9	0.136	0.213
10	0.114	0.039
G1D		
(N)	**65**	**115**
1	0.162	0.248
2	0.500	0.496
3	0.023	0.104
4	0.285	0.109
5	0.031	0.043
MU23		
(N)	**66**	**115**
1	0.197	0.117
2	0.008	0.022
3	0.023	0.000
4	0.205	0.252
5	0.030	0.096
6	0.250	0.165
7	0.212	0.122
8	0.076	0.226

Table 8. (Continued)

Locus	Treatment Area	Control Area
MU50		
(N)	**65**	**115**
1	0.031	0.039
2	0.054	0.113
3	0.185	0.170
4	0.015	0.048
5	0.223	0.057
6	0.000	0.022
7	0.008	0.030
8	0.123	0.065
9	0.362	0.457
G10P		
(N)	**64**	**115**
1	0.000	0.004
2	0.008	0.004
3	0.227	0.387
4	0.008	0.017
5	0.250	0.309
6	0.141	0.057
7	0.367	0.222

Hardy-Weinberg Equilibrium and Linkage Disequilibrium

For the hair and tissue samples on the treatment area, I detected evidence of non-random mating for 4 loci (G10B, $P = 0.035$; MU50, $P = 0.012$; G10L, $P = 0.033$; MU23, $P = 0.048$) when tested at $\alpha = 0.05$. However, after applying the sequential Bonferroni adjustment ($\alpha = 0.005$), no tests were significant. Deviations from Hardy-Weinberg equilibrium also were detected on the control area for 2 loci (G1D, $P = 0.034$; G10L, $P = 0.037$), but no tests were significant after the sequential Bonferroni correction.

I observed associations among genotypes at different loci between 19 pairs of loci out of 45 comparisons for the treatment area ($\alpha = 0.05$). However, only 5 were significant after the sequential Bonferroni adjustment ($\alpha = 0.0011$). On the control area, 15 of 45 comparisons were significant at $\alpha = 0.05$, but none were significant after sequential Bonferroni adjustment ($\alpha = 0.0011$).

Heterozygosity

Average heterozygosity on the treatment area was 0.667 (SE = 0.053), or 66.7%. On the control area, average heterozygosity was 0.664 (SE = 0.057), or 66.4%. The 2 levels of heterozygosity did not significantly differ from each other.

Genetic Relatedness

Individual pair-wise distances were calculated based on D_{SA}. Pair-wise genetic distances between bears T32 and C76 and T34 and C24 were 0, indicating that 2 individuals were sampled on both study areas during the hair-sampling period. Therefore, these bears were reassigned to the most appropriate population, based on

relatedness with other bears from these populations (T. L. King, U.S. Geological Survey, personal communication). The average genetic distance among individuals within the treatment area was 0.895 (range = 0.051–2.197). The average genetic distance of individuals within the control area was 0.863 (range = 0.051–2.303). For both areas combined, the average genetic distance of individuals was 0.905 (range = 0.051–2.996). The D_{SA} values were used to construct an unrooted tree for both the treatment and control areas and for the north and south populations within the treatment and control areas (Figures 14, 15, and 16). Figure 14 shows clustering of bears within their respective populations, indicating that some structure exists between the 2 populations. Likewise, bears captured north and south of the proposed highway seem to be clustered within the respective areas (Figure 15). On the control area, however, clustering of north and south populations is less obvious, with only a few noticeable clusters occurring on the left side of the tree (Figure 16).

I calculated pair-wise distances between populations and among subpopulation clusters (Figures 17 and 18) based on D_{CH}. The genetic distance between the 2 study area populations was 0.192. The genetic distance between the north and south populations within the treatment and control areas were 0.249 and 0.135, respectively. The average genetic distance among subpopulation clusters within the treatment area was 0.440 (range = 0.269–0.587) and 0.380 (range = 0.221–0.571) within the control area. For both areas combined, the average genetic distance among subpopulation clusters was 0.424 (range = 0.221–0.723). I used D_{CH} to construct unrooted trees for the treatment and control area subpopulations combined and for the north and south subpopulation clusters within the treatment and control areas (Figures 19, 20, and 21). Figure 19 shows that treatment area

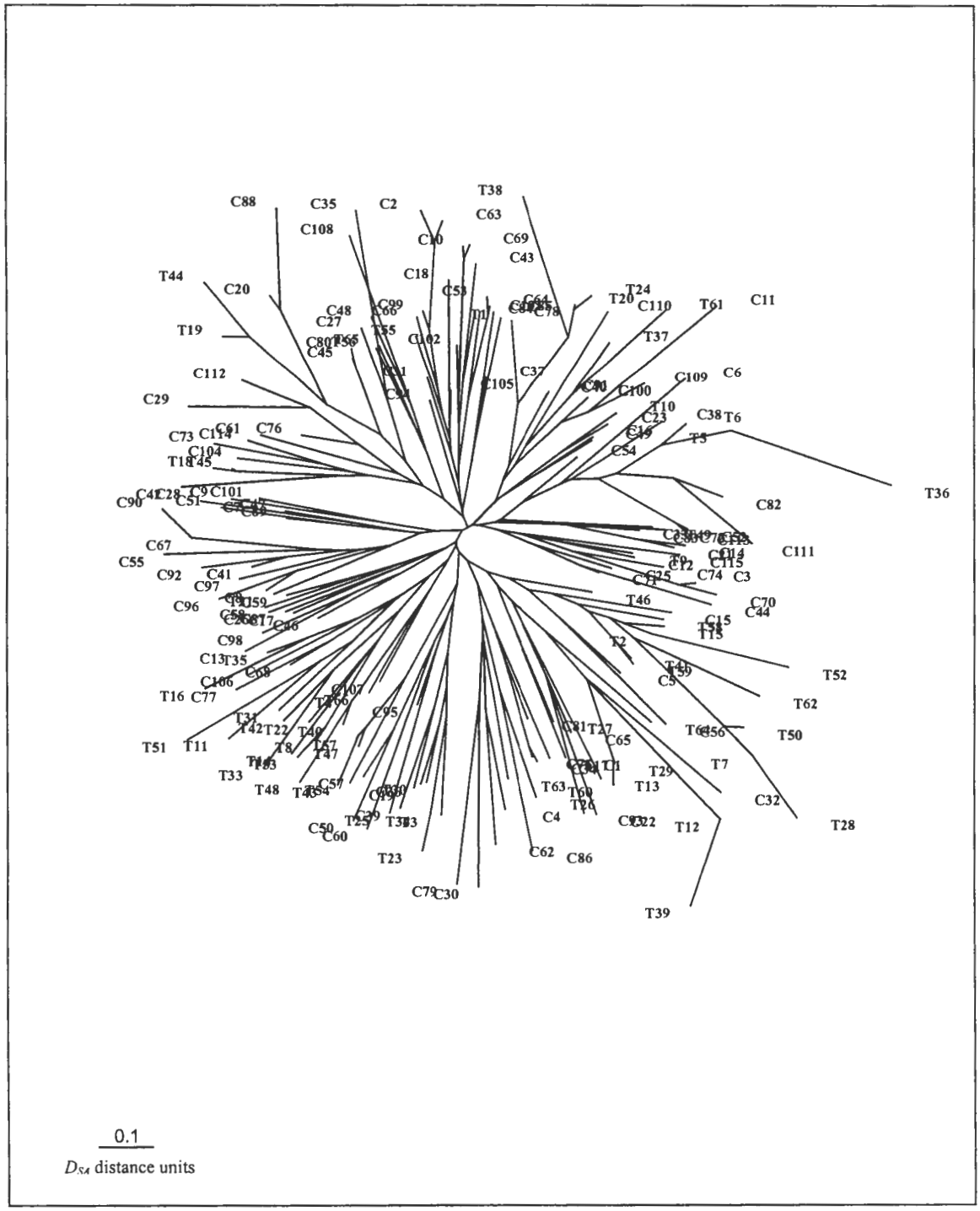

Figure 14. Unrooted neighbor-joining tree based on the proportion of shared alleles (D_{SA}) for individuals captured during the live-trapping and hair-sampling periods on the treatment (T) and control areas (C), Washington County, North Carolina, 2000.

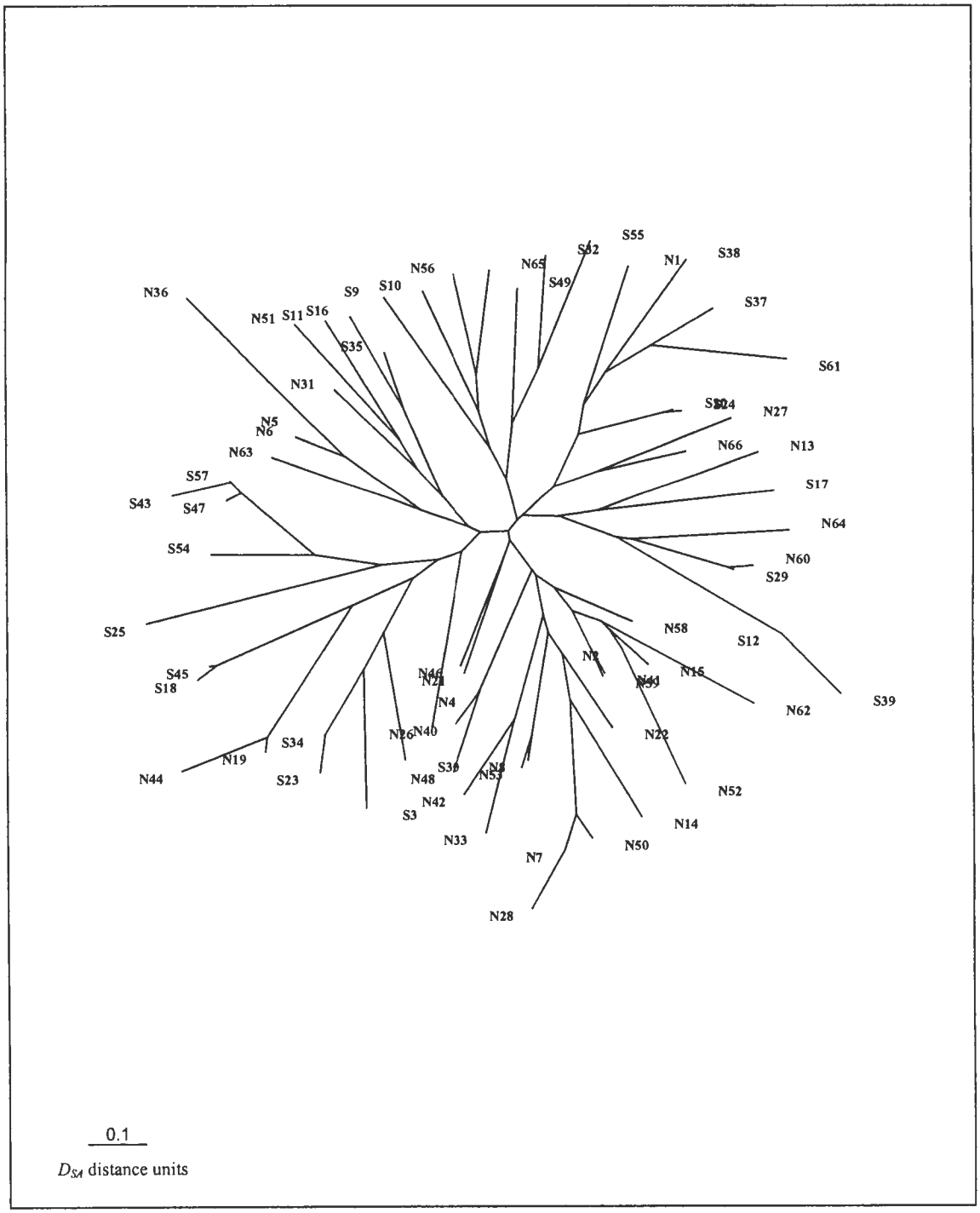

Figure 15. Unrooted neighbor-joining tree based on the proportion of shared alleles (D_{SA}) for individuals captured during the live-trapping and hair-sampling periods north (N) and south (S) of the proposed highway on the treatment area, Washington County, North Carolina, 2000.

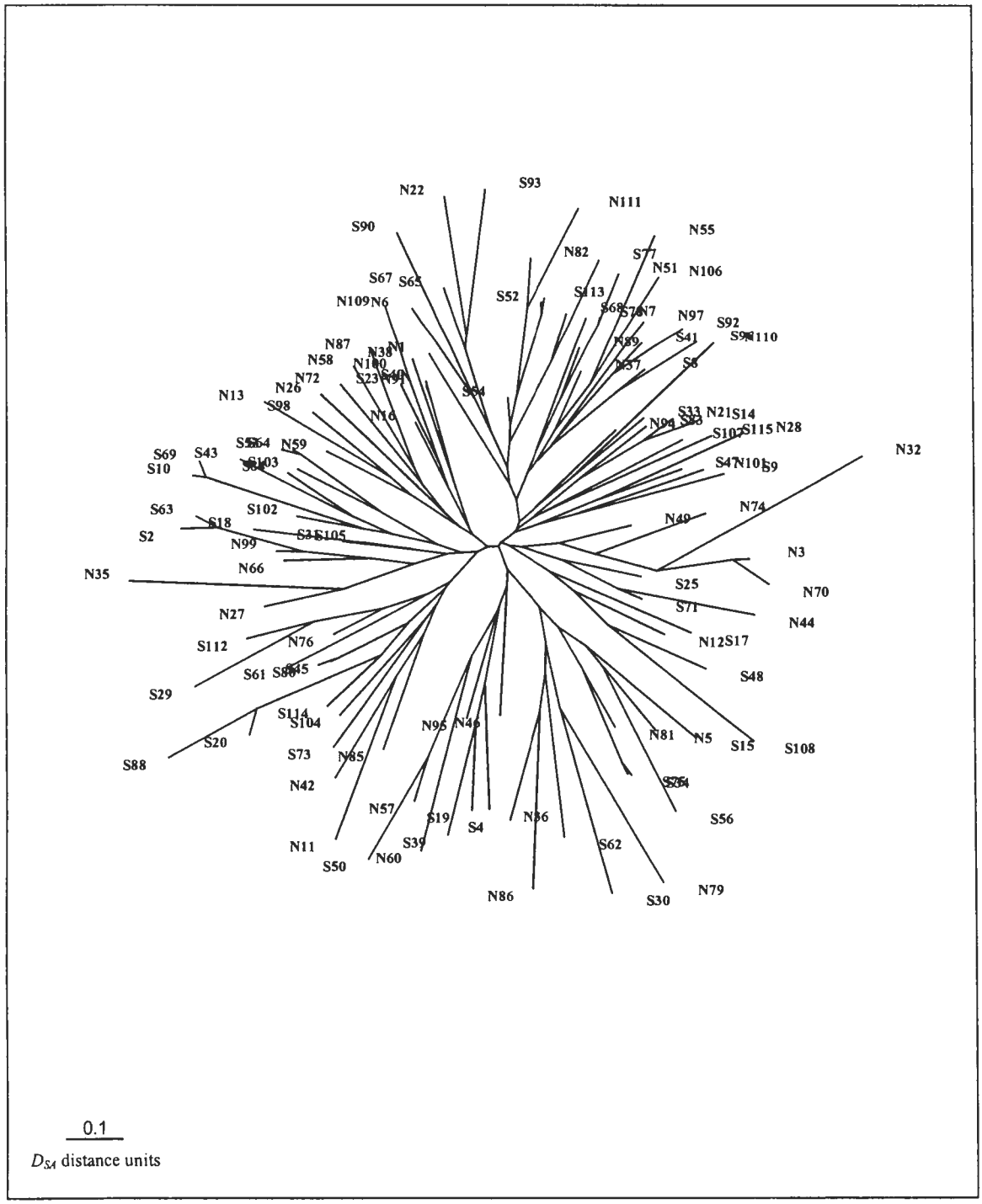

Figure 16. Unrooted neighbor-joining tree based on the proportion of shared alleles (D_{SA}) for individuals captured during the live-trapping and hair-sampling periods north (N) and south (S) of the fictitious highway on the control area, Washington County, North Carolina, 2000.

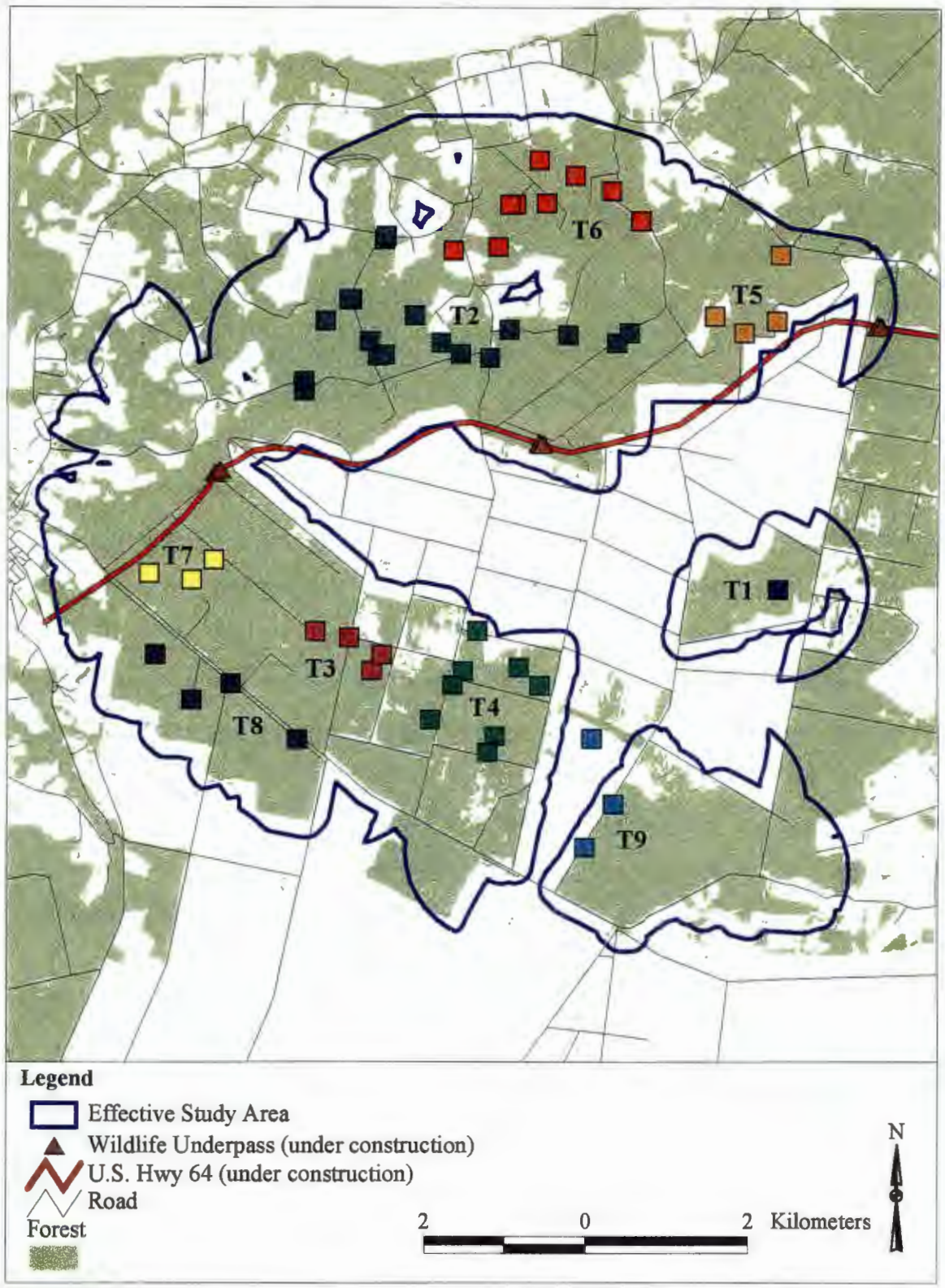

Figure 17. Subpopulation clusters of black bears identified from hair samples and live captures on the treatment area, Washington County, North Carolina, 2000.

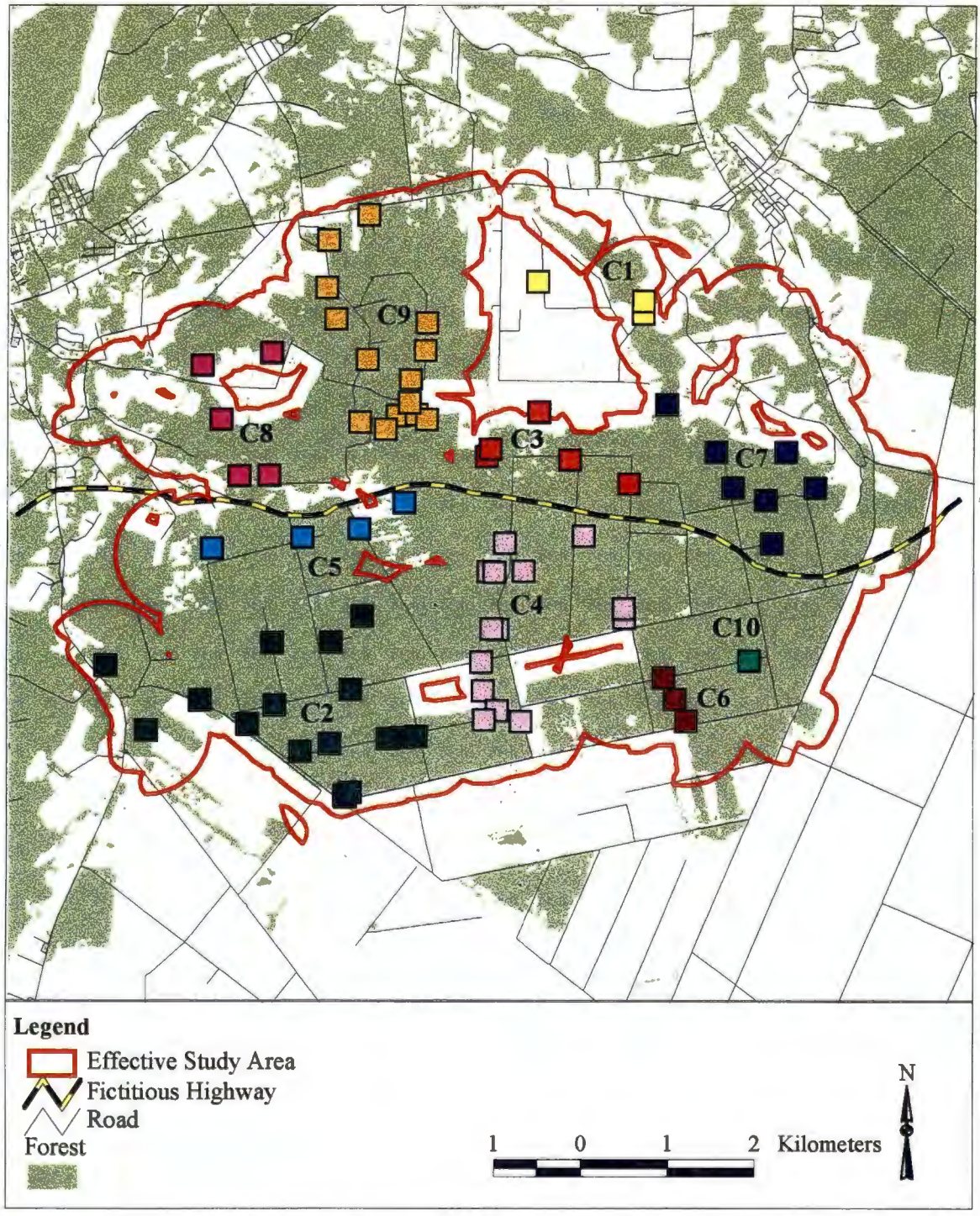

Figure 18. Subpopulation clusters of black bears identified from hair samples and live captures on the control area, Washington County, North Carolina, 2000.

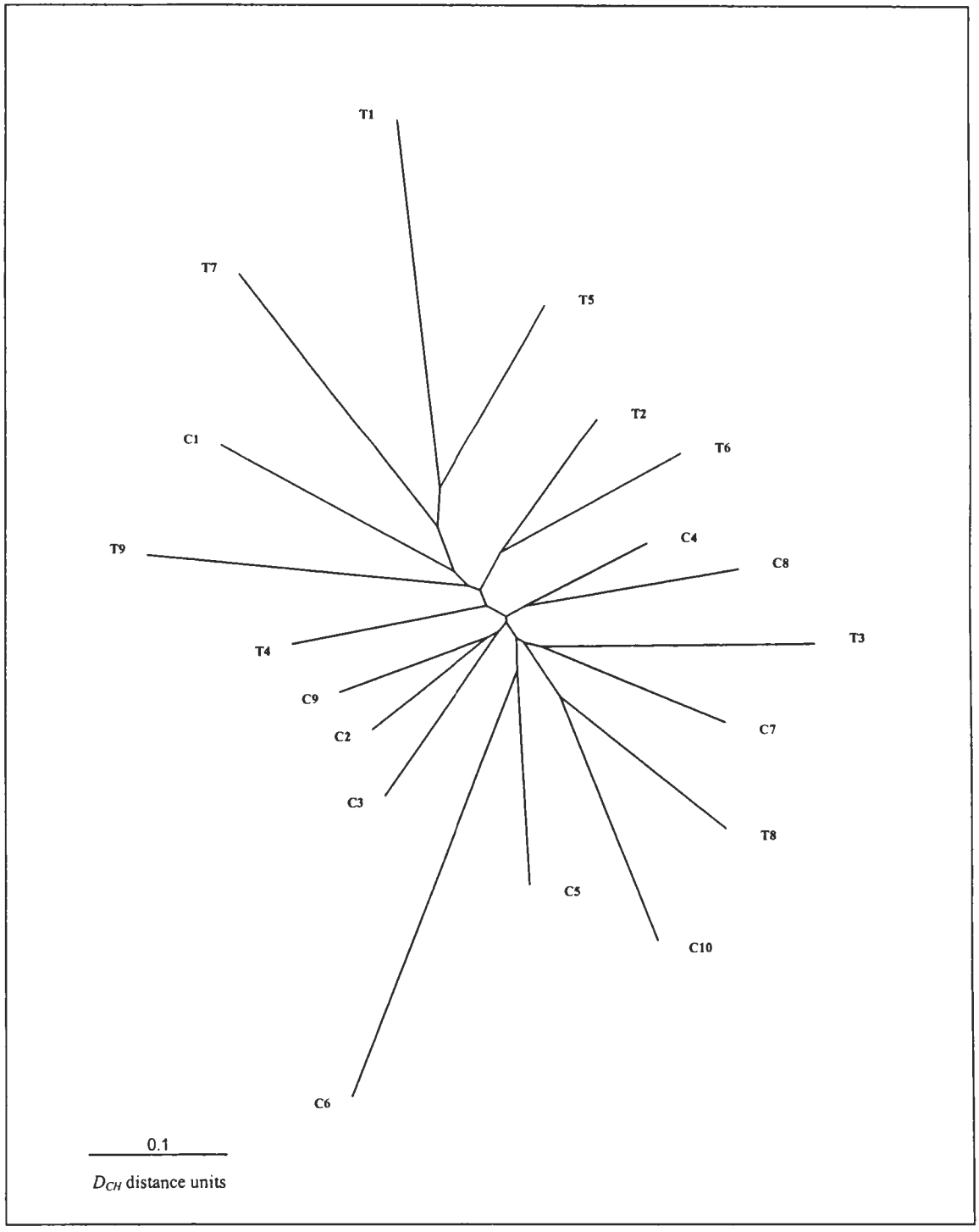

Figure 19. Unrooted neighbor-joining tree based on the chord distance (D_{CH}) among subpopulations sampled during the live-trapping and hair-sampling periods on the treatment (T) and control areas (C), Washington County, North Carolina, 2000.

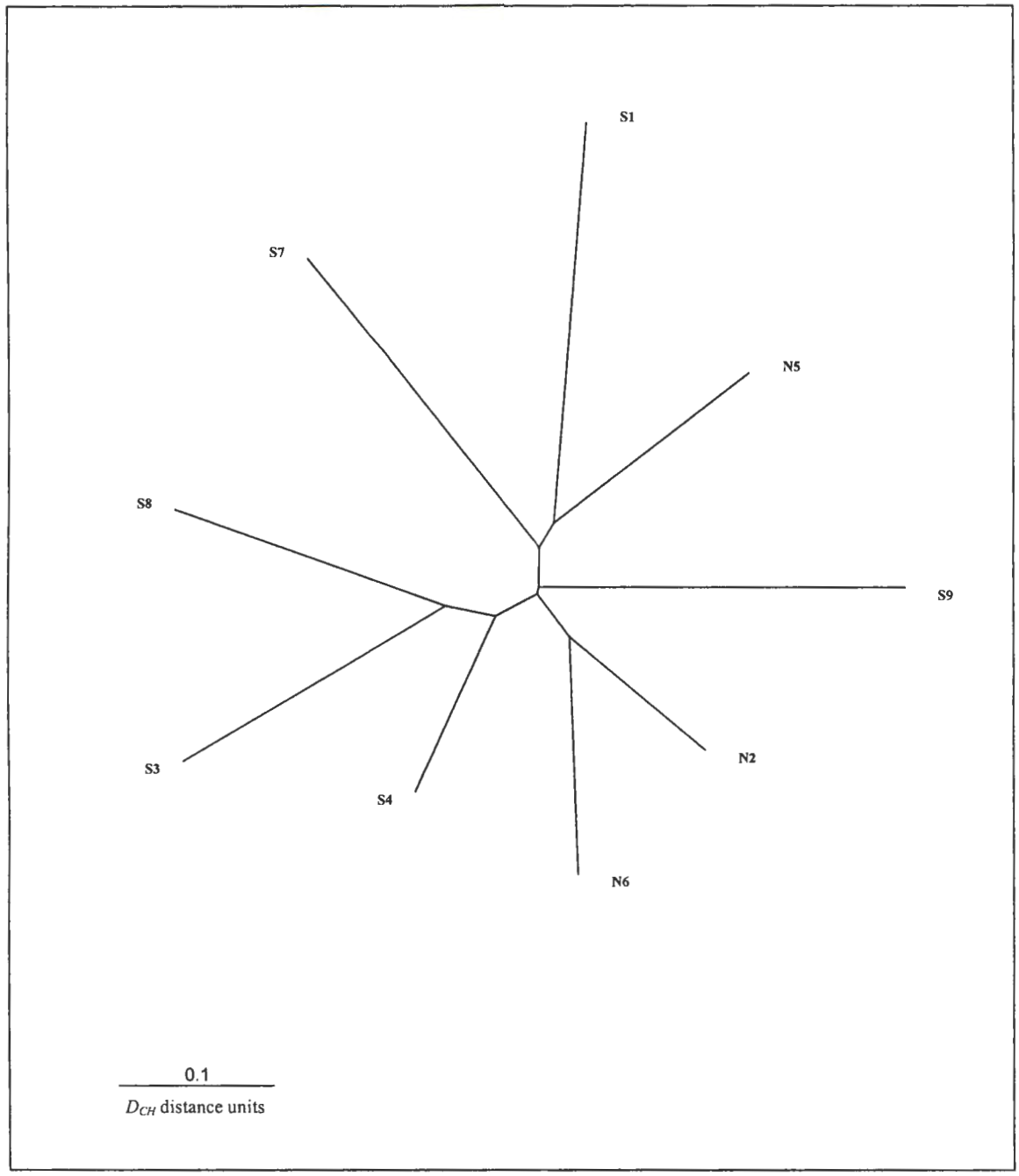

Figure 20. Unrooted neighbor-joining tree based on the chord distance (D_{CH}) among subpopulations sampled during the live-trapping and hair-sampling periods north (N) and south (S) of the proposed highway on the treatment area, Washington County, North Carolina, 2000.

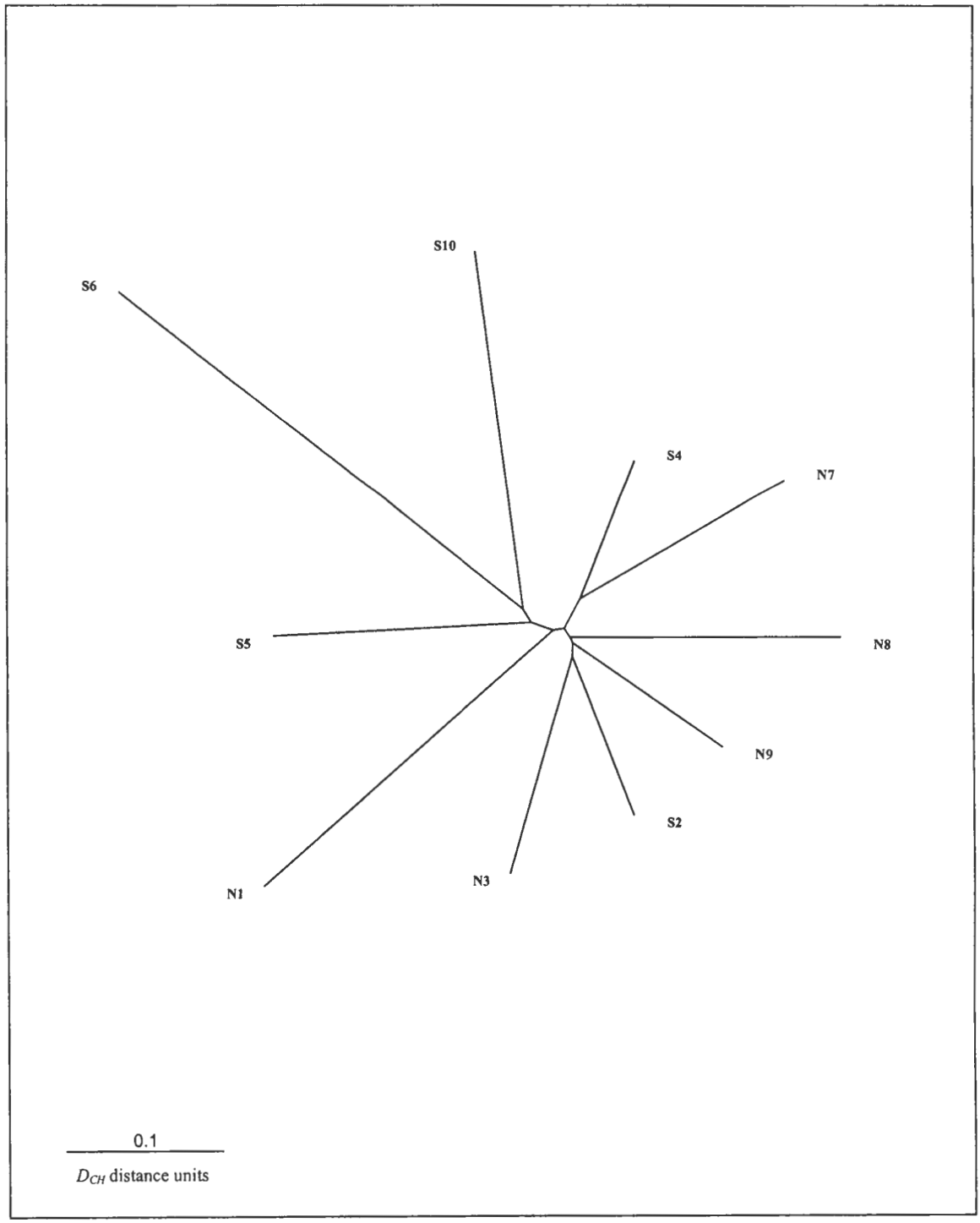

Figure 21. Unrooted neighbor-joining tree based on the chord distance (D_{CH}) among subpopulations sampled during the live-trapping and hair-sampling periods north (N) and south (S) of the fictitious highway on the control area, Washington County, North Carolina, 2000.

bears were somewhat clustered, where 2 pairs of subpopulations (T1, T5 and T2, T6) were neighbors. However, 2 subpopulations (T3 and T8) were clearly more related to control area subpopulations (C7 and C10). Although 2 of the 3 subpopulations from the northern portion of the treatment area were close neighbors (N2 and N6), the low sample size ($n = 3$) made it difficult to detect distinct associations among the northern subpopulations (Figure 20). On the control area, S5, S6, and S10 were clustered, but no overall pattern was evident (Figure 21).

Population Structure and Geneflow

Assignment Test.--The assignment test computed for the treatment and control areas indicated that 134 of 179 individuals (74.9%) were correctly assigned to their respective source populations. For individuals captured north and south of the proposed highway within the treatment area, 52 of 65 individuals (80.0%) were correctly assigned. Within the control area, 72 of 114 individuals (63.2%) were correctly assigned to the north and south populations.

Heterogeneity Test.--Results of the heterogeneity test indicated that allele frequencies differed between the treatment and control areas at all of the 10 loci examined when tested at $\alpha = 0.05$. After applying sequential Bonferroni adjustments, only locus G10C was not significant ($P = 0.019$) at $\alpha = 0.0056$. Therefore, allele frequencies differed between the 2 study areas at 9 of the 10 loci. For individuals captured north and south of the proposed highway within the treatment area, allele frequencies differed at 7 of the 10 loci when tested at $\alpha = 0.05$. Loci G1A ($P = 0.523$), G1D ($P = 0.796$), and MU50 ($P = 0.073$) did not differ between the 2 areas. However,

after sequential Bonferroni adjustments, only 4 of the 10 tests were significant (G10C, $P = 0.001$; G10B, $P < 0.001$; G10X, $P = 0.002$; G10L, $P = 0.001$) at $\alpha = 0.0083$. Allele frequencies differed at only 1 of the 10 loci (MU50, $P = 0.001$) for individuals captured north and south of the fictitious road within the control area, which remained significant after the sequential Bonferroni adjustment ($\alpha = 0.005$).

F_{ST}.--I detected marginal population structure between the 2 study areas and within the treatment area. The F_{ST} value for the treatment and control areas was 0.029 ($P < 0.001$). For the north and south populations within the treatment and control areas, the F_{ST} values were 0.04 ($P < 0.001$) and 0.006 ($P = 0.015$), respectively.

Gene Flow Rates (Nm).--The effective number of migrants per generation between the treatment and control areas was 4.6. The number of effective migrants for the north and south populations within the treatment and control areas were 3.3 and 7.6 individuals per generation, respectively.

Isolation-by-Distance

Mantel tests indicated correlations between the genetic distances of individuals (based on D_{SA}) and geographic distances for the treatment ($r = 0.11$, $n = 65$, $Z = 8.11 \times 10^7$, $P = 0.001$) and control areas ($r = 0.06$, $n = 114$, $Z = 2.56 \times 10^7$, $P = 0.025$) (scales 1 and 2). The correlation of genetic and geographic distances among individuals for both study areas combined (scale 3) was $r = 0.16$ ($n = 179$, $Z = 1.35 \times 10^8$, $P = 0.001$). The same correlations for subpopulation clusters (based on D_{CH}; Figures 22 and 23) were $r = 0.31$ ($n = 9$, $Z = 9.23 \times 10^4$, $P = 0.033$) and $r = 0.27$ ($n = 10$, $Z = 1.02 \times 10^5$, $P = 0.060$) for the treatment and control areas, respectively (scales 4 and 5). The correlation

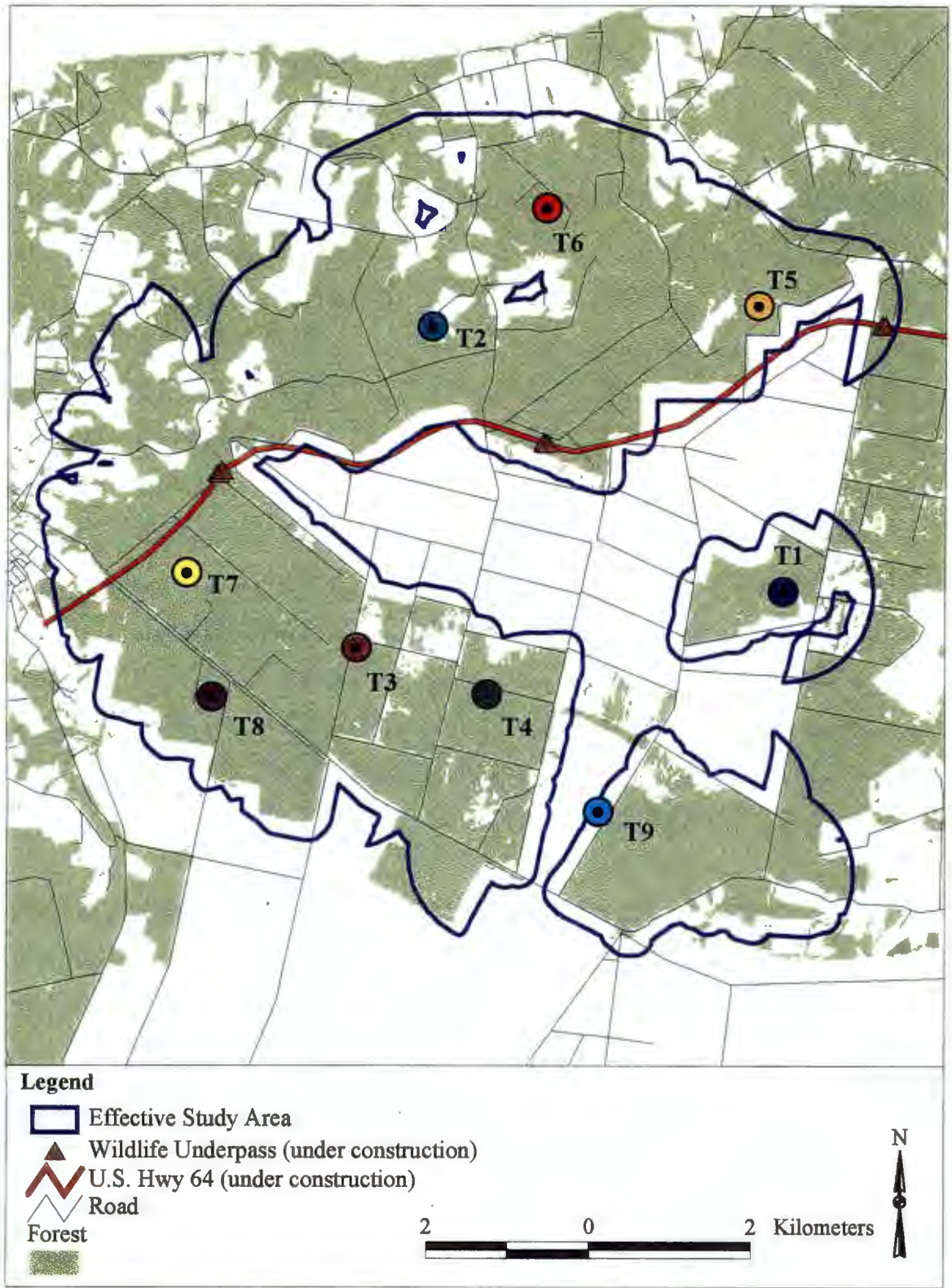

Figure 22. Average subpopulation locations (center points) of individual black bears identified from hair samples and live captures on the treatment area, Washington County, North Carolina, 2000.

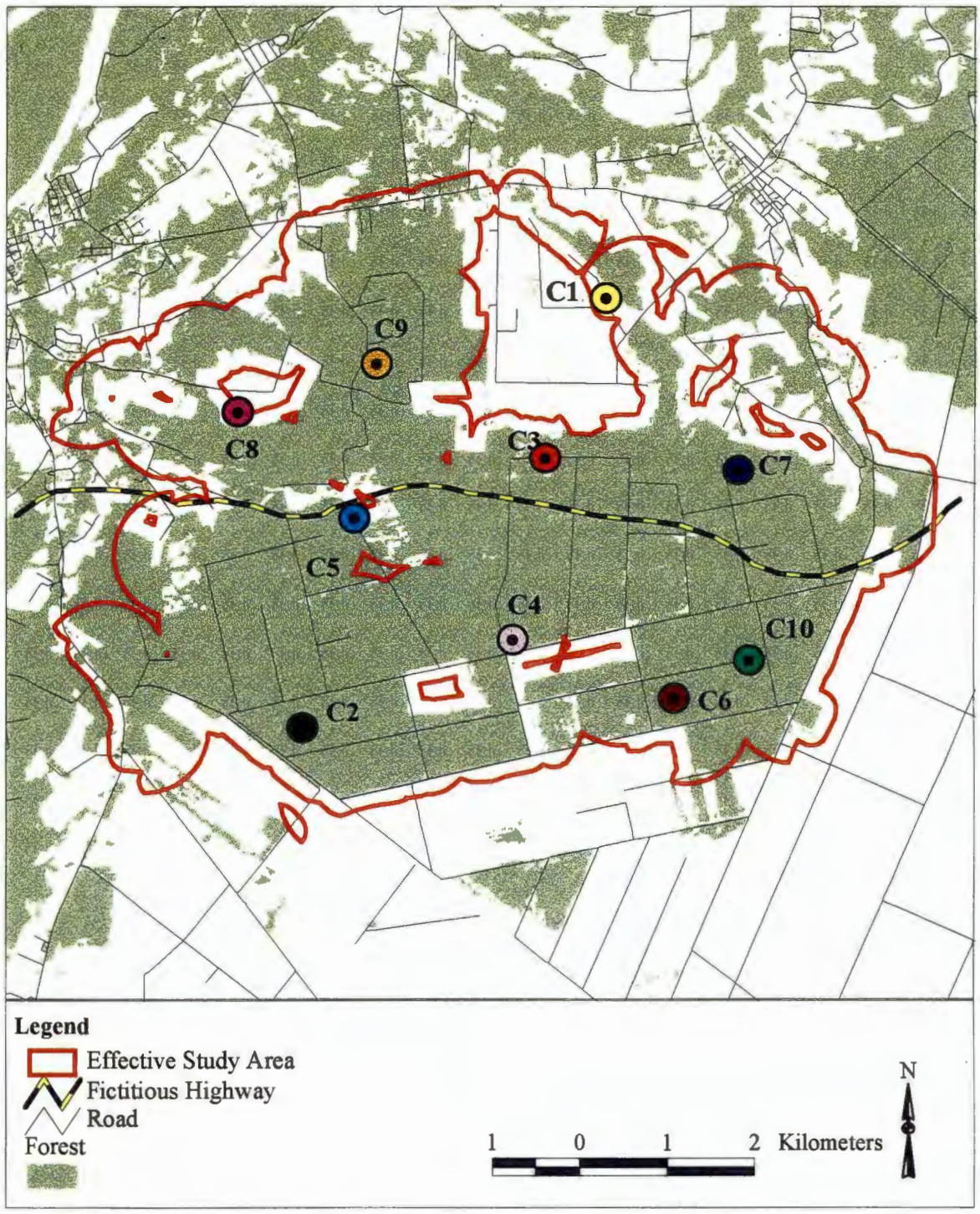

Figure 23. Average subpopulation locations (center points) of individual black bears identified from hair samples and live captures on the control area, Washington County, North Carolina, 2000.

among subpopulation clusters for both areas combined (scale 6) was $r = 0.17$ ($n = 19$, $Z = 6.80 \times 10^5$, $P = 0.057$). Finally, correlations of genetic and geographic distances of live-captured females (Figure 24), based on D_{SA}, were $r = 0.38$ ($n = 10$, $Z = 1.91 \times 10^5$, $P = 0.013$) for the treatment area, $r = 0.09$ ($n = 16$, $Z = 3.59 \times 10^5$, $P = 0.160$) for the control area, and $r = 0.26$ ($n = 26$, $Z = 2.84 \times 10^6$, $P = 0.002$) for both areas combined (scales 7, 8, and 9). The distance correlation for live-captured males (Figure 25) for both study areas combined (scale 10) was $r = 0.06$ ($n = 9$, $Z = 3.85 \times 10^5$, $P = 0.330$).

Allelic Diversity

The number of different alleles sampled at the hair-sampling sites within the treatment and control areas were interpolated throughout both study areas (Figure 26). A moderate relationship between forest density (Figure 27) and allelic diversity was detected ($r = 0.29$, $P = < 0.001$). No relationship between allelic diversity and connectance or cohesion were detected ($r = -0.09$, $P = 0.265$ and $r = 0.001$, $P = 0.989$, respectively).

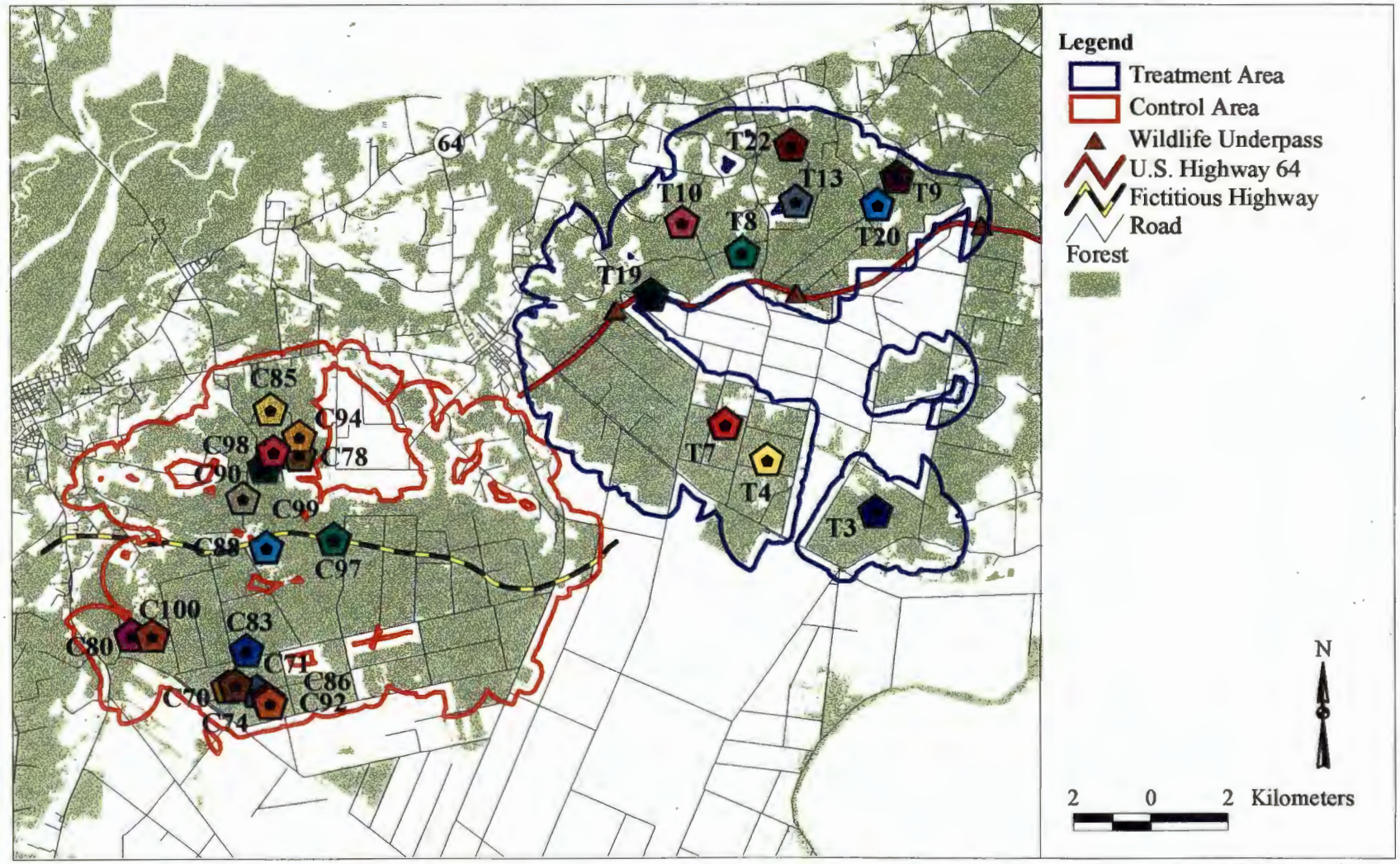

Figure 24. Home range centers for female black bears livecaptured on the treatment and control areas, Washington County, North Carolina, 2000.

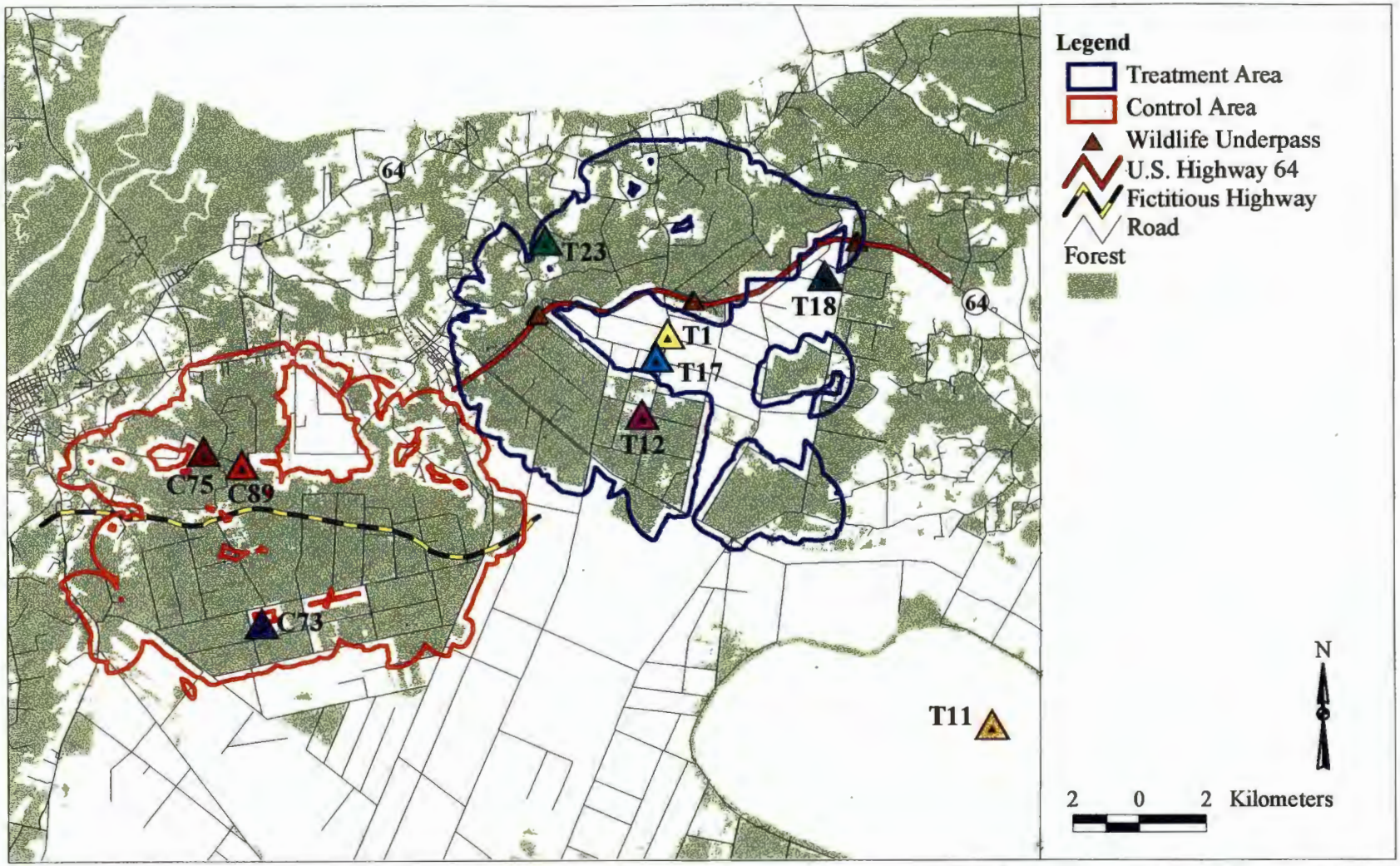

Figure 25. Home range centers for male black bears livecaptured on the treatment and control areas, Washington County, North Carolina, 2000.

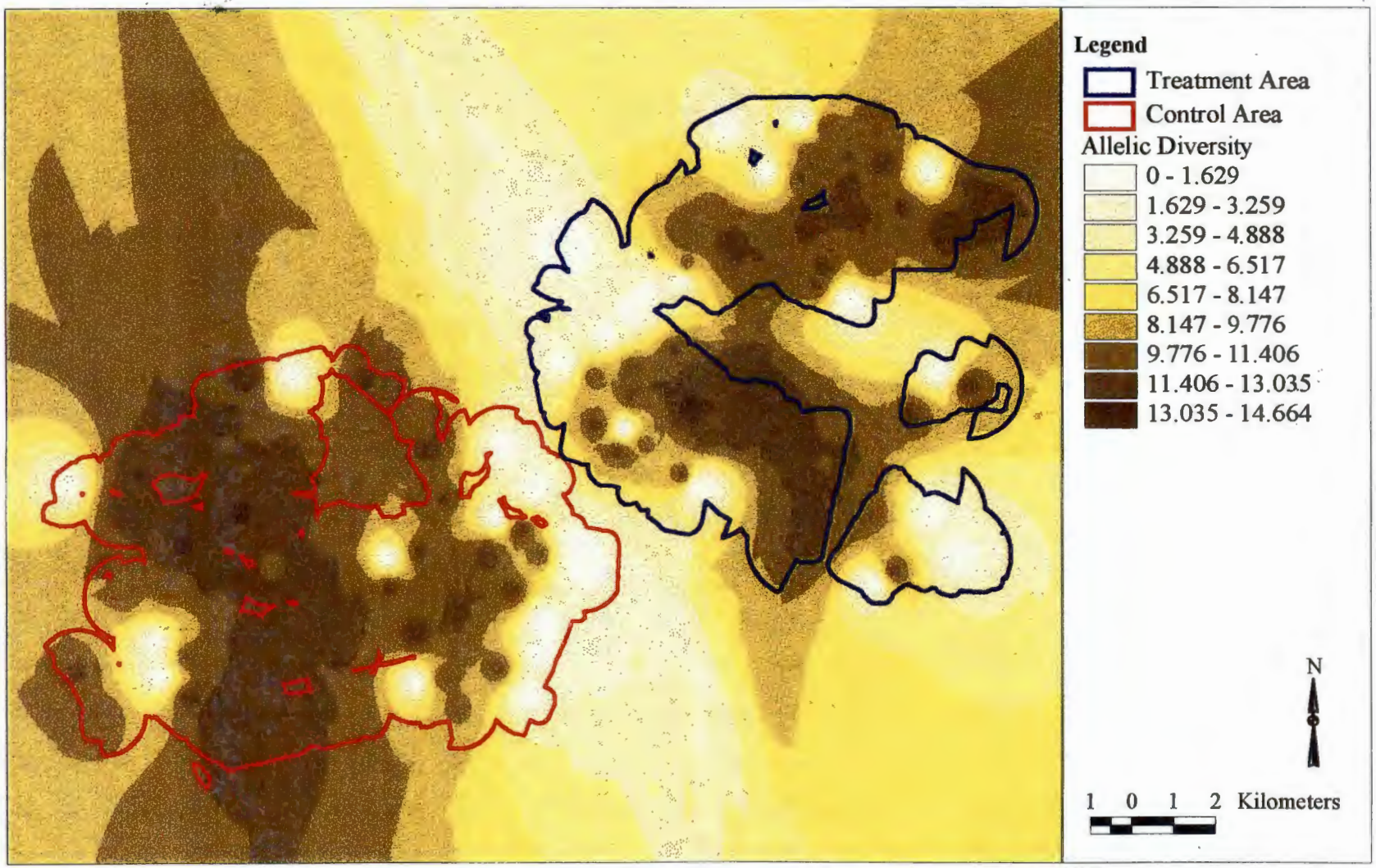

Figure 26. Allelic diversity of black bears, based on DNA collected at hair-sampling sites, treatment and control areas, Washington County, North Carolina, 2000

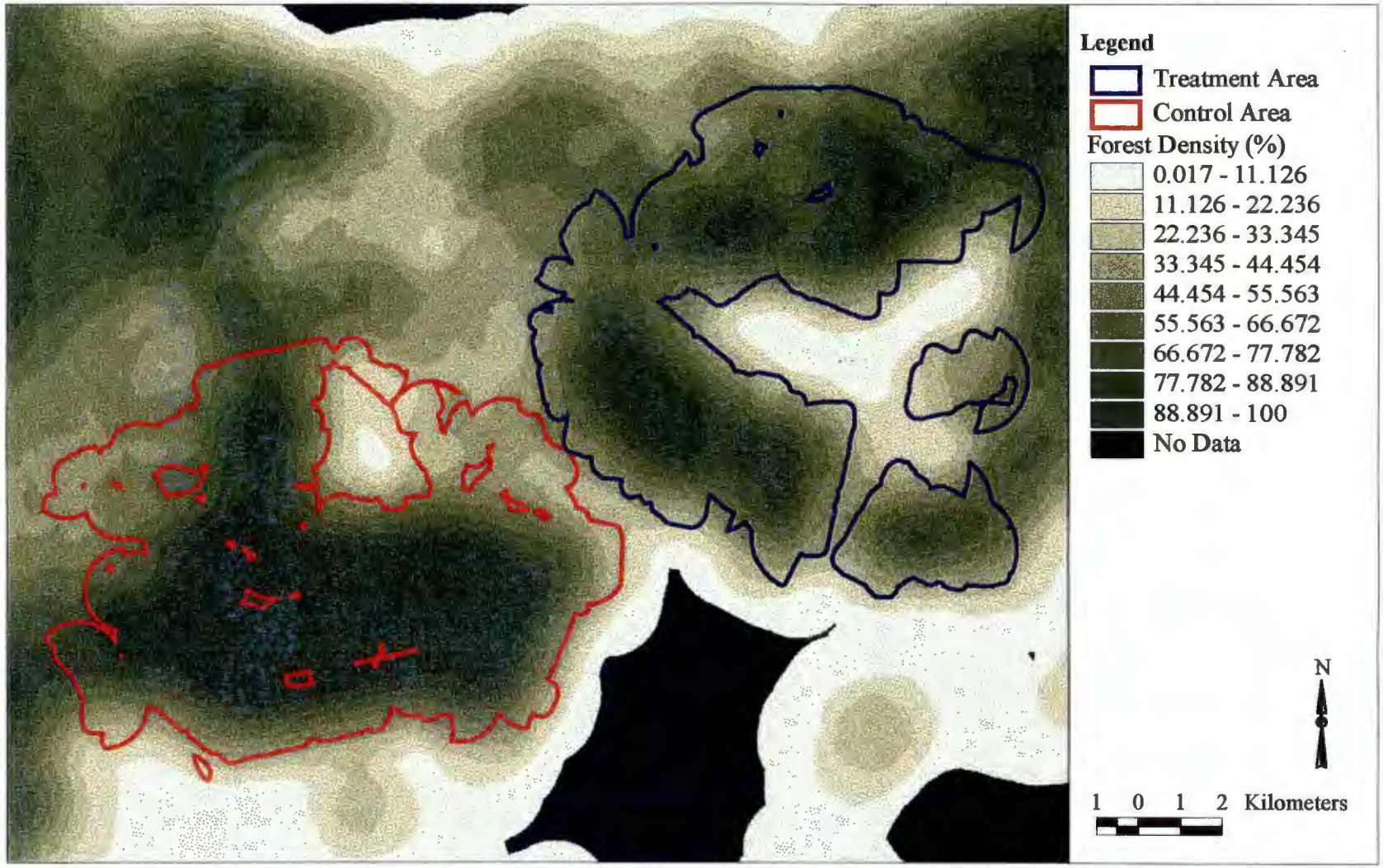

Figure 27. Percent forest density, treatment and control areas, Washington County, North Carolina, 2000.

CHAPTER V

DISCUSSION

POPULATION ABUNDANCE

Like many other carnivores, black bears can be difficult to study because they exhibit secretive behavior, are distributed at low densities, and often occupy remote and inaccessible habitats (Pelton et al. 1978). Therefore, obtaining mark-recapture data for reliable estimates of population abundance can be challenging. Past studies commonly relied on mark-recapture methods using live captures to estimate population abundance (e.g., Clark and Smith 1994). Although reliable population estimates can be achieved with live-capture data, many disadvantages exist. Capture and handling of animals is intrusive (Poole et al. 2001) and it is difficult and time-consuming to properly sample large study areas (Mowat and Strobeck 2000). Furthermore, sample sizes often are small (Mills et al. 2000) and capture probabilities may be affected by potential behavioral responses (e.g., trap shyness). Such trap-response biases may substantially affect the probability of recaptures, which tends to reduce accuracy of abundance estimates (Pollock et al. 1990). The collection of DNA from hair samples provides an alternative method for estimating population abundance with fewer biases (Woods et al. 1999). The hair-sampling techniques used in my study were non-intrusive and likely reduced "trap shyness" because bears presumably did not have a negative experience when visiting sampling sites. Positive trap response bias ("trap happiness") likely was small as well because only a small amount of bait was used (G. Mowat, Aurora Wildlife Research, British Columbia, personal communication). Finally, a sufficient number of traps were

established so that at least 1 trap was present in every female bear home range throughout the duration of the hair-sampling period, which likely reduced biases due to unequal trap access.

When sampling bears, Mowat and Strobeck (2000) indicated that sampling periods as short as 6–10 weeks will minimize any biases due to lack of closure. The closure tests in program CAPTURE indicated that the closure assumption was upheld for all pooling configurations of sampling periods on both study areas. I attribute this result to the short sampling season (7 weeks). During this short time period in the fall, no births occurred, and it is unlikely that any deaths occurred that were not documented during the intensive field research. Also, the study areas were somewhat confined, surrounded mostly by agricultural lands and improved roads and highways. Core habitat, as identified by the NCWRC, existed within approximately 2 km of the study areas; however, only a few narrow corridors provided connection to core habitat. If bear movements into or out of the study area occurred, they likely were random and only temporary, which would not bias estimates of population abundance (Kendall 1999). Furthermore, results of the genetic distance analysis indicated that only 2 animals out of 145 were sampled on both study areas during the hair-sampling season, suggesting that very few closure violations occurred. Therefore, I conclude that violation of the closure assumption was not a major factor during the short hair-sampling season.

Initially, I considered the Bailey's triple catch estimator because it allows for immigration and, thus, may be less sensitive to any closure violation. However, estimates produced with that estimator were imprecise, with CVs of 44% and 46% for the treatment and control areas, respectively. Because the closure assumption was not a

primary concern, I did not consider the estimates produced by the Bailey's triple catch estimator to be appropriate for the data.

The modified Lincoln-Petersen estimator and the multiple mark-recapture models produced precise results, with CVs <20%. However, the modified Lincoln-Petersen estimator is based on the assumption that all individuals have an equal opportunity to be captured (Pollock et al. 1990). That is, biased estimates may be produced if variation, such as heterogeneity or behavioral responses, exists among the capture probabilities of different sampling periods. Program CAPTURE results indicated strong heterogeneity among the capture probabilities on both study areas. Menkens and Anderson (1988) determined from a simulation study that estimates derived with the modified Lincoln-Petersen model tend to be biased low in the presence of heterogeneity. Thus, I concluded that the multiple mark-recapture models were most appropriate for my data because they allow for the relaxation of the assumption of equal catchability in the presence of heterogeneity (Otis et al. 1978).

Although the Chao models are appropriate for low capture probabilities, they are generally less precise than other multiple mark-recapture models (Chao 1987, Boulanger and Krebs 1996). Indeed, abundance estimates based on the Chao M_h model for the 7 separate sampling periods had high CVs (≥26%). Population estimates produced by the heterogeneity model (M_h) for the 7 separate weeks of hair sampling had low CVs (<20%), but estimates were inflated (n = 122 and n = 255 for the treatment and control areas, respectively) compared with estimates derived from the different pooling configurations. Moreover, capture probabilities (treatment area: 11%; control area: 7%) were lower than my initial objective of ≥20%. Rosenberg (1995) determined that the

heterogeneity model tends to be unreliable when capture probabilities are low. Therefore, I determined that pooling the data and estimating population abundance via the heterogeneity model was most appropriate. Of the 7 pooling configurations on the treatment area, the pooling configuration of weeks 1–2, weeks 3–5, and weeks 5–7, which provided an estimate of 85 bears, was most appropriate for the data (Table 6). On the control area, the most appropriate pooling configuration was weeks 1–2, weeks 3–4, and weeks 5–7, providing an estimate of 165 bears (Table 7).

The treatment and control area densities were 1.20 bears/km^2 and 1.78 bears/km^2, respectively. Densities were calculated based on the effective study area size, which was determined by delineating all forested areas (including a 250-m buffer) within the effective sampling area of hair-sample sites as defined by the radius of an average female home range. Although the average sizes of male home ranges (treatment area: 29.4 km^2; control area: 14.2 km^2) were larger than those of females (treatment area: 5.4 km^2; control area: 3.2 km^2; J. L. Kindall, University of Tennessee, Knoxville, unpublished data), I concluded that the radius based on female home ranges was most appropriate. Expanding the effective study area by incorporating male home ranges would have included mostly agricultural areas and water, but very little bear habitat. Furthermore, home ranges were based on data collected for an entire year, whereas movements during the 7-week hair-sampling season likely were more restricted. Restricting the effective study area to forested tracts, including a 250-m buffer from forest edges, also was important in determining the effective study area sizes. The treatment and control areas mostly were surrounded by large agricultural fields and busy roads, with only a few narrow corridors providing connections with other bear habitat (Figure 2). Such areas

received almost no bear use so it was important to eliminate them from the effective sampling area to reduce the potential of underestimating population density.

The treatment area had a moderately high density (1.20 bears/km^2) compared with other densities reported in North Carolina, including Big Pocosin (0.53 bears/km^2; Martorello 1998) and Alligator River National Wildlife Refuge (0.86 bears/km^2; Allen 1999). Black bear density on the control area (1.78 bears/km^2), however, was considerably greater than that of the treatment area. Although one should be cautious comparing population densities determined by different sampling methods and from study areas with different spatial extents and habitat quality, the control area density is among the highest reported in the Southeast. Other density estimates comparable with the control area are from Gum Swamp, North Carolina (1.35 bears/km^2; Martorello 1998) and the Deltic tracts in the Tensas River Basin, Louisiana (1.43 bears/km^2; Beausoleil 1999). These areas are similar to the control area in that they are both coastal plain populations with large, juxtaposed tracts of forest and agricultural crop fields. However, the observed differences in bear densities between the 2 study areas suggest that factors other than landscape composition may affect bear densities.

Although the treatment and control areas had similar areas of agricultural and forested lands, the layout of these landcover types were somewhat different; most of the forested tracts on the treatment area were on the periphery with large tracts of agricultural fields on the interior, reducing access to important edge habitats (Figure 2). Large, contiguous agricultural fields reduce edge, essentially making the interior agricultural fields inaccessible to bears. Furthermore, the forested tracts on the treatment area were more fragmented by residential areas and frequently traveled roads. Conversely, the

control area consisted mostly of large, contiguous forest tracts with small agricultural fields interspersed in the center of the study area and larger agricultural tracts on the periphery. The smaller agricultural fields within the large forested tracts produced more edge and likely were beneficial to bears by providing excellent access to agricultural crops.

I also considered whether the density difference could be related to food abundance. Both study areas had large areas of wheat and corn fields, which provide the 2 most important agricultural crops for black bears in eastern North Carolina. However, no major differences in the area of these 2 crops or the crop rotation were observed between the 2 study areas. Also, those crops were harvested at similar times on both areas; wheat in early summer and corn in mid to late summer. Thus, the disparity in density between the 2 areas may be more related to a difference in access of bears to agricultural foods rather than abundance of those foods.

A third possible reason for the marked difference in bear density between the 2 areas may be related to hunting pressure. Documented mortalities during the 2000–2001 hunting seasons indicated that hunting pressure likely was much greater on the treatment area. For example, 8 of the 23 bears marked on the treatment area in 2000 were killed during the 2000 or 2001 hunting seasons, whereas only 1 of the 32 bears on the control area was killed during that same period. Also, hunt clubs on the control area tended to be conservative in harvesting bears and, unlike the treatment area, no commercial outfitters used lands on the control area (personal observations and J. L. Kindall, University of Tennessee, personal communication). Based on anecdotal evidence of animals killed, hunting likely is the most important factor in the difference in densities. As such, the

control and treatment areas are potentially functioning as source-sink populations. That is, moderately high bear densities on the treatment area may be sustained, even with relatively high harvest rates, because of immigration of bears from the control area.

GENETIC STRUCTURE

Population structure measures the degree to which individuals or populations are genetically different. Researchers have used a variety of methods to determine population structure. For example, Paetkau et al. (1995) used genetic distance and the assignment test to determine inter- and intra-population structure of polar bears (*Ursus maritimus*) in Canada. For my study, I used several methods to determine population structure for the treatment and control areas and for areas north and south of the proposed highway on the treatment area.

Genetic relatedness is a useful measure to determine genetic relationships and provides an assessment of the amount of population structure. Genetic distance results for the treatment and control area populations and the north and south populations within the treatment and control areas indicated that the north and south populations within the treatment area were less related to each other (0.249) than the entire treatment and control area populations (0.192) and the north and south populations within the control area (0.135). The unrooted neighbor-joining trees based on genetic distance among individuals (D_{SA}) further supported this notion, with less clustering of individuals within the north and south populations of the control area (Figure 16) than in the treatment and control area populations (Figure 14) or the north and south populations of the treatment area (Figure 15). The unrooted neighbor-joining trees based on the Chord distance (D_{CH})

of subpopulation clusters, however, were less clear. I speculate that the confounding results were a reflection of the small sample size of subpopulation clusters and the clustering of subpopulations, which was based on somewhat arbitrary spatial criteria.

Another method I used to detect population structure was the assignment test. Although Cornuet et al. (1999) indicated that the assignment test has a variety of uses (e.g., comparing dispersal rates), it is appropriate for determining population structure (Paetkau et al. 1995). Test results showed that 74.9% of the individuals sampled on the treatment and control areas were correctly assigned to the sampled population. Correct assignment of individuals sampled north and south of the proposed highway on the treatment area was even greater (80.0%). However, correct assignment of individuals sampled north and south of the fictitious highway within the control area was lower (63.2%) than between the 2 study areas and within the treatment area. Although all percentages were substantial, the north and south populations within the control area exhibited less structure, supporting the results of the genetic distance analysis. The heterogeneity tests, however, provided somewhat different results. For example, allele frequencies differed at 9 of the 10 loci examined between the treatment and control areas, 4 of the 10 loci differed for individuals captured north and south of the proposed highway within the treatment area, and 1 of the 10 loci differed for individuals captured north and south of the fictitious highway within the control area. These results suggest that more population structure exists between the treatment and control areas than within the treatment area, which contradicts the genetic distance and assignment test results. However, the heterogeneity tests are less accurate when data are collected from only 1 year (T. L. King, U.S. Geological Survey, personal communication). The F_{ST} values

(0.029 between the treatment and control areas, 0.04 between the north and south populations within the treatment area, and 0.006 between the north and south populations within the control area) were supportive of the genetic distance and assignment test results, indicating that there is slightly more structure within the treatment area than between the 2 study areas or within the control area. The estimates of gene flow also support this notion, with fewer migrants traveling north and south of the proposed highway (3.3 migrants per generation) than traveling between the treatment and control areas (4.6 migrants per generation) or between the north and south populations within the control area (7.6 migrants per generation). Thus, with exception of results of the heterogeneity tests, it seems that population structure is somewhat greater for individuals captured within the treatment area than between the treatment and control areas or within the control area.

Differences in population structure for the 3 scales may be related to landscape characteristics. Both the treatment and control areas and areas north and south of the proposed highway within the treatment area exhibited distinct habitat boundaries. However, no distinct habitat boundary exists within the control area. The large area of agricultural fields within the interior of the treatment area, in conjunction with intensive farming practices, likely poses a greater movement barrier than the disturbances and fragmented areas between the treatment and control areas or within the control area (Figure 2).

Although I detected differences in the amount of population structure, I suggest that the overall amount of genetic differentiation was small. The F_{ST} estimates, although different from 0, were low. Furthermore, the assignment tests indicated that correct

assignment of individuals was high, but 100% correct assignment can be achieved when F_{ST} estimates are as low as 0.10 (Cornuet et al. 1999). This suggests that correct assignment of individuals at the 3 scales likely would have been greater if the populations exhibited strong genetic differentiation. Finally, gene flow estimates measured at the 3 scales indicated that exchange of individual bears occurs occasionally. Because only 1 migrant is required to homogenize subpopulations (Mills and Allendorf 1996, Slatkin 1985), it is very unlikely that strong population structure would exist.

I used the Mantel test to examine isolation-by-distance by determining relationships between the genetic and geographic distances of individuals and populations. The results showed significant relationships for 6 of the 10 scales. Some relationships exhibited substantial isolation-by-distance (treatment area females, $r = 0.38$; treatment area subpopulation clusters, $r = 0.31$), particularly given the small scale of the study areas (T. L. King, U.S. Geological Survey, personal communication). All isolation-by-distance analyses calculated for the treatment area were significant, whereas only 1 on the control area was marginally significant (distance among individuals, $r = 0.06$, $P = 0.022$). I speculate that this difference may be related to the density disparity between the 2 study areas. For instance, control area bears may disperse greater distances because of the high population density, thereby reducing genetic differentiation over a larger extent compared with the treatment area. Another notable finding was that the correlation based on female home range centers was strong on the treatment area ($r = 0.38$), but very weak ($r = 0.09$) on the control area. The live-trapping locations may provide an explanation for this observation. Bears were sampled from a large portion of the treatment area (Figure 3) and home ranges of female bears showed little overlap and

were evenly distributed throughout the study area (Figure 24). In contrast, bears were sampled in 2 small areas on the control area (Figure 4) and female home ranges were more clustered (Figure 24). Therefore, variation in geographic distances among female home ranges likely was too small to detect a correlation with genetic distance. The correlation based on male home ranges also was weak ($r = 0.06$). However, I speculate that this reflects the large home ranges of males relative to the scale of the study areas, making relationships between genetic and geographic distances difficult to detect.

The strongest isolation-by-distance relationship was observed for treatment area females. I speculate that the spatial scale of the study was large enough to detect differentiation among females but too small to detect differentiation among males, which may explain why the overall degree of population structure was low. If so, the small differences in the observed levels of genetic differentiation may partly be due to family structure rather than population structure (T. L. King, U.S. Geological Survey, personal communication). Indeed, the unrooted trees constructed from the genetic distance analyses showed more distinct clustering of individuals (i.e., family groups; Figures 14, 15, and 16) than clustering of subpopulations (Figures 19, 20, and 21).

Microsatellites can be used for a wide range of applications, including the evaluation of genetic processes on a landscape scale. Researchers have only recently begun to explore this area of research. For example, Mech and Hallett (2001) used a genetic approach to evaluate the effectiveness of corridors for red-backed voles (*Clethrionomys gapperi*) and deer mice (*Peromyscus maniculatus*). I used microsatellite data to determine relationships between allelic diversity and landscape features and found a positive correlation with forest density ($r = 0.29$). This finding likely reflects that

larger, contiguous populations typically are more genetically diverse than small and isolated populations (Lacy 1987). Given the small spatial scale of the study, this correlation is noteworthy. Although I found no relationship between allelic diversity and the 2 connectivity variables, this does not imply that connectivity was not an important factor. Forest density is a measure of habitat area and was correlated with forest cohesion ($r = 0.65$, $P < 0.001$). Thus, it is likely that both area and cohesiveness of habitat are important to maintain allelic diversity among black bears.

The level of genetic variation, or heterozygosity, typically reflects the amount of allelic diversity. As populations become more isolated because of fragmentation, allelic diversity is lost to genetic drift, subsequently reducing levels of genetic variation and causing populations to diverge genetically (Fleischer et al. 1995). Average heterozygosity was 66.7% and 66.4% for the treatment and control areas, respectively. Results of the Hardy-Weinberg equilibrium tests for the hair and tissue samples combined showed that observed heterozygosity levels did not differ from the expected heterozygosity, indicating that recent inbreeding or genetic bottlenecks have not occurred in the treatment and control area populations. Similar estimates of heterozygosity have been reported for black bears in the Southeast based on 8 of the 10 microsatellite loci used in my study (excluding MU23 and MU50), including the Okefenokee National Wildlife Refuge in Georgia (66.3%) and Osceola National Forest in Florida (67.9%) (Dobey 2002). Boersen (2001) reported 47.7% heterozygosity for the Tensas River National Wildlife Refuge in Louisiana, whereas Edwards (2002) reported 31.6% heterozygosity for the Mobile River area in Alabama. These latter estimates are much lower than those of my study and Okefenokee National Wildlife Refuge/Osceola

National Forest because they represent bear populations on the last large forest fragments within their respective regions. Consequently, those 2 populations are relatively small and isolated from other black bear populations, which can substantially reduce the level of genetic variation (Fleischer et al. 1995).

Gene flow estimates indicated that black bears move within and between the treatment and control areas. Results of the linkage disequilibrium tests indicated that some loci were associated, which may be an indicator that there were immigrants to the population (T. L. King, U.S. Geological Survey, personal communication). Thus, gene flow from surrounding populations seems to contribute to the genetic variation among black bears on the 2 study areas, thereby preventing genetic isolation. Moreover, these results also indicate that demographic exchange has occurred. Given the high bear densities observed in my study, it is likely that the treatment and control areas play an important role in the stability of the regional black bear population.

CHAPTER VI

MANAGEMENT AND RESEARCH IMPLICATIONS

Black bear population densities observed in my study are relatively high compared with other studies in the Southeast. The study areas were comprised mostly of agricultural fields and large, managed pine forest, with sparse patches of hardwood areas. The key aspect of this landscape is that it provides bears access to highly productive and consistent food sources adjacent to large and contiguous forest habitats. Bears can occur in this highly managed landscape because human and improved road densities are low and forest tracts are large; any changes in those conditions, however, could severely affect bear population densities. Therefore, if one management objective is to maintain current bear densities, it is important to assess the potential impacts of new road construction projects, such as the rerouting of U.S. Highway 64, and other developments.

The 2 adjoining populations in my study provide a striking example of how population densities can vary, even in similar habitats. Although differences in hunting pressure and food access are likely explanations for the 2-fold difference in population density, alternative explanations should be evaluated. A further assessment of the cause of this density difference will be important once the data from phase II are incorporated into the final analysis.

The genetic structure analyses indicated substantial gene flow within and between the treatment and control areas. The high levels of genetic variation within both populations also indicate sufficient gene exchange with surrounding populations. However, isolation of populations due to landscape fragmentation may change breeding

patterns among bears, even at small scales, as shown by the gene flow rates within the treatment area. Over time, such altered breeding patterns may increase the probability of inbreeding and genetic drift. Although the 2 study areas were somewhat isolated, the few narrow corridors connecting them to other bear habitat patches seemed sufficient to sustain adequate gene flow and support high levels of genetic variation. Preserving these small corridors throughout the North Carolina coastal range will be important to maintain black bear populations, both locally and regionally.

The objectives of my study were to measure population abundance and genetic structure before highway construction so that results can be compared with those collected after completion of the highway. One concern with that study design could be that changes in some or all of the parameters may be difficult to detect if pre-construction estimates are imprecise. The results of my analyses indicate that abundance and density estimates were relatively precise. Thus, any demographic changes likely would be detectable after study phase II has been completed. Almost all genetic structure parameters were consistent and, thus, sufficient to detect biological changes. However, results likely would be more discernable if phase II data were collected several generations after highway construction is completed (J. B. Wolf, University of Tennessee, Knoxville, personal communication).

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APPENDICES

Appendix A. Live-trapping Results for the Treatment and Control Areas, Washington County, North Carolina, 2000

Table A.1. Black bear live-trapping results for the treatment area, Washington County, North Carolina, 2000.

Bear ID	Date	Sex	Capture Type	Weight (kg)	Age (Years)
WT 001	16-Jun-00	Male	Initial	61.2[a]	3
WT 002	17-Jun-00	Male	Initial	77.1	3
WT 003	19-Jun-00	Female	Initial	47.6	2
WT 004	21-Jun-00	Female	Initial	70.3[a]	5
WT 005	23-Jun-00	Male	Initial	127.0[a]	3
WT 006	5-Jul-00	Male	Initial	45.4[a]	1
WT 007	9-Jul-00	Female	Initial	38.6	1
WT 008	11-Jul-00	Female	Initial	61.2	4
WT 009	12-Jul-00	Female	Initial	70.3	3
WT 002	13-Jul-00	Male	Recapture	77.1	3
WT 010	14-Jul-00	Female	Initial	52.2[a]	3
WT 011	14-Jul-00	Male	Initial	158.8[a]	3
WT 012	18-Jul-00	Male	Initial	124.7[a]	6
WT 013	27-Jul-00	Female	Initial	65.8	7
WT 014	30-Jul-00	Male	Initial	99.8[a]	2
WT 015	31-Jul-00	Male	Initial	79.4[a]	2
WT 016	5-Aug-00	Female	Initial	27.2	1
WT 017	13-Aug-00	Male	Initial	90.7[a]	2
WT 018	16-Aug-00	Male	Initial	132.9	3
WT 019	18-Aug-00	Female	Initial	72.6	3
WT 020	18-Aug-00	Female	Initial	47.6	2
WT 021	20-Aug-00	Male	Initial	68.0	1
WT 017	24-Aug-00	Male	Recapture	127.0	2
WT 022	25-Aug-00	Female	Initial	79.4[a]	7
WT 008	27-Aug-00	Female	Recapture	83.9	4
WT 023	4-Sep-00	Male	Initial	113.4	2
WT 010	6-Sep-00	Female	Recapture	72.6	3

[a] Estimated with regression equation (T. H. Eason and F. T. van Manen, University of Tennessee, Knoxville, unpublished data)

Table A.2. Black bear live-trapping results for the control area, Washington County, North Carolina, 2000.

Bear ID	Date	Sex	Capture Type	Weight (kg)	Age (Years)
WT 100	10-Jul-00	Female	Initial	79.4[a]	
WT 099	12-Jul-00	Female	Initial	56.7[a]	5
WT 098	12-Jul-00	Female	Initial	56.7[a]	3
WT 097	15-Jul-00	Female	Initial	59.0	7
WT 096	16-Jul-00	Female	Initial	24.0	1
WT 095	16-Jul-00	Female	Initial	24.9[a]	2
WT 094	16-Jul-00	Female	Initial	77.1[a]	5
WT 093	18-Jul-00	Male	Initial	72.6	1
WT 092	19-Jul-00	Female	Initial	74.8	4
WT 091	20-Jul-00	Female	Initial	35.4	2
WT 090	20-Jul-00	Female	Initial	63.5	9
WT 089	21-Jul-00	Male	Initial	166.9[a]	
WT 088	21-Jul-00	Female	Initial	65.8	6
WT 087	22-Jul-00	Female	Initial	36.3	1
WT 086	22-Jul-00	Female	Initial	62.6	3
WT 085	22-Jul-00	Female	Initial	54.4	5
WT 084	23-Jul-00	Female	Initial	31.8	2
WT 083	23-Jul-00	Female	Initial	49.0	2
WT 082	23-Jul-00	Male	Initial	52.2	1
WT 081	23-Jul-00	Female	Initial	36.3	2
WT 080	24-Jul-00	Female	Initial	79.4	5
WT 079	25-Jul-00	Female	Initial	27.2	1
WT 078	25-Jul-00	Female	Initial	70.3	4
WT 077	29-Jul-00	Female	Initial	24.9	1
WT 076	30-Jul-00	Male	Initial	36.3	2
WT 075	31-Jul-00	Male	Initial	176.9	8
WT 074	31-Jul-00	Female	Initial	65.8	7
WT 073	31-Jul-00	Male	Initial	97.5	2
WT 072	31-Jul-00	Male	Initial	86.2	1
WT 084	4-Aug-00	Female	Recapture		2
WT 071	6-Aug-00	Female	Initial	74.8	
WT 070	7-Aug-00	Female	Initial	47.6	9
WT 069	7-Aug-00	Male	Initial	40.8	1

[a] Estimated with regression equation (T. H. Eason and F. T. van Manen, University of Tennessee, Knoxville, unpublished data)

Appendix B. Laboratory Protocol for Microsatellite Analysis

MICROSATELLITE ANALYSIS

DNA Isolation

DNA was extracted from hair follicles using the InstaGene Matrix (Bio-Rad Laboratories, Hercules, California, USA). Specifically, follicles were incubated in the InstaGene Matrix in the presence of Proteinase K at 65° C overnight. This mixture was boiled (100° C) for 8–10 minutes, followed by centrifugation at 10,000–12,000 rpm. The resulting supernatant was used in PCR reactions.

Microsatellite Amplification and PCR

Microsatellite DNA amplification was performed for 10 microsatellite loci using the PCR primers described by Paetkau and Strobeck (1994) and Paetkau et al. (1995). These loci were: G1A, G1D, G10B, G10C, G10L, G10M, G10P, G10X, MU23, and MU50.

Each PCR reaction consisted of 1.5 μl of genomic DNA extract, 0.875 X PCR buffer (59 mM Tris-HCl, pH 8.3; 15 mM $(NH_4)_2SO_4$; 9mM β-mercaptoethanol; 6 mM ETDA), 2.25 mM $MgCL_2$, 0.2 mM dNTPs, 0.15-0.43 μM of each primer (forward primer fluorescently labled with TET, FAM, or HEX; Applied Biosystems (ABI), Foster City, California, USA), 1.2 units of Taq polymerase (ABI), and deionized water added to achieve the final volume of 15 μl. The amplification cycle consisted of an initial denaturing at 94° C for 2 minutes followed by 35 cycles of 94° C denaturing for 30 seconds, 56° C annealing for 30 seconds, and 72° C extension for 1 minute. Cycling culminated with a 5-minute extension at 72° C. Thermal cycling was performed in an MJ

DNA Engine PTC 200 (MJ Research, Watertown, Massachusetts, USA) configured with a heated lid.

Fragment Analysis

Generally, 1 μl of PCR product was diluted 1:1 with deionized water and thoroughly mixed. One μl of this dilution was added to 12 μl of deionized formamide and 0.5 μl of the internal size standard GENESCAN-500 (ABI). Alternatively, PCR products of separate multiplexed reactions (2–3 loci each) and multiple separate reactions (2–4) were combined and analyzed without dilution. Loci were identified in these multiplexed samples by virtue of their characteristic molecular mass and attached fluorescent label. The size standard contained DNA fragments fluorescently labeled with the dye phosphoramidite TAMRA (red). This PCR product/size standard/formamide mixture was heat denatured at 95° C for 3 minutes and placed immediately on ice for at least 5 minutes. The mixture was subjected to capillary electrophoresis on an ABI PRISM 310 Genetic Analyzer (i.e., automated sequencer). Fluorescently labeled DNA fragments were analyzed, and genotype data generated using Genescan software (ABI). GENOTYPER v. 2.0 (ABI) DNA fragment analysis software was used to score, bin, and output allelic (and genotypic) designations for each bear sample.

VITA

Laura Marie Thompson, daughter of Robert and Nancy Thompson, was born in Maryville, Tennessee on December 20, 1976. She attended Maryville High School in Maryville, Tennessee and graduated in 1995. She received a Bachelors of Science degree in Wildlife and Fisheries Science from the University of Tennessee, Knoxville in December of 1999. Laura then began her graduate research in June of 2000 in the Department of Forestry, Wildlife and Fisheries at the University of Tennessee in Knoxville. She received her Masters of Science degree in Wildlife and Fisheries Science in May 2003.