

Geostatistical analysis of allele presence patterns among American black bears in eastern North Carolina

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Abstract: Highways are one of the leading causes of wildlife habitat fragmentation and may particularly affect wide-ranging species, such as American black bears (*Ursus americanus*). We initiated a research project in 2000 to determine potential effects of a 4-lane highway on black bear ecology in Washington County, North Carolina. The research design included a treatment area (highway construction) and a control area and a pre- and post-construction phase. We used data from the pre-construction phase to determine whether we could detect scale dependency or directionality among allele occurrence patterns using geostatistics. Detection of such patterns could provide a powerful tool to measure the effects of landscape fragmentation on gene flow. We sampled DNA from roots of black bear hair at 70 hair-sampling sites on each study area for 7 weeks during fall of 2000. We used microsatellite analysis based on 10 loci to determine unique multi-locus genotypes. We examined all alleles sampled at ≥ 25 sites on each study area and mapped their presence or absence at each hair-sample site. We calculated semivariograms, which measure the strength of statistical correlation as a function of distance, and adjusted them for anisotropy to determine the maximum direction of spatial continuity. We then calculated the mean direction of spatial continuity for all examined alleles. The mean direction of allele frequency variation was 118.3° ($SE = 8.5$) on the treatment area and 172.3° ($SE = 6.0$) on the control area. Rayleigh's tests showed that these directions differed from random distributions ($P = 0.028$ and $P < 0.001$, respectively), indicating consistent directional patterns for the alleles we examined in each area. Despite the small spatial scale of our study (approximately 11,000 ha for each study area), we observed distinct and consistent patterns of allele occurrence, suggesting different directions of gene flow between the study areas. These directions seemed to coincide with the primary orientation of the best habitat areas. Furthermore, the patterns we observed suggest directions of potential source populations beyond the 2 study areas. Indeed, nearby areas classified as core black bear habitat exist in the directions indicated by our analysis. Geostatistical analysis of allele occurrence patterns may provide a useful technique to identify potential barriers to gene flow among bear populations.

Key words: allele mapping, American black bear, anisotropy, geostatistics, highways, North Carolina, semivariogram, *Ursus americanus*

Ursus 16(1):59–69 (2005)

Habitat fragmentation has become a focal issue for the management and conservation of mammalian carnivores throughout the world. Although the sources of habitat fragmentation can be natural or anthropogenic, the most substantial threats to population stability and persistence

often are anthropogenic in nature (Wilcox 1980). Highway networks, in particular, represent one of the primary concerns for wildlife managers. Movements of animals across the landscape can be important to supplement declining populations, maintain gene exchange, and recolonize habitat patches after local population extinctions. However, highways often are barriers to such movements, thereby undermining those

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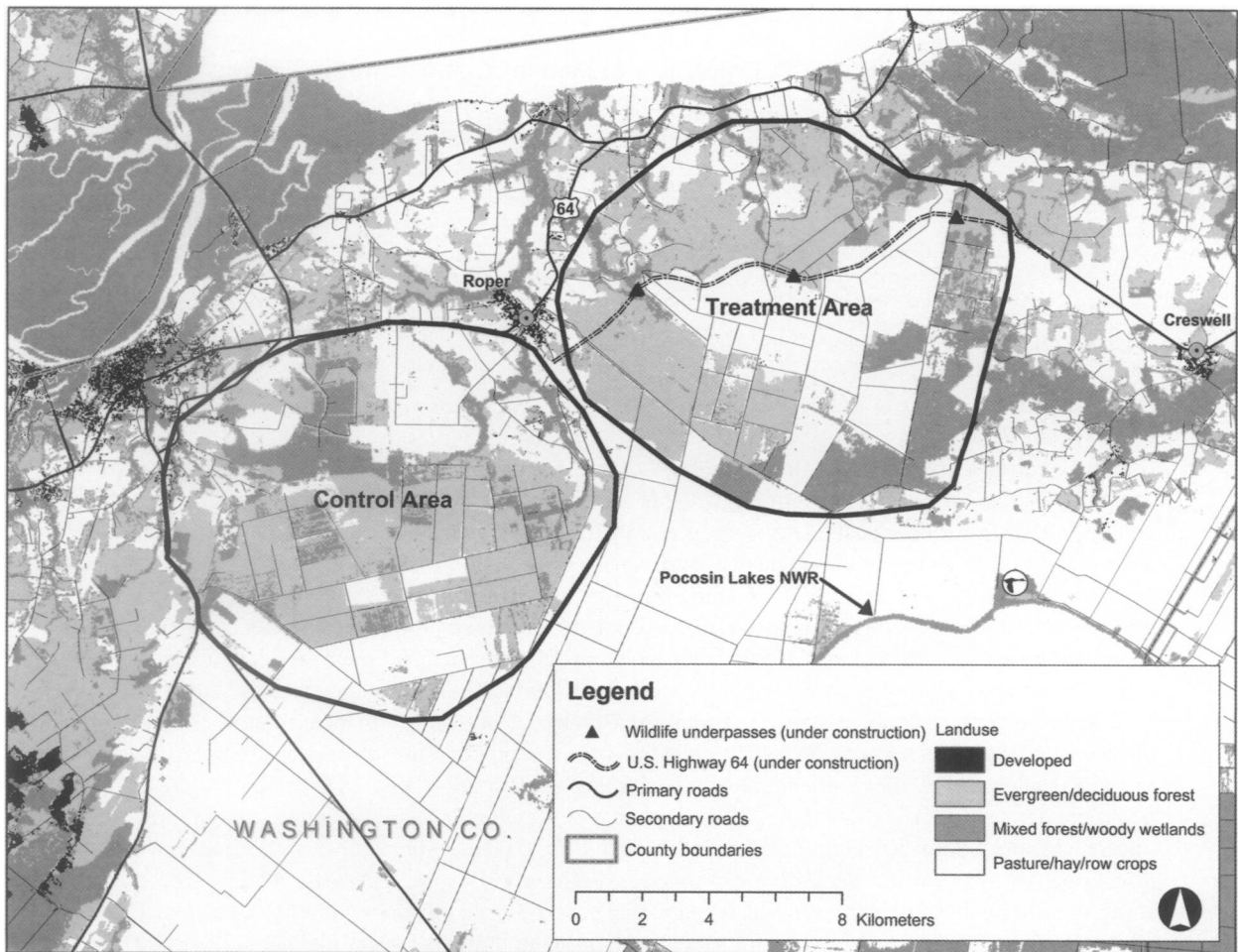


Fig. 1. Treatment and control areas to study potential impacts of a 4-lane highway on black bears, Washington County, North Carolina, USA.

ecological processes (Wilcox 1980, Beringer et al. 1990, Clevenger et al. 2001).

In recent years, researchers have increasingly emphasized studying 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 depend on regional landscapes (Ruediger 1998). American black bears occur in relatively low densities and use their habitat on a landscape scale (Schoen 1990). As such, this species can provide valuable ecological insights regarding the effects of highways on wildlife.

The North Carolina Department of Transportation is expanding US Highway 64 between Raleigh and the Outer Banks. The present 2-lane highway will be

upgraded to a 4-lane highway in segments within the next 10–15 years. A 19.3-km segment of US Highway 64 in Washington County is presently under construction and is the focus of our research. A new route was identified south of current US Highway 64 between the towns of Roper and Creswell, bisecting important black bear habitat (Fig. 1). The new highway route borders large tracts of forested land, potentially restricting bear movements to adjacent agricultural fields, which are important for foraging. The North Carolina Department of Transportation incorporated 3 wildlife underpasses into the highway construction design to reduce vehicle collisions and to mitigate the potential effects of the highway on movements of bears and other wildlife, both locally and regionally. The locations of the wildlife underpasses were based on surveys of bear sign

conducted by the North Carolina Wildlife Resources Commission in 1999 (Scheick and Jones 1999).

Our study is one component of a project designed to determine the impacts of the 4-lane highway with wildlife underpasses on black bear ecology (van Manen et al. 2001). Few studies have documented ecological responses of wildlife to highways because data often cannot be collected before construction. This project was designed to collect data before highway construction (phase I) for comparison with post-construction data (phase II). The design included a control area to ensure that observed effects on the treatment area are not due to factors other than the presence of the highway.

Potential barrier effects of the highway may manifest themselves in terms of demographic changes and changes in spatial use patterns of bears. With the recent advancements in DNA analysis technology, the consequences of habitat fragmentation also may be measured at the genetic level (Proctor et al. 2002). However, detecting spatial changes in genetic patterns is contingent on the ability to observe and interpret such spatial patterns. Therefore, we examined whether geostatistical analysis techniques may be useful to determine such spatial patterns.

Applied geostatistics is a relatively new branch of geography and statistics that involves the detection, modeling, and estimation of spatial dependence, or spatial continuity (Rossi et al. 1992). Conceptually, spatial continuity can best be described by considering pairs of measurements. Because of spatial autocorrelation, 2 nearby measurements are more likely to be similar than are 2 distant measurements. The distance beyond which no spatial autocorrelation exists may be biologically important: measurements within that distance are scale-dependent, whereas measurements at greater distances are scale-independent (Trani-Griep 2002). Geostatistical techniques were developed in the field of geology and mining, but also have a history in forestry and are finding increasing use in the field of ecology (Rossi et al. 1992, Le Corre et al. 1998).

We used geostatistics to provide insights on the direction and extent of allele frequency patterns among black bears within and between the 2 study areas and to evaluate their usefulness to assess the potential impacts of the highway after construction has been completed. The specific objectives of our study were to: (1) examine whether allele occurrences are scale-dependent, and (2) determine the directionality of allele occurrence and whether those directional patterns were consistent among alleles.

Study area

The 2 study areas were located in Washington County in eastern North Carolina (Fig. 1). The center of the overall study area was located at approximately 35°87' north latitude, 76°65' west longitude. The treatment and control areas each were slightly over 10,750 ha. Both areas were bordered to the north by the current US Highway 64. The new 19.3-km segment of highway will pass through the center of the treatment area and will contain 3 wildlife underpasses (Fig. 1). The control area was chosen to closely resemble the landscape of the treatment area, particularly the juxtaposition and area of agricultural and forested lands.

Washington County is hot and humid in summer and winters typically are cool. The average minimum and maximum temperatures are 25°C and 31°C, respectively, during summer and 0°C and 6.7°C, respectively, during winter (Soil Conservation Service 1979). Average annual precipitation ranges from 115.0 to 127.5 cm, with maximum precipitation occurring between April and September.

Managed pine forests (primarily loblolly pine [*Pinus taeda*]) comprised the majority of forested lands, whereas hardwoods were restricted to natural drainages. Most drainages were composed of trees in the 40- to 120-year age classes and were dominated by oaks (*Quercus* spp.), baldcypress (*Taxodium distichum*), and blackgum (*Nyssa sylvatica*). Human development was limited to a few large farms within the study areas and several small towns near the boundaries of the study area. A commercial forest products company was the largest landowner in 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 land in crop production. Major agricultural crops included corn, wheat, soybeans, peanuts, and cotton.

Methods

Field data collection

We used ArcView® (ESRI, Redlands, California, USA) geographic information system (GIS) software to generate random location coordinates for 70 hair-sampling sites each on the treatment and control areas (Fig. 2). Coordinates for hair-sampling sites were generated only within forested habitats. The number of sites was chosen so that all bears had access to >1 sampling site based on minimum home-range estimates (Kindall 2004).

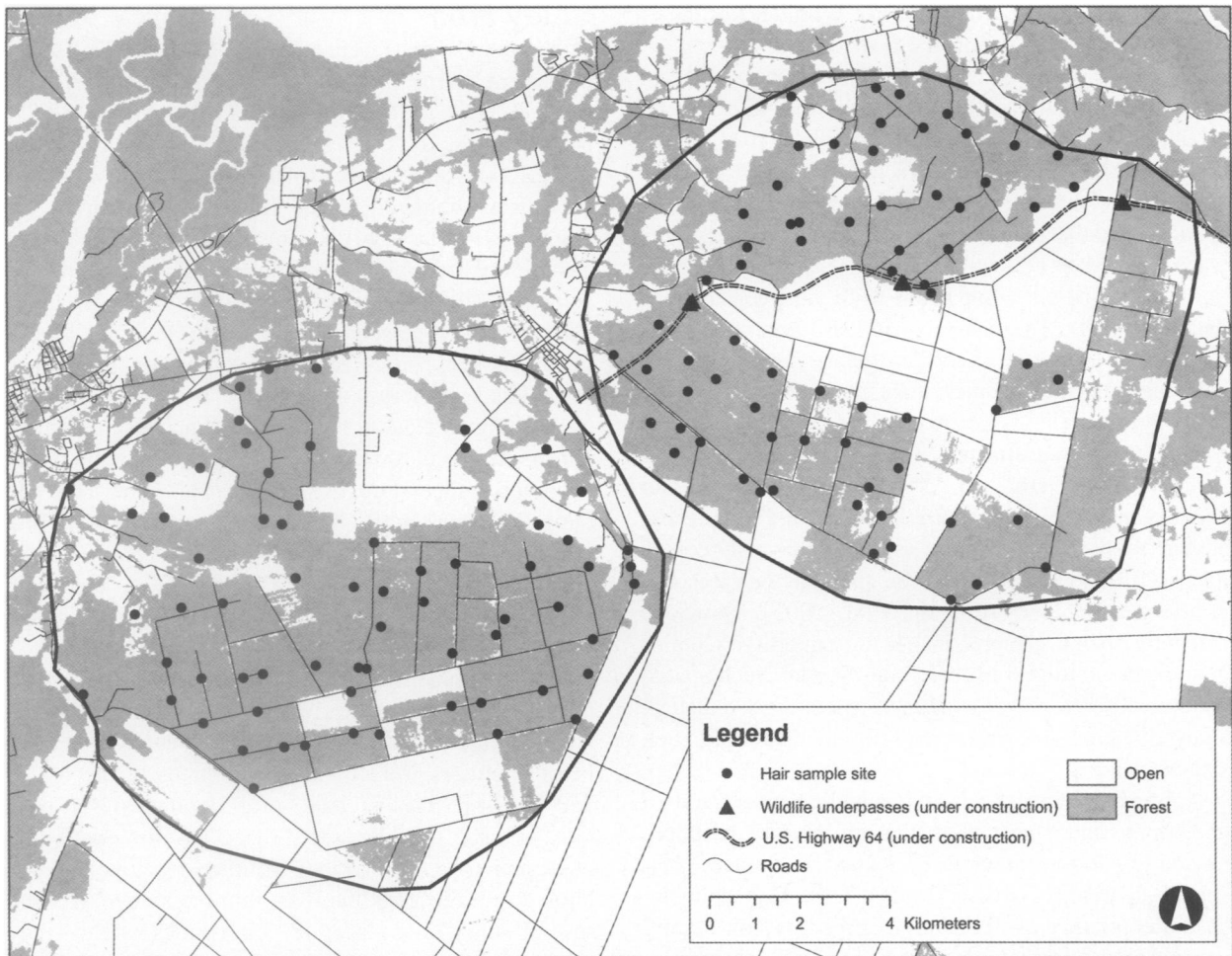


Fig. 2. Location of hair-sample sites on the treatment and control areas, Washington County, North Carolina, USA, 2000.

Each hair-sample site consisted of a barbed-wire enclosure (Woods et al. 1999, Mowat and Strobeck 2000) that was baited with a small amount of bakery products and scented with raspberry extract. Starting on 1 October 2000, sites were monitored for hair samples weekly for 7 weeks. 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.

We randomly selected 25 hair-sample sites for each sampling period and randomly chose 1 hair sample from each of those sites for microsatellite analysis. That number of samples was chosen to meet the objectives of another study component (a conservative estimate of

samples needed to achieve a coefficient of variation of $\leq 20\%$ for population abundance estimates). We prepared the selected samples for microsatellite DNA analysis by clipping approximately 0.6 cm of 5–10 hair roots for each sample and placing those hair roots in a 1.5-ml centrifuge tube.

Microsatellite analysis

DNA was extracted from hair roots using the InstaGene Matrix (Bio-Rad Laboratories, Hercules, California, USA) as described in Boersen et al. (2003). Microsatellite analysis was conducted by the US Geological Survey, Leetown Science Center (Kearneysville, West Virginia, USA). Ten microsatellite loci were analyzed for each selected hair sample at the following markers: G1A, G1D, G10B, G10L, G10C, G10M, G10P,

G10X, MU23, and MU50 (Paetkau and Strobeck 1994, Paetkau et al. 1995). Amplification conditions are described in Boersen et al. (2003). Fluorescently labeled DNA fragments were analyzed and genotype data were generated using GeneScan[®] Analysis Software (Applied Biosystems, Foster City, California, USA). We used DNA fragment analysis software (Genotyper[®], version 2.0; Applied Biosystems, Foster City, California, USA) to score, bin, and output allelic (and genotypic) designations for each black bear hair sample.

The multilocus genotype generated for each individual was analyzed to determine the uniqueness of each hair sample. All samples possessing multilocus genotypes differing by 1 or 2 alleles were genotyped a second time to minimize the likelihood of polymerase chain reaction or genotyping errors that can result in the false identification of unique genotypes, which inflate census estimates. Individual pair-wise genetic distances, using the proportion of shared alleles algorithm, were calculated using the program Microsat (version 1.5d; E. Minch, Stanford University, Stanford, California, USA). Pair-wise genetic distances of zero indicated identical multilocus genotypes (i.e., recaptures).

Allele mapping

We first determined which individual genotypes and which alleles were sampled at the 70 hair-sample sites on each study area. Rather than mapping all alleles, we selected a subset of relatively common alleles for the geostatistical analysis. Because the basis for our analysis was the spatial distribution of sampled alleles, we examined how many sites with allele presence would be needed to ensure adequate fit of the geostatistical models. We determined that alleles occurring at ≥ 25 sites would meet that goal. Because microsatellites tend to have multiple common alleles per locus, the number of alleles that met that criterion was relatively large ($n = 19$ and $n = 26$ for the treatment and control area, respectively). As such, the most important alleles were represented in the analysis. Next, for each of the selected alleles we determined whether it was present at each hair-sample site. We used occurrence rather than allele frequencies to avoid bias due to potential differences in bear density or multiple visits by the same bear.

Geostatistical analysis

We first performed an exploratory spatial data analysis, which is an important step before the calculation of geostatistics (Rossi et al. 1992). One assumption in the application of geostatistics is that spatial measurements have a constant mean and variance, which is referred to as

stationarity (Isaaks and Srivastava 1989). Global stationarity (entire dataset) is unlikely in most environmental datasets; however, local stationarity often is a viable assumption (Isaaks and Srivastava 1989). We tested for local stationarity based on Voronoi diagrams that were constructed using the Geostatistical Analyst in ArcGIS[™] (version 8.2, ESRI, Redlands, California, USA; Aurenhammer 1991).

We used semivariograms (usually referred to as variograms) as our primary geostatistical measure. A variogram determines the average degree of similarity between locations separated by a given distance (h) in space (Le Corre et al. 1998). The variogram can be estimated with semivariance, $\hat{\gamma}(h)$, as follows:

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i) - z(x_i - h)]^2,$$

where $N(h)$ is the number of pairs of locations separated by distance h and z is the measure of interest at location x_i (Rossi et al. 1992). Variogram values tend to increase as the distance between pairs of locations increases but often will reach a plateau where pairs are no longer spatially correlated (Isaaks and Srivastava 1989; Fig. 3). The distance before the variogram reaches this plateau is referred to as the 'range', whereas the height of the plateau is referred to as the 'sill' (Fig. 3). Finally, a discontinuity, or 'nugget effect', may occur at the origin of the variogram, where the intercept is not 0 (Fig. 3), which represents errors due to sampling and spatial variability at scales too small to measure (Isaaks and Srivastava 1989, Rossi et al. 1992).

We calculated variograms based on allele presence (coded as "1") or absence (coded as "0"), which are referred to as indicator variograms (Rossi et al. 1992). We first calculated omnidirectional variograms (variograms that do not incorporate directionality) using the Geostatistical Analyst in ArcGIS[™] for the selected alleles. We calculated those variograms for the treatment and control areas separately. We chose distance classes to calculate the variograms (Barbujani 1988) based on the common geostatistical rule that specifies that the number of distance classes (lags) multiplied by the size of the distance classes (lag size) should be less than half the maximum distance between pairs of locations (Le Corre et al. 1998). The maximum distance between pairs of hair-sample sites was slightly greater than 12,000 m on both the treatment and control areas. Thus, we calculated the variograms for 10 lag classes of 600 m.

Various models (e.g., spherical, exponential, Gaussian) can be used to calculate a variogram. In our

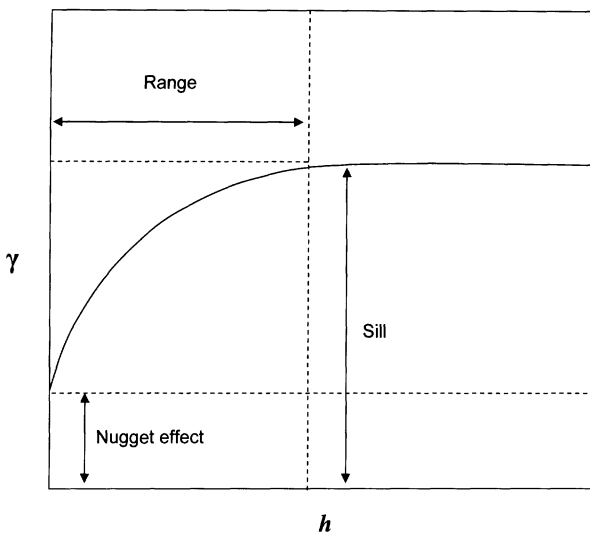


Fig. 3. A typical semivariogram (variogram). Semi-variance values (γ) increase as a function of distance between pairs (h) until pairs of locations are no longer correlated and plateau at the sill. The distance to the sill is the range. A combination of small-scale variability and measurement error can cause a discontinuity at the origin (nugget effect).

analysis, we chose the best model for each allele by examining the residuals based on cross-validation and a visual assessment of model fit to the data (Isaaks and Srivastava 1989). Unbiased residuals of the predicted values have a standardized mean (mean divided by the standard error) near 0 and a root-mean-squared standardized error close to 1.

Once we selected appropriate variograms for each allele, we used them to determine whether allele patterns were spatially dependent (i.e., the variogram values increase with distance). A linear variogram (no sill) would indicate spatial correlation at all sampled scales. However, the presence of a sill indicates that allele patterns tend to differ beyond a certain distance. Variograms that exhibit no spatial dependence are flat ('pure' nugget effect) and indicate random patterns of variation (Isaaks and Srivastava 1989, Rossi et al. 1992, Le Corre et al. 1998).

For each allele, we also determined the relative nugget effect, which is the ratio of the nugget effect to the sill (Isaaks and Srivastava 1989). A large relative nugget effect indicates that only a small proportion of the total variance is available to model spatial dependence from the available sampling grid (Rossi et al. 1992).

If spatial dependence and a sill were present, we next determined the directionality, if any, of those patterns. A variogram model that shows similar spatial continuity in all directions is referred to as isotropic (Isaaks and Srivastava 1989, Rossi et al. 1992). However, change in allele frequencies can be more progressive in particular directions, causing the variogram to reach the sill more quickly. That process is referred to as anisotropy. We adjusted the omnidirectional variograms for anisotropy and determined the maximum direction of spatial continuity for each of the selected alleles, using the Geostatistical Analyst in ArcGIS™. Because a variogram calculated in a particular direction will be identical to the variogram calculated in the opposite direction (Isaaks and Srivastava 1989), we determined the directional axis for each allele based on a 0–180° scale. We used the program Oriana (version 2.00; Kovach Computing Services, Anglesey, Wales) to determine the mean directional axis for all alleles on the treatment and control areas. Finally, we tested whether those mean directional axes were different from random using Rayleigh's test for directional data and whether those axes differed between the 2 areas using the Mardia-Watson-Wheeler test (Batschelet 1981).

Results

Field data collection

On the treatment area, 55 sampling sites were visited by bears during the 7 hair-sampling periods, resulting in 686 hair samples (range = 0–26 per site per period). Of the 686 hair samples, 162 were selected for microsatellite analysis. On the control area, 64 different sample sites were visited, which resulted in 1,240 hair samples (range = 0–25 per site per period); 175 samples were selected for microsatellite analysis.

Microsatellite analysis and allele mapping

Microsatellite analysis was unsuccessful on 30 hair samples from the treatment area and 22 hair samples from the control area due to insufficient quantity of DNA. Therefore, 132 and 153 hair samples were analyzed for the treatment and control areas, respectively. Microsatellite analysis identified 53 unique multilocus genotypes (individuals) on the treatment area and 92 individuals on the control area; 2 of these genotypes were sampled on both areas. We sampled a total of 71 alleles on the 2 study areas (61 and 68 alleles on the treatment and control areas, respectively). Nineteen alleles met the sample size criterion of ≥ 25 sites for the

Table 1. Variogram models, direction and range of maximum spatial continuity, and relative nugget effect of allele occurrences of black bears on the treatment and control areas, Washington County, North Carolina, USA, 2000.

Allele	No. sites sampled		Variogram model		Direction of maximum continuity (°) ^a		Relative nugget effect (%) ^b	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
G10B-157	41	54	hole effect	exponential	90.1	176.3	39.0	23.7
G10B-159	28	29	exponential	hole effect	146.3	117.7	53.4	82.7
G10C-114	51	55	exponential	circular	80.5	174.7	39.5	60.8
G10L-151	30		exponential		127.8		32.7	
G10L-155		39		spherical		169.5		66.6
G10L-157		34		exponential		171.6		43.2
G10M-209		28		exponential		111.5		70.2
G10M-213	40	47	Gaussian	circular	171.1	149.9	72.6	73.4
G10M-219		35		spherical		26.5		71.9
G10P-158		35		exponential		169.8		46.0
G10P-162	35	48	hole effect	exponential	127.3	19.2	61.0	48.8
G10P-166	40	35	hole effect	Gaussian	64.7	23.7	67.8	87.9
G10X-142		25		circular		159.0		67.7
G10X-144	44	48	Gaussian	Gaussian	90.1	157.0	61.7	71.0
G10X-154	32	27	spherical	hole effect	160.8	130.2	68.7	55.4
G1A-187	30		Gaussian		160.1		63.0	
G1A-189	31	38	hole effect	circular	150.2	144.6	69.8	75.7
G1A-191	38	37	circular	Gaussian	101.7	3.2	81.4	86.1
G1A-193		35		exponential		176.5		43.7
G1D-172		38		Gaussian		57.5		71.4
G1D-176	45	49	spherical	exponential	91.0	165.0	73.9	41.5
G1D-180		25		circular		33.6		81.9
G1D-184	32		circular		164.6		78.1	
MU23-161	33	29	hole effect	exponential	65.2	126.3	76.8	81.5
MU23-167	26	32	hole effect	spherical	106.6	173.6	58.6	69.7
MU23-169	26	26	Gaussian	hole effect	135.8	18.0	83.8	86.8
MU23-171		36		circular		28.3		81.2
MU50-206		27		Gaussian		177.1		83.6
MU50-210	31		exponential		126.8		42.1	
MU50-222	33	40	circular	circular	88.3	162.0	65.5	73.6

^aValues reflect a bidirectional axis, but are reported on a 0–180° scale.

^bThe nugget effect reflects errors due to spatial variability and sampling errors; the relative nugget effect is expressed as the ratio of the nugget effect to the sill (Fig. 3).

treatment area, whereas 26 alleles met that criterion on the control area (Table 1). Voronoi diagrams indicated that local means and variances of allele occurrence were constant on both study areas; therefore, we assumed local stationarity (Isaaks and Srivastava 1989).

Geostatistical analysis

The best-fitting variogram models produced standardized mean residuals ranging from –0.008 to 0.047 and root-mean-squared standardized errors between 0.961 and 1.056, indicating a good fit of the models. All variograms showed some spatial dependence and increased to a sill (Fig. 4).

Anisotropy was evident among all variograms with a mean direction of 118.3° (SE = 8.5) on the treatment

area and 172.3° (SE = 6.0) on the control area (Table 1; Fig. 5). The results of Rayleigh's tests indicated that those directions differed from random for the treatment area ($Z = 3.52$, $n = 19$, $P = 0.028$; Fig. 5) and the control area ($Z = 8.46$, $n = 26$, $P < 0.001$). Moreover, the directions were different between the treatment and the control areas (Mardia-Watson-Wheeler test: $W = 10.23$, $n_1 = 19$, $n_2 = 26$; $P = 0.006$). The average minor range of scale dependence was 3,259 m (SE = 249.7, range = 977–5,813 m) for the treatment area and 3,504 m (SE = 264.2, range = 977–5,955) for the control area (Fig. 4). On both areas, the major range of scale dependence was near the maximum of the distance classes we examined (5,580–5,955 m; Fig. 4). Some variograms were best fitted to a hole effect model, indicating that their semivariance

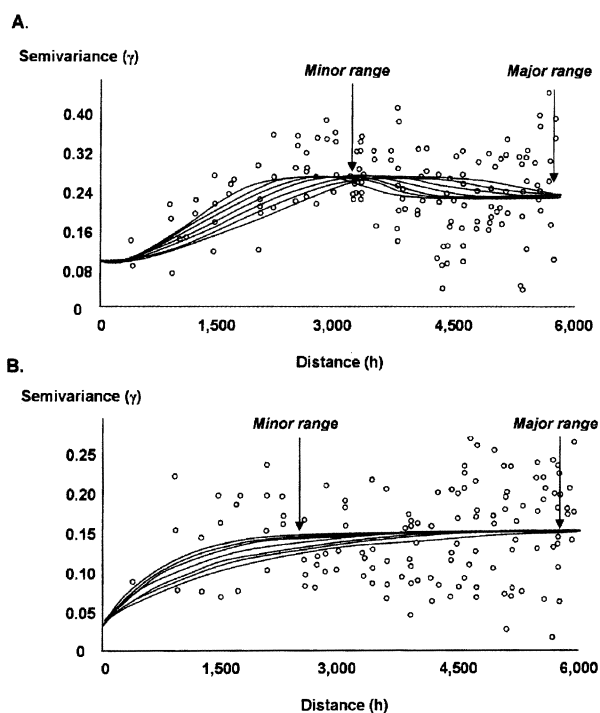


Fig. 4. Example variograms for the G10B-157 allele on the treatment area (A) and the control area (B), Washington, County, North Carolina, USA, from a fall 2000 study. Distance (h) units are in meters. The circles represent empirical data. Individual lines represent the fitted variogram model at a different direction. Minor and major ranges reflect the directions at which the shortest and longest distances of spatial continuity occur, respectively.

values decreased after reaching a sill (Table 1; Fig. 4A), which may indicate a repetitive spatial pattern. The 2 datasets had relative nugget effects ranging from 23.7% to 87.9% (Table 1).

Discussion

Microsatellite DNA is inherently polymorphic (Paetkau and Strobeck 1998), with many different alleles occurring at a given locus and often with similar frequencies, a characteristic that could dilute any signal present in allele occurrence patterns. Consequently, if few alleles are chosen for analysis, the power of statistical techniques to detect patterns could be limited. Therefore, we included the most important alleles by choosing those that occurred at many sites. We observed distinct spatial dependency, consistent directions, and consistent scales of autocorrelation among allele occur-

rence patterns within the 2 study areas, suggesting that our findings may be biologically meaningful.

Spatial directionality in allele occurrence patterns may be caused by directionality in migration (Epperson 2003), or, for black bears, dispersal routes. However, because of the relatively small sizes of the study areas, we considered whether the directional patterns simply may be a result of bear movements during the study period. Therefore, we examined the distribution of sites that were visited by the same bears (visits of multiple sites could have contributed to the directions we found). However, the vast majority of multiple visits occurred among neighboring sites, reflecting local movement patterns within home ranges. Of 20 bears with visits to multiple sites on the treatment area, only 2 (3.7% of all sampled bears) had multiple locations that coincided with the allele pattern directions we observed. On the control area, that proportion was 6.5% ($n = 6$). Thus, we suggest that bear movements during the 7-week sampling period were not responsible for the allele patterns we observed.

Although several of the variograms had large relative nugget effects (Table 1), indicating that limited spatial variability was modeled, the patterns we observed were consistent. Variograms increased to a sill in most directions; however, for the direction of maximum spatial continuity (major range), many variograms likely reached the sill at ranges extending beyond the maximum distance we examined. Thus, except for those directions, alleles generally were not spatially correlated beyond a certain distance. In genetic theory, this process could be interpreted as isolation-by-distance (Piazza et al. 1981), which occurs when dispersal limitations produce genetic correlations among spatially close animals; these correlations are gradually reduced as distances increase (Rohlf and Schnell 1971, Falconer and Mackay 1996, Epperson 2003). The range distances at the minimum direction of continuity were fairly consistent among alleles (3–4 km), but were substantially less than the major range distances. Hence, the minor range distances likely indicated that dispersal in those directions was limited by something other than distance, such as habitat quality or patch size.

Because of the small scale of the study areas, we hypothesize that the anisotropic patterns we observed were mostly due to short female dispersal distances. Females in both study areas had relatively small home ranges (4.3 km²) and, as has been observed in other areas (Rogers 1987), female offspring seemed to remain close to the home range of their mother. We believe this resulted in local areas where occurrence patterns were similar; on both study areas we observed overlap among

home ranges of several radiocollared females whose genotypes were closely related, indicating female philopatry. These observations may explain the different directions of allele patterns between the control and the treatment area. The treatment area consisted of 2 large areas of forested tracts separated by a large expanse of agricultural areas. The 2 forest tracts were oriented in east–west and southeast–northwest directions (Fig. 1) and the directional patterns coincided with those. The directionality of allele patterns on the control area was almost perpendicular to that of the treatment area. We speculate that the north–south orientation of the best habitat areas on the control area (i.e., areas with agriculture–forest edges with nearby woody wetlands and mixed forests stands; Kindall 2004; Fig. 1) may have contributed to this direction. Because of the configuration of the best habitat areas (Fig. 1), female offspring would be more likely to establish home ranges to the southeast or northwest of their mother on the treatment area and to the north and south on the control area. This interpretation is supported by pair-wise genetic distances: bears within the northern tract on the treatment area were more closely related to each other than they were to bears in the southern tract, and vice versa. In contrast, genetic distances between bears in the northern and southern portions of the control area did not show clear structure (Thompson 2003).

Additionally, common paternity may have contributed to the patterns we observed. Dominant male bears typically establish large home ranges that overlap with those of multiple females, thereby optimizing breeding opportunities. Such a breeding strategy could result in discrete lenses (local areas) where allele occurrence patterns are distinctly similar. Observations from the treatment area may support this notion; 6 alleles on treatment area were best fitted to a hole-effect model, indicating that allele patterns were repeated after some distance (Isaaks and Srivastava 1989, Rossi et al. 1992). Males mating in both the north and south forest tracts of the treatment area (Fig. 1) could have contributed to such a pattern. Indeed, telemetry observations of several adult males indicate they were active in both forest tracts during the mating season.

Epperson (2003) suggested that allele frequency patterns tend to follow environmental factors, so we

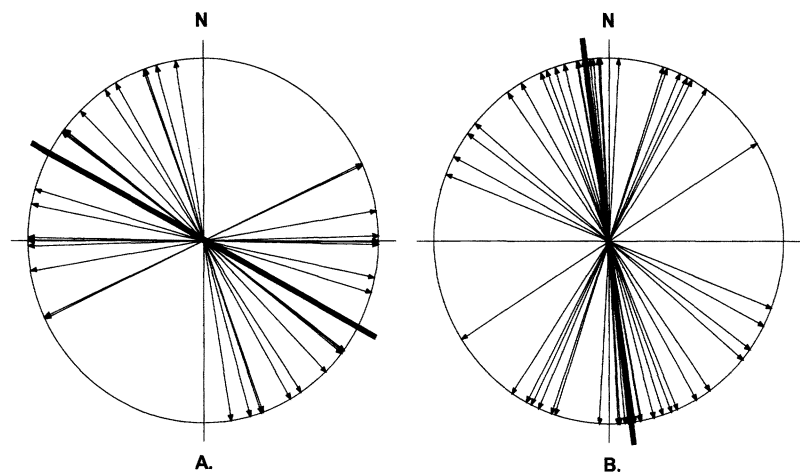


Fig. 5. Directions of maximum continuity for black bear alleles on the treatment area (A) and the control area (B) in Washington County, North Carolina, USA, from a fall 2000 study. The bold line represents the mean direction of maximum continuity over all alleles.

also considered the broader landscape context to interpret our observations. Both study areas were mostly bordered by agricultural fields with the exception of several forested linkages with core black bear areas (North Carolina Wildlife Resources Commission, unpublished data) to the north-northwest of the control area and east to southeast of the treatment area (Fig. 1). The mean direction of allele frequencies on the control area was 172.3° , which aligns with the direction of core habitat areas and habitat linkages north-northwest of the control area. The direction of maximum continuity of the alleles on the treatment area also may have been influenced by processes originating outside the study area. The treatment area experienced relatively high harvest pressure, suggesting the possible existence of a source–sink dynamic between the 2 study areas (Thompson 2003). Indeed, black bear density on the control area (1.78 bears/ km^2) was greater than on the treatment area (1.20 bears/ km^2 ; Thompson 2003). Areas classified as core black bear habitat (M.D. Jones, North Carolina Wildlife Resources Commission, Raleigh, North Carolina, USA, unpublished data) to the east and southeast (e.g., Pocosin Lakes National Wildlife Refuge) of the treatment area also may serve as source areas (Fig. 1). Thus, the layout of the landscape may have contributed to the southeast–northwest allele occurrence patterns that we observed on the treatment area.

Research implications

One of the objectives of our analysis was to determine whether allele occurrence patterns exhibited scale-

dependency within the relatively small study areas. Microsatellites tend to be polymorphic, so we did not anticipate finding strong patterns of scale dependence at the small spatial scale. However, our results suggest that microsatellite DNA sampling can be effectively used to examine small-scale spatial autocorrelation. Directional variograms may provide a powerful tool to assess whether highways or other potential sources of landscape fragmentation affect gene flow patterns among bears.

Our results indicated distinct and consistent patterns of scale-dependence among the alleles we examined. Furthermore, the directions we observed in the 2 study areas were different and seemed to conform to black bear ecology, different habitat patterns within the study areas, and habitat linkages with adjacent areas. We suggest that the use of geostatistical measures, in combination with microsatellite DNA data, provides a potentially promising approach to examine how anthropogenic changes in the landscape, such as highways, affect bear ecology.

As with any statistical technique, users should be aware of the potential pitfalls of geostatistical measures. For example, the assumption of local stationarity should be tested. Also, many GIS programs provide geostatistical capabilities with a wide array of options, and we caution users not to use such features indiscriminately; biological considerations should be used to develop and examine variograms.

Acknowledgments

The North Carolina Department of Transportation provided funding for this project through the North Carolina Wildlife Resources Commission. M.D. Jones, D. Cox, D. Evans, J. Folta, T.L. Riddick, C. Smith, and W. Wescott provided valuable logistical assistance during the study. We thank J.L. Kindall and the many technicians for help with data collection. We are very grateful to S. Julian for conducting the microsatellite analysis. B. Ralston provided much insight and guidance on the interpretation of the geostatistical results. Finally, we thank J.E. Clark for reviewing an earlier draft of the manuscript.

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Received: 5 March 2004

Accepted: 21 September 2004

Associate Editor: G. Shields