Evaluation of Genetic Structure and Population Estimate In New Jersey Black Bears (*Ursus americanus*)

Jane E. Huffman, MS, PhD, MPH Project Director Terri Ombrello, BS Drew Zellner, MS Northeast Wildlife DNA Laboratory Applied DNA Sciences Fast Stroudsburg University

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INTRODUCTION

History of Black Bears In New Jersey

Black bears are native to New Jersey and are the largest land mammal in NJ. They are an integral part of the state's natural heritage and a vital component of healthy ecosystems. Since the 1980s the New Jersey black bear population has been increasing and expanding its range both southward and eastward from the forested areas of northwestern New Jersey. Within the most densely populated state in the nation, black bears are thriving and there are now confirmed bear sightings in all 21 of New Jersey's counties.

The growing population has primarily been due to increased black bear habitat as agriculture lands reverted to mature forests, protection afforded by game animal status, and increasing bear populations in Pennsylvania and New York which pushed animals into the state. A New Jersey black bear management plan concluded in 1997 that habitat fragmentation and human population growth *has made it untenable to continue maintaining a black bear population at its present level (450 to 550).* They suggested it be reduced and maintained at 272 to 340. In the first hunting season in 30 years, in 2003, more than 300 bears were killed and the population in the *hunt area* was estimated to be 1,777 (Brown, 2009).

To better manage the growing black bear population and respond to black bear complaints, the Division formed the Black Bear Project Team in 2001. The Division and Black Bear Project Team use a comprehensive approach to managing black bears and fostering coexistence between people and bears.

Expansion of Black Bear Populations into NJ

In New York State the Catskill region in the Southern Bear Range contains a population of 1,500 to 2,000 black bears.

The Southern Black Bear Range is made up of much of the Allegany Plateau, the Catskill Mountains and the Taconic Highlands. This range extends from the southwest corner of the state, easterly along the Pennsylvania border, through south central New York in the Binghamton area, into the Catskill Mountains and to southern Orange and Rockland Counties at the New Jersey border. Although the actual numbers are not known, it is clear that a portion of the bear population in this range is an expansion of Pennsylvania's bear population. There is considerable evidence of bears freely passing from one state to another. Some significant parcels of forested property stretch from the New Jersey border and follow northeast along Interstate 87 to the Hudson River. This is a primary corridor for bear immigration from and into New Jersey.

The immigration and emigration of bears to and from adjoining states is an important consideration for black bear management. Bears immigrate from New York and Pennsylvania into New Jersey

Biologists have monitored black bear (*Ursus americanus*) populations using annual Lincoln-Petersen (L-P) estimates of population size derived from the fraction of marked

bears recovered in the harvest (Diefenbach et al. 2004).

In 2001 the Pennsylvania black bear population was estimated at 12,000 (Ternent 2005). New Jersey projected that as of 2003 there were 3,278 adult bears residing in the state (Carr and Burguess 2002).

Currently, only fourteen states use DNA analysis to complete population estimates for their black bear populations along with mark – recapture techniques (Table 1).

Table 1: List of Methods used to calculate population estimates and to conduct other be	ear
research for each U.S. state.	

State	Methods of Population Estimation	Article		
AL	Mark/recapture (Lincoln-Peterson), future			
AK	biomarking, DNA	(Peacock 2002)		
AZ	not found	not found		
AR	hunter harvested estimations	(Estridge and Henderdon 2006)		
	cementum annuli analysis, hunter surveys,			
CA	& harvest data	(CA Dept. Fish and Game 1998)		
CO	GPS and Tracking	(Baruch-Mordo, S. 2007)		
ст	Trap-mark-release	Protection		
DE	believed to be extirpated	n/a		
E 1	habitat assessment, nuisance complaints,	(Eason T.H. 2003)		
	DNA conture (release and redia collere	(Easoli, T.H. 2003)		
GA	DNA capture/release and radio collars	South Carolina Dept. of Natural Resources		
HI	no bears found in Hawaii	n/a		
ID	harvest data	Idaho Fish and Game		
IL	believed to be extirpated	n/a		
IN	believed to be extirpated	n/a		
IA	believed to be extirpated	n/a		
KS	believed to be extirpated	Kansas Department of Wildlife and Parks		
кү	live trapping, mark/recapture and "hair snare"	Kentucky Department of Fish and Wildlife Resources		
LA	DNA Analysis	(Cook, R.L. 2005)		
ME	Bio-markers, mark/recapture, hunter harvest information	Maine Department of Inland Fisheries and Wildlife		
MD	DNA Studies (hair), mark/recapture	(Spiker and Bittner 2004)		
MA	not found	n/a		
мі	DNA, capture-mark-recapture (hair), harvest bears, annuli analysis	(Dreher, B.P. et al. 2007)		
MN	Hunter harvested tetracycline tooth analysis	Minnesota Department of Natural s Resources		
MS	Research Trapping and sighting reports	(Young, B. 2006)		
MO	not found	not found		
мт	DNA analysis (mostly grizzly work)	Grizzly Bear and Black Bear Ecology (NMRSC Research)		

NE	believed to be extirpated	n/a
	GPS, GIS, mark/recapture, physical data	
NV	recorded	(Lansford, K.C. 2007)
		New Hampshire Fish and Game
NH	hunter surveys	Department
NJ	mark/recapture future: DNA analysis	(Carr and Burguess 2002)
NM	capture, GIS, and Annuli analysis	(Costello, C. et. al 2001)

State	Methods of Population Estimation and Research	Article	
NY	bear harvests, non-hunting mortality, and nuisance complaints	(Bureau of Wildlife 2007)	
NC	hunter and automobile killed bear information and teeth collection	North Carolina Wildlife Resources Commission	
ND	believed to be extirpated	n/a	
ОН	not found	n/a	
ОК	DNA Analysis, mark/recapture, radio collars	(Cook, R.L. 2005)	
OR	not found	n/a	
PA	harvest data, mark-recapture	(Ternent 2006)	
RI	not found	n/a	
SC	DNA mark/recapture	South Carolina Dept. of Natural Resources	
SD	DNA mark/recapture	South Carolina Dept. of Natural Resources	
TN	DNA mark/recapture	South Carolina Dept. of Natural Resources	
тх	Collecting hair and tissue samples for future DNA analysis	(Cook, R.L. 2005)	
UT	Current: harvest; Future: cementum annuli analysis and track transects	(Black 2000)	
VT	radio collars; hunter harvest information	Vermont Fish and Wildlife Department	
VA	harvest, nuisance reports, and structure	(Virginia Department of Game and Inland Fisheries 2002)	
WA	current project- using DNA analysis	(Koehler, G. 2001)	
wv	mark/recapture and radio transmitters	West Virginia Department of Natural Resources	
WI	harvest information, and bait station visitation calculations	(Rolley, R.E. and M.P. Woodford 2007)	
WY	Current:hunter harvest information, Future research: DNA mark/recapture	(Trophy Game Section (WY) 2007)	

Genetic Fingerprinting to Estimate Bear Population Numbers

The recent development of using genetic fingerprinting to estimate size of wildlife populations has provided bear biologists with a more efficient tool to estimate bear numbers than traditional mark-recapture techniques. The technique was pioneered with grizzly bears and black bears in British Columbia (Woods et al. 1999).

Genetic analyses offer important insights into the population structure and connectivity among wide-ranging animals such as bears. Genetic data provide information about historic and current levels of gene flow among populations, as well as information about genetic diversity and fitness, relatedness, and movement patterns within populations (Queller *et al.* 1993; Paetkau *et al.* 1998; Schenk *et al.* 1998; Woods *et al.* 1999). Genetic information is essential to estimating population viability and evaluating possible management decisions

Population genetics provides a finer scale perspective, requiring genetic markers of finer resolution, or greater variability, than phylogeography. Microsatellites have been widespread in wildlife population genetics research (Snow & Parker 1998). The distribution of allele frequencies can be informative regarding definition of genetic populations, the geographic ranges of populations, changes in population size and detection of recent population bottlenecks (Deyoung & Honeycutt 2005). Population assignment tests use allele frequencies to detect population structure and determine the natal population of an individual (Paetkau *et al.* 1995).

Measuring levels of genetic variation is an important aspect of conservation genetics. The informativeness of such measurements is related to the variability of the genetic markers used; a particular concern in species, such as bears, which are characterized by low levels of genetic variation resulting from low population densities and small effective population size.

Population Genetics

The detection and location of genetic discontinuities is important to managing natural wildlife populations, and is an important first step in more complex analyses of the processes affecting genetic patterns (Manel *et al.*, 2003; Scribner *et al.*, 2005). Genetic patterns are characterized by the distribution of alleles within populations or across space (Epperson, 2003; Manel *et al.*, 2003). These patterns may depart from randomness, panmxia, due to various forms of isolation: geographic distance (Wright, 1943), dispersal barriers (Manni *et al.*, 2004), landscape resistance (Cushman *et al.*, 2006), behavior factors (Deyoung, Honeycutt, 2005), or temporal factors (Vandewoestijne, Baguette, 1999). Identifying genetic patterns and correlating them with influential landscape features can provide ecological information, reveal cryptic population structure and secondary contact between previously isolated populations (Manel *et al.*, 2003). There are many applied examples. Genetic structure has been used to infer metapopulation dynamics (Manier, Arnold, 2005). Differences in spatial genetic structure related to landscape patches has been used to determine effects of anthropogenic landscape change on dispersal and gene flow (Banks *et al.*, 2005).

Objectives

We used genetic data to estimate the effective population size of black bears in New Jersey and to evaluate population structure, and landscape-genetic relationships.

Specific objectives and research questions addressed included:

Objective 1: Describe population genetic structure and diversity of black bears in NJ. Determine effective population size .

Objective 2: Relate population genetic patterns to the landscape of New Jersey.

Is there evidence for increasing genetic isolation with geographic distance?

MATERIALS AND METHODS

Sample Collection and DNA Extraction

All samples of black bear DNA were provided by the New Jersey Division of Fish and Wildlife (NJDFW). Tissue samples were collected from hunter harvested bears and research trapped bears. The tissue samples consisted of ear punches. Bears were sampled from the 4 bear management zones in NJ (Figure 1).



Figure 1 Bear management zones in NJ.

Samples were preserved in 95% ethanol and stored at -80°C. Three hundred samples were chosen to represent the greatest geographical distribution. A 2-3mm portion of the ear punch was used for extraction of DNA. DNA was extracted using the MO BIO Tissue Extraction Kit (MO BIO Laboratories, Inc. Carlsbad, CA.), using standard protocols. DNA was quantified using a Quantech fluorometer (Barnstead International, Dubuque IA.) and was amplified using PCR (Polymerase Chain Reaction).

PCR was performed on a 20uL solution consisting of 10uL of Promega Master Mix (Promega, Madison, WI.), 2.5uL of both forward and reverse primers, 50ng of DNA, and distilled water. The primers used for PCR amplification were the following six microsatellite loci (Table 2): G10B, G10X, G10M, G1D, G10C, G1A, G10J, G10L, G10P, Mu50, G10H, G10O, Mu59, and G10U (Paetkau and Strobeck 1994, Paetkau and Strobeck 1995, Paetkau et al., 1998, and Taberlet et al., 1997). Amplification was performed on a Thermo Scientific Thermal Cycler (Thermo Fisher Scientific Inc., Waltham, MA.) using the following times and temperatures; 5 minutes at 95°C, then 35 cycles of 1 minute at 95°C, 1 minute at 57°C, 58°C, or 60°C depending on the microsatellite (Table 2), and 1 minute at 72°C. The extension reaction was set at 72°C for 5 minutes. The PCR products were then kept at 4°C until used within 1 to 3 days.

PCR Product Analysis

Microsatellite analysis was completed on the Applied Biosystems Genetic Analyzer 3130 using the Genemapper 3.7 program (Applied Biosystems, Foster City, CA.). The primers used in PCR were dyed (Table 3) so that certain markers could be multiplexed. Four pools were made using particular quantities of the PCR products shown in Table 2. The pooled samples were then prepped for the Applied Biosystems Genetic Analyzer 3130 (AB3130) by taking 9uL of High Dye, 0.25uL of Liz Standard, and 0.5uL of pooled sample and running PCR using standard protocol.

Pool 1	Pool 2	Pool 3	Pool 4			
G10B: 2.5uL	G1A: 2.5uL	Mu50: 5uL	G10X: 10uL			
G10M: 5uL	G10L: 10uL	G10H: 10uL	G10J: 10uL			
G1D: 5uL	G10P: 10uL	G10O: 10uL	Mu59: 10uL			
G10C: 5uL		G10U: 10uL				

Table 2: Pooling Quantities for the Applied Biosystems Genetic Analyzer 3130 which were determined by this study.

Statistical analysis of the microsatellite data was performed using GenAlEx v6 (Peakall and Smouse 2001), and Microsatellite Toolkit v3.1 (Park 2001). This provided the expected heterozygosity, polymorphism information content, allelic frequency, and probability of identity to be calculated for each of the markers. These calculations determined the rank and value of the individual markers that would be best for future population estimate and genetic identification projects.

Analysis

Genotyping Error Estimation

Accurately identifying individual's genotypes is critical for population estimation because genotyping errors can bias population estimates (Waits and Leberg 2000; Creel et al. 2003). To minimize errors, we carefully scrutinized the data as Paetkau (2003) suggested, reexamining, and reamplifying where necessary, samples that differed by 1 or 2 alleles to verify the difference was real.

RESULTS

Objective 1: Describe population genetic structure and diversity of black bears in NJ. Determine the effective population size of black bears in New Jersey.

The effective population size is the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration.

In 2001 a mark recapture population estimate was calculated to be 1777 black bears (Carr and Burguess 2003). The effective population size was calculated for bears in management zones 1 and 3. The sample size from zones 2 and 4 were small. The animals from these zones were used for the calculation of allelic frequencies. These estimates have always been linked with a mark-recapture estimate (Lincoln-Peterson Index). Figure 1 shows the effective population size for New Jersey black bears from 2001 (1), 2003 (2), 2005 (3), and 2009 (4). The decrease in effective population size for MeW Jersey black bears from 46.7 to 35.3 may be the result of the bear hunt conducted in 2003.



Figure 1 Effective population size for NJ black bears from 2001 (1), 2003 (2), 2005 (3) and 2009 (4).

Table 1 shows the effective population size for management zones 1 and 3, with the sample size utilized for the genotyping.

Year	Management	Effective	Population	Study	Sample
	Zone	Population	Estimate M/R		Size -
		Size			genotyping
2001	1&3	22.6	1777	(Carr and	36
		95%CI(16.1-		Burguess	
		33.8)		2003)	
2003	1&3	46.7	(333 animals		69
		95%CI(33.4-	harvested)		
		70.1)			
2005	1&3	35.3	(298 animals		175
		95%CI(29.4-	harvested)		
		42.4)			
2009	1&3	69.1 95%	3438	This study	345
		CI(68.5-81.9)			

Table 1. Effective population size for management zones 1 and 3. Sample size for genotyping is indicated along with mark recapture data.

The data indicates a steady increase in the effective population size of black bears. NJ Fish & Wildlife conducted a black bear hunt in 2003 and 2005. A total of 631 animals were harvested during the two hunts. Assuming a population estimate of 1146 animals after the two hunts and a 3 fold increase in EPS from 2001 to 2009 the number of bears may be assumed to be 3438.

What is the optimal sampling design for future population monitoring? The optimal sampling design would be to include DNA analysis along with Mark – Recapture techniques. These techniques should include both research trapping for tagging animals, hair snaring, and a yearly harvest to obtain genetic and mark recapture information.

Obtaining accurate population estimates for black bear (*Ursus americanus*) is important when implementing adaptive management principles in harvest strategies. Most state and provincial black bear population estimates are based on known harvest rates or extrapolation from small mark-recapture studies (Garshelis and Hristienko 2006).

Objective 2: Relate population genetic patterns to the landscape of New Jersey.

ALLELE	No.	Zone 1 (97)	Zone 2(16)	Zone 3(248)	Zone 4 (14)
G10L					
	138	0.351	0.375	0.315	0.250

143	0.160	0.094	0.268	0.214
148	0.149	0.313	0.218	0.071
152	0.021	0.031	0.008	0.000
157	0.242	0.125	0.161	0.321
161	0.077	0.063	0.030	0.143

Mu50

113	0.010	0.000	0.010	0.000
119	0.041	0.000	0.016	0.000
123	0.526	0.281	0.411	0.643
127	0.031	0.094	0.016	0.036
135	0.041	0.063	0.024	0.000
143	0.345	0.563	0.520	0.321
150	0.005	0.000	0.000	0.000
153	0.000	0.000	0.002	0.000

G10P

166	0.000	0.000	0.002	0.000
170	0.129	0.125	0.063	0.071
174	0.031	0.000	0.044	0.000
178	0.165	0.063	0.163	0.321
182	0.629	0.750	0.688	0.393
186	0.046	0.063	0.038	0.214
188	0.000	0.000	0.002	0.000

G10H

143	0.015	0.000	0.002	0.000
214	0.005	0.000	0.000	0.000
223	0.052	0.000	0.077	0.036
244	0.237	0.094	0.159	0.321
248	0.479	0.688	0.546	0.429
256	0.155	0.094	0.155	0.143
264	0.057	0.125	0.058	0.071
273	0.000	0.000	0.004	0.000

G100

158	0.165	0.125	0.159	0.143
178	0.000	0.000	0.000	0.036
190	0.062	0.031	0.024	0.036
205	0.356	0.438	0.244	0.214
209	0.320	0.219	0.258	0.286
221	0.093	0.188	0.313	0.286
250	0.000	0.000	0.002	0.000
258	0.005	0.000	0.000	0.000

86	0.675	0.563	0.706	0.607
90	0.031	0.000	0.012	0.036
101	0.211	0.375	0.224	0.286
105	0.077	0.063	0.056	0.071
112	0.000	0.000	0.002	0.000
120	0.005	0.000	0.000	0.000

There is no evidence for increasing genetic isolation with geographic distance in New Jersey. The animals are not restricted in their movements.

APPENDIX

Table A comparison of alleles located for the thirteen microsatellite markers when looking at New Jersey and Pennsylvania black bear populations. Alleles are highlighted in blue (alleles that are unique to Pennsylvania black bears) and yellow (alleles that are unique to New Jersey black bears) to better visualize differences between the two states. Allelic frequencies are listed to show how often the alleles appear in the population. The number of unique alleles, and how often they appear throughout the population, is calculated at the bottom of the tables.

Loci	PA Alleles	AF	NJ Alleles	AF
G10J	~		80	0.01
	~		82	0.01
	84	0.17	84	0.29
	86	0.38	86	0.35
	88	0.01	~	
	90	0.04	~	
	98	0.07	98	0.09
	100	0.09	100	0.06
	102	0.06	102	0.02
	104	0.10	104	0.12
	106	0.03	106	0.05
	108	0.01	~	
	112	0.03	~	
# of Unique Alleles:		4.00		2.00
% of Pop. Found in:		9%		2%

G10J

Loci	PA Alleles	AF	NJ Alleles	AF
G1D	175	0.04	~	
	179	0.46	179	0.57
	181	0.09	181	0.06
	183	0.01	~	
	185	0.06	185	0.01
	187	0.20	187	0.27
	189	0.09	189	0.06
	191	0.05	191	0.03
# of Unique Alleles:		2.00		0.00
% of Pop. Found in:		5%		0%

Loci	PA Alleles	AF	NJ Alleles	AF
G1A	183	0.06	~	
	185	0.05	185	0.05
	187	0.29	187	0.53
	189	0.16	189	0.08
	191	0.14	~	
	193	0.3	193	0.34
# of Unique Alleles:		2.00		0.00
% of Pop. Found in:		20%		0%

Loci	PA Alleles	AF	NJ Alleles	AF
MU59	~		231	0.01
	233	0.09	~	
	235	0.03	~	
	~		237	0.02
	~		239	0.12
	241	0.13	241	0.14
	243	0.44	243	0.43
	245	0.03	245	0.14
	247	0.10	247	0.14
	249	0.17	~	
	251	0.01	~	
	~		257	0.02
# of Unique Alleles:		4.00		4.00
% of Pop. Found in:		30%		17%

Loci	PA Alleles	AF	NJ Alleles	AF
G10B	156	0.02	156	0.02
	158	0.31	158	0.36
	160	0.02	160	0.02
	162	0.29	162	0.43
	164	0.11	~	
	166	0.26	166	0.12
	~		168	0.05
# of Unique Alleles:		1.00		1.00
% of Pop. Found in:		11%		5%

Loci	PA Alleles	AF	NJ Alleles	AF
MU50	111	0.04	~	
	117	0.07	2	
	119	0.19	119	0.31
	121	0.16	121	0.10
	123	0.19	123	0.14
	125	0.02	2	
	131	0.04	~	
	133	0.06	133	0.01
	137	0.03	137	0.03
	139	0.12	139	0.26
	141	0.03	141	0.10
	143	0.05	143	0.05
# of Unique Alleles:		4.00		0.00
% of Pop. Found in:		17%		0%

Loci	PA Alleles	AF	NJ Alleles	AF
G10L	135	0.06	135	0.09
	137	0.14	137	0.12
	139	0.12	139	0.06
	143	0.01	143	0.02
	145	0.08	145	0.13
	~		147	0.32
	149	0.02	ł	
	151	0.06	~	
	153	0.06	153	0.02
	155	0.19	155	0.24
	157	0.07	2	
	159	0.12	ł	
	161	0.02	ł	
	163	0.03	2	
# of Unique Alleles:		6.00		1.00
% of Pop. Found in:		32%		32%

Loci	PA Alleles	AF	NJ Alleles	AF
G10H	~		216	0.01
	220	0.03	220	0.03
	222	0.01	222	0.01
	234	0.01	2	
	238	0.01	2	
	240	0.02	240	0.02
	242	0.09	242	0.25
	244	0.09	244	0.04
	246	0.21	246	0.32
	248	0.09	248	0.19
	250	0.11	2	
	252	0.03	252	0.04

	254	0.07	254	0.03
	256	0.08	~	
	258	0.02	258	0.02
	260	0.04	260	0.03
	262	0.02	262	0.01
	264	0.05	~	
	269	0.01		
	270	0.02	~	
# of Unique Alleles:		7.00		1.00
% of Pop. Found in:		29%		1%

Loci	PA Alleles	AF	NJ Alleles	AF
G10P	166	0.01	~	
	168	0.01	168	0.01
	170	0.04	170	0.06
	172	0.05	~	
	174	0.18	174	0.12
	176	0.04	~	
	178	0.35	178	0.32
	180	0.11	180	0.19
	182	0.16	182	0.21
	184	0.04	184	0.08
# of Unique Alleles:		3.00		0.00
% of Pop. Found in:		10%		0%

Loci	PA Alleles	AF	NJ Alleles	AF
G10O	156	0.14	156	0.05
	158	0.05	158	0.03
	190	0.03	~	
	~		200	0.01
	204	0.17	204	0.24
	206	0.16	206	0.08
	208	0.27	208	0.29
	212	0.01	~	
	214	0.01	~	
	218	0.01	~	
	220	0.06	220	0.29
	222	0.09	~	
# of Unique Alleles:		5.00		1.00
% of Pop. Found in:		15%		1%

Loci	PA Alleles	AF	NJ Alleles	AF
G10M	194	0.06	194	0.04
	196	0.03	196	0.14
	198	0.08	~	
	200	0.64	200	0.61
	202	0.14	202	0.12
	204	0.03	204	0.08

	206	0.02	~	
# of Unique Alleles:		2.00		0.00
% of Pop. Found in:		10%		0%

Loci	PA Alleles	AF	NJ Alleles	AF
G10C	108	0.09	108	0.18
	~		110	0.01
	112	0.01	2	
	114	0.23	114	0.30
	116	0.22	116	0.37
	118	0.37	118	0.14
	120	0.08	2	
# of Unique Alleles:		2.00		1.00
% of Pop. Found in:		9%		1%

Loci	PA Alleles	AF	NJ Alleles	AF
G10X	~		185	0.08
	~		187	0.04
	189	0.12	189	0.04
	191	0.09	191	0.04
	193	0.41	193	0.63
	195	0.09	~	
	197	0.03	~	
	199	0.03	~	
	201	0.06	~	
	205	0.09	~	
	207	0.03	~	
	209	0.06	209	0.17
# of Unique Alleles:		6.00		2.00
% of Pop. Found in:		33%		12%

Examining Two Possible New Jersey Black Bear Populations

There has been some speculation over the idea that the highways of New Jersey form boundaries that split the bear population in half. Instead of simply having New Jersey bears, the bear population is instead categorized as two populations known as the New Jersey/New York bear population and the New Jersey/Pennsylvania population. The NJ/NY population is thought to be isolated from the NJ/PA population by Route 80 and Route 94.

To test this theory, the New Jersey bear samples were split into two separate populations defined by the stated highways, and any other man-made or natural obstacle that would dissuade the black bear from migrating into the opposite territory. The two populations consisted of 26 samples that made up NJ/NY population and 24 samples that made up the NJ/PA population. The alleles from the two groups were compared for each microsatellite marker and are shown in Table 1. G10M and G1A showed no alleles to be unique between the two possible populations. The other eleven microsatellite markers however found differences between the NJ/PA, and NJ/NY bears. A total of 14 alleles were found to be unique to the NJ/PA population, and a total of 10 alleles were unique to the NJ/NY population. The true test was to see if the New Jersey bear samples could be distinguished as having come from the NJ/PA or NJ/NY populations. Therefore, the unique alleles were located in the New Jersey samples and out of those 50 samples, 21 or 42% were able to be distinguished as NJ/PA or NJ/NY black bears.

It would require more than 50 samples to adequately prove or disprove this hypothesis. However, with the information provided by the samples, the results seem to disprove the NJ/PA, NJ/NY split. A forty-two percent ability to distinguish between the two possible populations is very low, especially when limited sample numbers were used. It appears more likely that instead of two individual New Jersey populations, there is one population inter-mixed throughout the state. This theory also appears to be backed by the previous comparison between Pennsylvania and New Jersey bear samples, which showed a significant difference between the two state bear populations. If indeed there was a NJ/PA population, then there should not be a significant difference between the NJ/PA and Pennsylvania bear populations which should translate into a lower difference between that this is not the case, and that New Jersey bears are a single population, a NJ/PA and Pennsylvania black bear comparison was done.

Table 1: A comparison of alleles located for the thirteen microsatellite markers when looking at New Jersey/New York and New Jersey/Pennsylvania black bear populations. Alleles are highlighted in blue (alleles that are unique to New Jersey/Pennsylvania black bears) and yellow (alleles that are unique to New Jersey/New York black bears) to better visualize differences between the two possible populations. Allelic frequencies are listed to show how often the alleles appear in the population. The number of unique alleles, and how often they appear throughout the population, is calculated at the bottom of the tables.

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
G10B	156	0.02	156	0.02
	158	0.23	158	0.48
	160	0.04	~	
	162	0.56	162	0.31
	166	0.06	166	0.17
	168	0.08	168	0.02
# of Unique Alleles:		1.00		0.00
% of Pop. Found in:		4%		0%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
G10M	194.00	0.07	194.00	0.02
	196.00	0.09	196.00	0.19
	200.00	0.59	200.00	0.63
	202.00	0.13	202.00	0.12
	204.00	0.13	204.00	0.04
# of Unique Alleles:		0.00		0.00
% of Pop. Found in:		0%		0%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
G10J	~		80	0.02
	82	0.02	~	
	84	0.39	84	0.19
	86	0.24	86	0.44
	98	0.04	98	0.13
	100	0.04	100	0.08
	102	0.02	102	0.02
	104	0.20	104	0.06
	106	0.04	106	0.06
# of Unique Alleles:		1.00		1.00
% of Pop. Found in:		2%		2%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
G1D	179	0.50	179	0.63
	181	0.06	181	0.06
	~		185	0.02
	187	0.31	187	0.23
	189	0.08	189	0.04
	191	0.04	191	0.02
# of Unique Alleles:		0.00		1.00
% of Pop. Found in:		0%		2%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
G10C	108	0.15	108	0.21
	110	0.02	~	
	114	0.26	114	0.33
	116	0.39	116	0.35
	118	0.17	118	0.12
# of Unique Alleles:		1.00		0.00
% of Pop. Found in:		2%		0%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
G10X	~		185	0.14
	187	0.10	~	
	189	0.10	~	
			191	0.07
	193	0.70	193	0.57
	209	0.10	209	0.21
# of Unique Alleles:		2.00		2.00
% of Pop. Found in:		20%		21%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
G1A	185	0.06	185	0.04
	187	0.58	187	0.48
	189	0.04	189	0.12

	193	0.31	193	0.37
# of Unique Alleles:		0.00		0.00
% of Pop. Found in:		0%		0%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
G10L	135	0.02	135	0.15
	137	0.13	137	0.12
	139	0.10	139	0.02
	143	0.02	143	0.02
	145	0.15	145	0.12
	147	0.25	147	0.38
	153	0.04	~	
	155	0.29	155	0.19
# of Unique Alleles:		1.00		0.00
% of Pop. Found in:		4%		0%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
G10P	168	0.02	2	
	170	0.11	170	0.02
	174	0.13	174	0.12
	178	0.33	178	0.31
	180	0.22	180	0.17
	182	0.07	182	0.35
	184	0.13	184	0.04
# of Unique Alleles:		1.00		0.00
% of Pop. Found in:		2%		0%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
MU59	231	0.02	2	
	~		237	0.04
	239	0.20	239	0.04
	241	0.14	241	0.13
	243	0.39	243	0.46
	245	0.11	245	0.15
	247	0.09	247	0.17
	257	0.05	ł	
# of Unique Alleles:		2.00		1.00
% of Pop. Found in:		7%		4%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
MU50	119	0.39	119	0.23
	121	0.09	121	0.12
	123	0.20	123	0.10
	~		133	0.02
	137	0.07	~	
	139	0.13	139	0.37

	141	0.07	141	0.13
	143	0.07	143	0.04
# of Unique Alleles:		1.00		1.00
% of Pop. Found in:		7%		2%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
G10H	~		216	0.02
	~		220	0.06
	222	0.02	222	0.08
	240	0.04	~	
	242	0.27	242	0.23
	244	0.02	244	0.06
	246	0.33	246	0.31
	248	0.19	248	0.19
	252	0.02	252	0.06
	254	0.04	254	0.02
	~		258	0.04
	260	0.06	2	
	~		262	0.02
# of Unique Alleles:		2.00		4.00
% of Pop. Found in:		10%		14%

Loci	NJ/PA Alleles	AF	NJ/NY Alleles	AF
G10O	156	0.08	156	0.04
	158	0.08	~	
	200	0.03	~	
	204	0.28	204	0.21
	206	0.13	206	0.04
	208	0.28	208	0.31
	220	0.15	220	0.40
# of Unique Alleles:		2.00		0.00
% of Pop. Found in:		11%		0%

Table 2 Unique alleles are highlighted and compared for each of the 13 microsatellite markers. The alleles were color coded for the different sample groups; PA (blue), NJ/PA (yellow), NJ/NY (green), PA & NJ/PA (purple), PA & NJ/NY (pink), and NJ/PA & NJ/NY (tan).

Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
G10B	156	0.02	156	0.02	156	0.02
	158	0.31	158	0.23	158	0.48
	160	0.02	160	0.04		
	162	0.29	162	0.56	162	0.31
	164	0.11				
	166	0.26	166	0.06	166	0.17

1		400	0.00	400	0.00
		100	0.06	100	0.02

Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
G10M	194	0.06	194	0.07	194	0.02
	196	0.03	196	0.09	196	0.19
	198	0.08				
	200	0.64	200	0.59	200	0.63
	202	0.14	202	0.13	202	0.12
	204	0.03	204	0.13	204	0.04
	206	0.02				

Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
G10J					80	0.02
			82	0.02		
	84	0.17	84	0.39	84	0.19
	86	0.38	86	0.24	86	0.44
	88	0.01				
	90	0.04				
	98	0.07	98	0.04	98	0.13
	100	0.09	100	0.04	100	0.08
	102	0.06	102	0.02	102	0.02
	104	0.10	104	0.20	104	0.06
	106	0.03	106	0.04	106	0.06
	108	0.01				
	112	0.03				

Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
G1D	175	0.04				
	179	0.46	179	0.50	179	0.63
	181	0.09	181	0.06	181	0.06
	183	0.01				
	185	0.06			185	0.02
	187	0.20	187	0.31	187	0.23
	189	0.09	189	0.08	189	0.04
	191	0.05	191	0.04	191	0.02
Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
G10C	108	0.09	108	0.15	108	0.21
			110	0.02		
	112	0.01				
	114	0.23	114	0.26	114	0.33
	116	0.22	116	0.39	116	0.35
	118	0.37	118	0.17	118	0.12
	120	0.08				

Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
G10X					185	0.14
			187	0.10		
	189	0.12	189	0.10		

191	0.09			191	0.07
193	0.41	193	0.70	193	0.57
195	0.09				
197	0.03				
199	0.03				
201	0.06				
205	0.09				
207	0.03				
209	0.06	209	0.10	209	0.21

Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
G1A	183	0.06				
	185	0.05	185	0.06	185	0.04
	187	0.29	187	0.58	187	0.48
	189	0.16	189	0.04	189	0.12
	191	0.14				
	193	0.30	193	0.31	193	0.37

Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
G10L	135	0.06	135	0.02	135	0.15
	137	0.14	137	0.13	137	0.12
	139	0.12	139	0.10	139	0.02
	143	0.01	143	0.02	143	0.02
	145	0.08	145	0.15	145	0.12
			147	0.25	147	0.38
	149	0.02				
	151	0.06				
	153	0.06	153	0.04		
	155	0.19	155	0.29	155	0.19
	157	0.07				
	159	0.12				
	161	0.02				
	163	0.03				

Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
G10P	166	0.01				
	168	0.01	168	0.02		
	170	0.04	170	0.11	170	0.02
	172	0.05				
	174	0.18	174	0.13	174	0.12
	176	0.04				
	178	0.35	178	0.33	178	0.31
	180	0.11	180	0.22	180	0.17
	182	0.16	182	0.07	182	0.35
	184	0.04	184	0.13	184	0.04

Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
MU59			231	0.02		

233	0.09				
235	0.03				
				237	0.04
		239	0.20	239	0.04
241	0.13	241	0.14	241	0.13
243	0.44	243	0.39	243	0.46
245	0.03	245	0.11	245	0.15
247	0.10	247	0.09	247	0.17
249	0.17				
251	0.01				
		257	0.05		

Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
MU50	111	0.04				
	117	0.07				
	119	0.19	119	0.39	119	0.23
	121	0.16	121	0.09	121	0.12
	123	0.19	123	0.20	123	0.10
	125	0.02				
	131	0.04				
	133	0.06			133	0.02
	137	0.03	137	0.07		
	139	0.12	139	0.13	139	0.37
	141	0.03	141	0.07	141	0.13
	143	0.05	143	0.07	143	0.04

Locus	PA Allele	PA	NJ/PA Allele	NJ/PA	NJ/NY Allele	NJ/NY
G10H					216	0.02
	220	0.03			220	0.06
	222	0.01	222	0.02		
	234	0.01				
	238	0.01				
	240	0.02	240	0.04		
	242	0.09	242	0.27	242	0.23
	244	0.09	244	0.02	244	0.06
	246	0.21	246	0.33	246	0.31
	248	0.09	248	0.19	248	0.19
	250	0.11				
	252	0.03	252	0.02	252	0.06
	254	0.07	254	0.04	254	0.02
	256	0.08				
	258	0.02			258	0.04
	260	0.04	260	0.06		
	262	0.02			262	0.02
	264	0.05				
	269	0.01				
	270	0.01				

Locus	PA Allele	AF	NJ/PA Allele	AF	NJ/NY Allele	AF
G10O	156	0.14	156	0.08	156	0.04
	158	0.05	158	0.08		
	190	0.03				
			200	0.03		
	204	0.17	204	0.28	204	0.21
	206	0.16	206	0.13	206	0.04
	208	0.27	208	0.28	208	0.31
	212	0.01				
	214	0.01				
	218	0.01				
	220	0.06	220	0.15	220	0.40
	222	0.09				

Allelic frequencies for the unique alleles found were analyzed. Because of the low number of individuals being looked at for the NJ/PA and NJ/NY populations, only the alleles that were highly frequent (found in at least 10% of the population) were considered significant. Looking at table 2, there are three microsatellite markers that show unique alleles that are highly significant, G10X, G10L, and Mu59. Although G10X has alleles that meet the criteria, the high frequency is more likely due to the low number of samples that showed alleles for this marker, than for the fact that the alleles are strongly unique. G10L shows the allele 147 to be strongly unique to both NJ/NY and NJ/PA being found in 38% and 25% of the respective populations, and 0% in the Pennsylvania bear samples. Mu59 also shows a unique allele, number 239, to be strongly unique to the NJ/PA population as being present in 20% of the population. Even though it does not appear significantly in the NJ/NY population, it does appear in 4% of it, and is not found at all in the larger Pennsylvania population. This gives more evidence to the belief that New Jersey black bears are likely to be one population that have slight differences from the Pennsylvania population. More samples from Zone 1 should be evaluated.

Highways may cause bears some hesitation upon crossing, but the high number of bear road kills should show that it will not stop a bear entirely. Also, bears may be able to swim across rivers and streams, but it may be possible that the Delaware River combined with route 209, 611, and 32 are what provide a larger barrier between the New Jersey and Pennsylvania populations. The alleles do show differences between New Jersey and Pennsylvania black bear populations, but in order to confirm that the New Jersey population is a single population requires more samples. It is important to compare the New Jersey samples to New York black bear samples as well to provide an overall tri-state bear assessment.

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