The biogeography of Montana black bear genetics
by William Allen Ostheimer

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences
Montana State University

Abstract:
Traditional studies of Island Biogeography focused on the diversity of species found on different island systems. The application of genetic diversity studies to island biogeography is a relatively recent development. Microsatellite DNA allele heterozygosity was used to assay the genetic diversity of black bears living on isolated mountain ranges in Montana, and compare those heterozygosity levels to levels found in contiguous black bear habitat that represented a mainland. Probability of identity was also calculated and the presence of unique alleles in some of the populations helped illuminate possible histories of those populations. Due to very small sample sizes (n=10, 8, 5, 5) a bootstrap approach was used to resample the larger mainland population (n=38) at the smallest sample size. There were no significant differences between the mainland populations and the mountain islands, with the heterozygosity levels of the mountain islands fitting the bootstrapped mainland population with 95 % confidence. Possible histories of black bear occupation of central Montana were discussed.

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William Allen Ostheimer

The thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date April 30 1998

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Traditional studies of Island Biogeography focused on the diversity of species found on different island systems. The application of genetic diversity studies to island biogeography is a relatively recent development. Microsatellite DNA allele heterozygosity was used to assay the genetic diversity of black bears living on isolated mountain ranges in Montana, and compare those heterozygosity levels to levels found in contiguous black bear habitat that represented a mainland. Probability of identity was also calculated and the presence of unique alleles in some of the populations helped illuminate possible histories of those populations. Due to very small sample sizes (n=10, 8, 5, 5) a bootstrap approach was used to resample the larger mainland population (n=38) at the smallest sample size. There were no significant differences between the mainland populations and the mountain islands, with the heterozygosity levels of the mountain islands fitting the bootstrapped mainland population with 95 % confidence. Possible histories of black bear occupation of central Montana were discussed.

INTRODUCTION

In 1967, Robert MacArthur of Princeton University and Edward Wilson of Harvard University published The Theory of Island Biogeography. MacArthur and Wilson's work has profoundly influenced the study of species and their relationship to the spatial structure of their environment (Rosensweig 1995). MacArthur and Wilson reported that the number of species found on an island was dependent upon that island's size and degree of isolation. Islands which were small and isolated had lower species diversity than larger islands occurring closer to a mainland. Species diversity was dependent on the interaction between immigration and extinction. Investigations of true islands (Adler 1992, Patterson 1987, Diamond 1975, Abbott & Black 1978) and habitat islands (Rosensweig 1995, Brown 1971, Thompson 1974, Picton 1979) have widely supported the tenants set forth in 1967.

Similar to the classical island biogeography studies which addressed community diversity on islands and isolated habitats, studies investigating genetic diversity of single species with respect to island biogeography have shown reduced genetic diversity in isolated populations of pool frogs (*Rana temporaria*) in Germany (Reh & Seitz 1990), black bears (*Ursus americanus*) on the island of Newfoundland (Paetkau & Strobeck 1994), and brown bears (*Ursus arctos*) on Kodiak Island (Paetkau & Strobeck, *in press*). The advent of genetic fingerprinting techniques has greatly increased the sensitivity of intraspecies analyses (Hill 1987, Weber & May 1989, Taberlet & Bouvet 1992a,). Genetic studies of black bears have successfully employed multilocus probes (Schenk & Kovacs 1996), mitochondrial sequences (Paetkau & Strobeck 1996), and microsatellites (Paetkau & Strobeck 1994).

Microsatellites are di, tri, tetra or pentanucleotide "words" (ie: GGCCG) repeated five to thirty times and occurring some 30,000 times throughout the genome of vertebrates, they constitute non-coding regions of nDNA and are not under the same correction procedures as coding regions and subsequently are subject to higher rates of mutation (Jeffreys 1985). Due to their high rate of mutation microsatellites exist in multiple alleles and prove to be indicators of genetic diversity and population markers in many organisms including bears (Craighead 1994, Paetkau & Strobeck 1994, 1995).

For this study, I investigated genetic diversity of black bears in Montana, and questioned if the isolated mountain ranges in central Montana act as genetic islands for black bears. Genetic diversity was assayed in six populations of black bears and was expected to decrease with increased isolation and decreased area. Specifically, I predicted the microsatellite allele heterozygosity of black bears would be greater in the "continental" portion of Montana than in the isolated mountains. Among the "island" mountains, the smaller, more isolated ranges would show the lowest heterozygosities. Heterozygosity would be related to the size of the mountain range and inversely related to the degree of isolation. In addition to testing black bears microsatellite heterozygosity in relation to size of occupied area and degree of isolation, I investigated the probability that two individuals from the same mountain range would share the identical sampled genotype. This measure, probability of identity (Paetkau & Strobeck 1994), has been used to estimate the ability to identify individuals. Knowledge of heterozygosity levels and probabilities of identity would be useful for identifying genetically depauperate populations that may need special management strategies. The allele distribution information presented here may provide information concerning the history of black bears in central Montana.

The distribution of microsatellite alleles in black bears occupying island habitats in central Montana may provide information about how those islands became occupied. There are two simplistic models to explain species diversity found in island systems. In the colonization (or stepping stone) model, species colonize islands either from the mainland or the next proximal island. In the refugia model, a once contiguous habitat is fragmented into island habitats. Species assemblages on colonized islands should be subsets of the mainland assemblage and subsets of any island that is closer to the mainland. The refugia islands should have the same assemblage as the mainland when the island was formed and over time the size of the island determines which species will persist (Brown 1971 & Lomolino et. al. 1989) (Fig. 1).

By comparing shared and unique alleles between the populations I investigated, I hoped to shed light on the history of those populations. If the isolated mountain populations are the result of colonization events, I would expect them to contain only alleles found in the mainland population, and more distant mountain island populations would contain only alleles found in the populations closer to the mainland. If

the isolated mountain populations are refugia, then the bears on them would have a random mix of alleles found on the mainland and other islands.

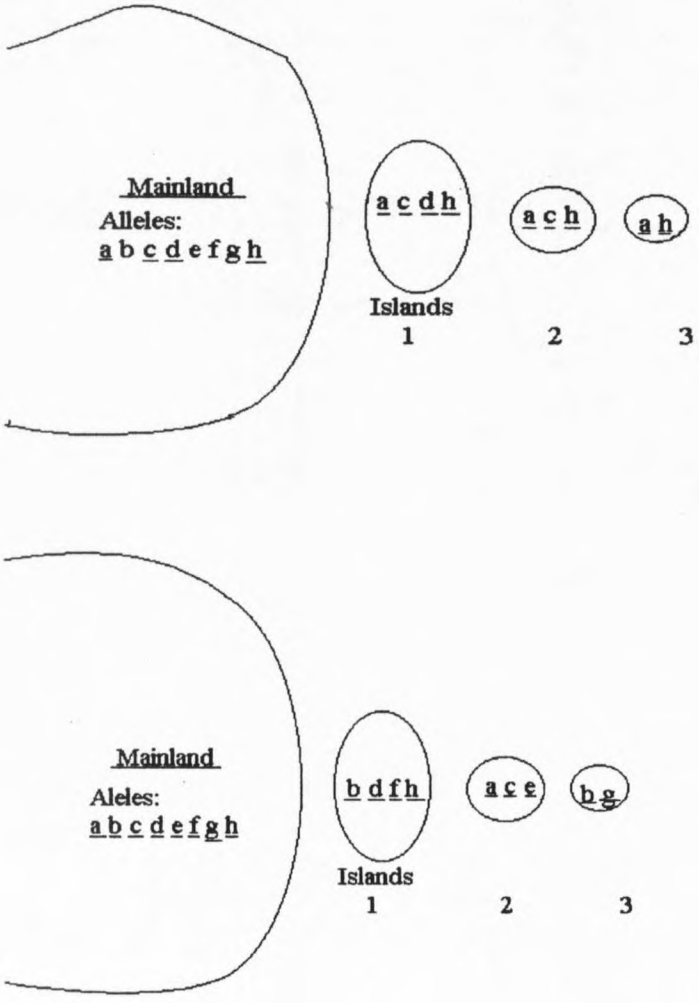

Figure 1.
Two island system models showing different assemblages of alleles. The top model depicts a colonization (or stepping stone) model where alleles are brought to the islands from the island closer to the mainland. ach distant island, 2 and 3, contain a population with a subset of the alleles found on the next proximal island, 1 and 2 respectivly. The bottom model depicts allele distributions on islands that are refugia of a past contiguous habitat. Alleles found on the islands are a random assortment of the alleles found on the mainland.

STUDY AREA

The study area encompasses the Madison mountain range, Gallatin mountain range, Absaroka-Beartooth mountain complex, Rocky Mountain Front and northwestern mountains, Bridger Mountains, Crazy Mountains, Little Belt Mountains, and Snowy Mountains of Montana. The Rocky Mountain Front is continuous with Glacier National Park and the Canadian Rocky Mountains. The Gallatin-Madison complex is continuous with Yellowstone National Park to the south and the Absaroka-Beartooth range to the east. This conglomeration of mountain ranges represents continuous black bear habitat extending north to Alaska and south to southern Wyoming, and acts as a mainland for this study.

To the east of the Rocky Mountain Front, and north-east of the Madison-Gallatin-Absaroka mountain complex, there are mountain ranges of varying size. The Little Belt Mountains occupy 3,200 km^2 and are 3 km east of the Big Belt mountains which are contiguous with the Rocky Mountain Front. The Bridger Mountains are 660 km^2 and are separated from the Madison-Gallatin mountain complex by Interstate 90. The Crazy mountains occupy 1,100 km^2 and are 21 km east of the Bridger mountains, and 28 km north of the Absaroka-Beartooth range. The Snowy mountains are the eastern most mountain range investigated, occupying 700 km^2 they are 65 km north east of the Crazy Mountains and 13 km east of the Little Belt Mountains (Fig. 2). Forested land was used to define suitable black bear habitat for computing areas.

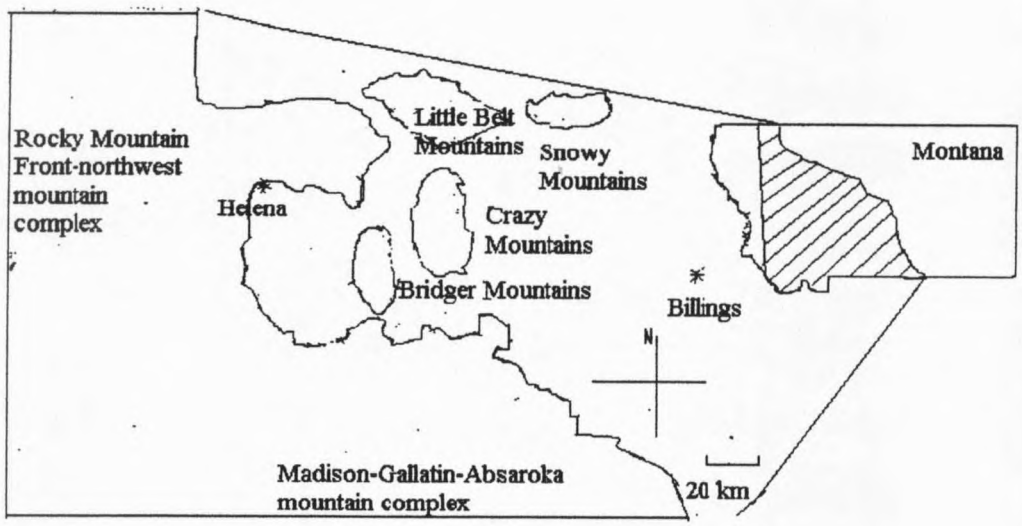

Figure 2. Western and central Montana, showing contiguous black bear habitat in the western and southern portion of the state, as well as the isolated mountain ranges containing black bear populations analyzed for genetic diversity at six microsatellite loci.

METHODS

All bears killed in the state of Montana require verification from the Montana Department of Fish Wildlife and Parks (FWP). Ninety four black bear samples were collected from hunter harvested bears in 1995, 1996 and 1997. Thirty eight samples came from the Madison-Gallatin-Absaroka- Beartooth mountain complex, 28 from the Rocky Mountain Front and Northwest mountains, 10 from the Bridger mountains, 8 from the Crazy mountains, 5 from the Little Belt mountains and 5 from the Snowy mountains. Fish Wildlife and Parks personnel collected samples from harvested bears and sent them to the FWP Wildlife Research Lab in Bozeman, Montana. In addition to hunter killed animals, Animal Damage Control agents that killed bears in response to bear - livestock conflict are required to turn in carcasses to FWP. Samples were stored frozen until DNA could be extracted. Whenever possible I used striated or cardiac muscle for DNA extraction. A few samples came from liver, kidney, or spleen, or hair.

In 1995 and 1996, DNA was extracted from tissue samples using phenol/chloroform. This procedure yielded DNA, however the presence of organic material, phenol, chloroform, or combinations of these, prohibited adequate amplification and resolution of genotypes. In 1997, all DNA was extracted with Qiagen ™ tissue kits following the protocol provided by the manufacturer. DNA extracted in 1995 and 1996 was reextracted in 1997 using these kits. After DNA extraction, microsatellite loci were amplified using six primers developed by David Paetkau (Table 1). These primers have proven very successful in providing genetic profiles of Ursids (Craighead 1994, Paetkau & Strobeck 1994, Paetkau et al. 1995). Primers were labeled with florescence which allowed for non-radioactive imaging. G1A and G10L were labeled with TET green, G10B,G10C, and G1D with 6FAM (ABI) blue, and G10P with HEX (ABI) yellow (Paetkau et al 1995).

Table 1. Locus designation and primer sequences for six Ursid microsatellites.

Locus	(GT) strand primer	(CA) strand primer
G1A	GACCCTGCATACTCCTCTGATG	GCACTGTCCTTGCGTAGAAGTGA
G1D	GATCTGTGGGTTTATAGGTTACA	CTACTCTTCCTACTCTTTAAGAG
G10B	GCCTTTTAATGTTCTGTTGAATTT	GACAAATCACAGAAACCTCCATCC
G10C	AAAGCAGAAGGCCTTGATTTCCT	GGGGACATAAACACCGAGACAGC
G10L	GTACTGATTTAATTCACATTTCCC	GGGGACATAAACACCGAGACAGC
G10P	AGGAGGAAGGAAAGATGGAAAAC	TCATGTGGGGAAATACTCTGAA

Amplification mixtures for the polymerase chain reaction are in the appendix. Amplified PCR products were run on a polyacrylamide gel (Weber & May 1989) in an ABI 373 automated sequencer and run for 6 hours (Paetkau & Srtobeck 1994). Each lane in the gel contained the PCR product and a standard (genescan 2500). Each gel could hold 36 samples but no more than 32 were loaded. The 373 used a laser to scan for each loci primer, biotintilated with yellow, green, blue and red, and the biotintilated primers were scored as they passed the laser. Alleles at different loci that were similar in size contained primers dyed different colors, allowing all six loci to be run in the same lane of the gel.

Immediately before loading the gel, 1.5 µl of the G1A, G10L mix were placed in the G10B, G10C, G1D tube and 1.5 µl of that mixture was added to the G10P tube. From the G10P tube mixture, 1.2 µl was mixed with 2.0 µl of formamide buffer, and 0.5 µl of an internal standard (Genescan 2500, biotintilated with ROX (ABI)). Each lane of the gel was loaded with this diluted DNA mixture from a single individual (Paetkau & Strobeck 1995). The computer accompanying the sequencer, using Genescan 672 software, scored each band with respect to the standard in each lane and formed an image of the whole gel as well as a suite of electropherograms for each lane. This information was then imported into Genotyper software and the bands were assigned as alleles. This process was be visually inspected to account for missed alleles, incorrectly assigned alleles, and non-alleles scored as alleles.

Chi square tests (X^2) were used to test for discrepancies between observed and expected numbers of heterozygous individuals for each population at each loci and for each population at all loci combined.

Expected heterozygosities and probabilities of identity were calculated using the formulae: $h=1-(n\Sigma p_i^2-1)/(n-1)$ (Nei & Roychoudhury 1974), and $I= \Sigma p_i^4+\Sigma\Sigma(2p_ip_j)^2$ (Paetkau & Strobeck 1995) respectively, where p_i and p_j are the i^{th} and j^{th} alleles in a population.

To investigate the impact of sample size on levels of heterozygosity or probability of identity, I resampled with replacement the Madison-Gallatin-Absaroka population 38 times at n=5. This process gave me a distribution of values that I could then compare with the mountain island populations. If the values for the mountain island population fit within the 95 % confidence they were considered not significantly different from the mainland Madison-Gallatin-Absaroka population.

RESULTS

I analyzed six microsatellite loci in 94 black bears from six geographic areas in Montana. Genotypes for all bears used for this study (as well as bears left out of the analysis due to location uncertainty or incomplete genotypes) can be found in the appendix. Allele distributions, frequencies, and number of alleles at each locus for each population are summarized in Table 2. Although both the Rocky Mountain Front-Northwest and Madison-Gallatin-Absaroka populations represent a mainland, I treated them separately in order to elicit possible allelic commonalties between them and the mountain island populations of the Bridger Mountains, Little Belt Mountains, Crazy Mountains and Snowy Mountains.

There were no significant differences between the expected and observed heterozygosities for each population: Madison-Gallatin-Absaroka complex $\chi^2 = 5.27$, $p = 0.38$, Rocky Mountain Front-northwest mountains $\chi^2 = 0.89$, $p = 0.97$, Bridger mountains $\chi^2 = 2.83$, $p = 0.73$, Little Belt mountains $\chi^2 = 0.95$, $p = 0.97$, Crazy Mountains $\chi^2 = 0.95$, $p = 0.97$, Snowy mountains $\chi^2 = 0.58$, $p = 0.99$. These values suggest no significant deviation from the expected Hardy-Weinberg equilibria. Null alleles, reported in some microsatellite analyses, were not present in significant numbers. Null alleles are alleles that do not amplify in the polymerase chain reaction, thereby causing a larger proportion of homozygotes (Craighead 1994, Paetkau & Strobeck 1995).

Heterozygosities ranged between 0.761 for the Rocky Mountain Front- North West, and 0.633 for the Snowy Mountains (Table 3). These values are similar to published microsatellite heterozygosities for mainland black bears in Canada (Paetkau & Strobeck 1994), where values were reported (at 4 of the loci used in this study G1A, G1D, G10B, and G10L) between 0.801 and 0.783. Probability of identity (Table 3) values ranged between 1 in 1.5 million for the Rocky Mountain Front- North West, to 1 in 6,289 for the Snowy Mountains. Heterozygosity levels for the isolated mountain ranges were not outside the 95%

confidence interval established for the resampled Madison-Gallatin-Absaroka population (0.632< mean = 0.682 <0.732). Although heterozygosity levels and probabilities of identity did not differ significantly between the sampled populations, linear regressions showed trends relating heterozygosity and probability of identity to area and distance to nearest suitable habitat (Fig. 3). The R^2 values ranged from 0.52, $p = 0.27$, for the probability of identity against area to a R^2 0.00, $p = 0.94$ for probability of identity against distance to nearest habitat. Values for Heterozygosity against area and distance to habitat were $R^2 = 0.07$, $p = 0.73$ and $R^2 = 0.12$, $p = 0.65$ respectively.

Table 2. Allele distributions, frequencies and number of alleles at six Ursid microsatellite primers for six black bear populations in Montana: Madison-Gallatin-Absaroka mountain complex n=38 (MGA), Rocky Mountain Front-north western mountain complex n=28 (NWF), Bridger Mountains n=10 (BR), Little Belt Mountains n=5 (LB), Crazy Mountains n=8 (CR), and Snowy Mountains n=5 (SN).

	Alleles																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	No. of alleles
Locus G10B																			
MGA				0.013						0.645	0.039	0.092	0.197	0.013					6
NWF				0.089						0.321	0.143	0.107	0.286	0.054					6
BR										0.700	0.050	0.050	0.200						4
LB								0.100		0.500		0.100	0.300						4
CR									0.125	0.750			0.125						3
SN									0.200	0.700			0.100						3
Locus G10C																			
MGA		0.368	0.013	0.500	0.066		0.023	0.013	0.013										8
NWF		0.375		0.482	0.054			0.036	0.036	0.018									6
BR		0.450		0.450					0.100										3
LB		0.900		0.100															2
CR		0.688		0.250	0.062														3
SN		0.400		0.400					0.200										3
Locus G10L																			
MGA	0.026	0.039	0.105		0.184				0.013	0.053	0.026	0.224		0.026	0.171	0.013	0.118		12
NWF	0.054	0.125	0.071	0.018	0.054				0.036			0.321	0.018	0.071	0.089		0.143		11
BR					0.250					0.050		0.200		0.050	0.300	0.050	0.100		7
LB			0.100		0.100					0.100		0.200			0.300		0.200		6
CR					0.062					0.250		0.125		0.125	0.062	0.062	0.250	0.062	8
SN	0.100	0.300														0.400	0.200		4