MtDNA Analysis of Sexual Dispersal Among Glacier National Park Grizzly Bears (Ursus arctos)

Joseph Chiovaro
Carroll College, Helena, MT
MtDNA Analysis of Sexual Dispersal Among Glacier National Park Grizzly Bears (*Ursus arctos*)

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Joseph C. Chiovaro
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This thesis for honors recognition has been approved for the Department of Natural Sciences.

_____________________________  4/11/02
Dr. Gerald Shields, Ph.D, Thesis Director  Date
Professor of Biology, Carroll College

_____________________________  4/11/02
Dr. John Addis, Ph.D, Thesis Reader  Date
Head of Natural Sciences Department, Carroll College

_____________________________  4/10/02
Murphy Fox, Thesis Reader  Date
Director of Honors Scholars Program, Carroll College
Abstract

This study compared 369 base pairs of the hypervariable control region of mitochondrial DNA in 137 brown bears (71 males, 66 females) of Glacier National Park to investigate dispersal patterns between the sexes. Since females establish home ranges close to their birth sites, we hypothesized a stronger phylogeographic pattern among females. Contrary to our expectations, males exhibited stronger phylogeographic patterning with one haplotype in males occurring exclusively in northern regions of the park, while a second occurred exclusively in the south. No phylogeographic patterning was observed among females. Male dispersal patterns could be influenced by gene flow from regions contiguous to the park. All brown bear haplotypes in Glacier National Park belong to clade IV of earlier studies and suggest a recent establishment of brown bears in this region.

Key words: brown bears, mtDNA, dispersal, phylogeography
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Introduction

Not only has the brown bear, *Ursus arctos*, been eradicated from 99% of its pre-contact range in the contiguous United States and Mexico, but also, total population size has been reduced from 100,000 to less than 1,000 individuals due to human encroachment (Allendorf and Servheen, 1986; Servheen, 1990). Moreover, remaining brown bears are restricted to small, fragmented populations between which gene flow is reduced (Servheen, 1990). Waits *et al.* (1998) demonstrated that the population of *U. arctos* in Wyoming possesses a low level of genetic variation that could be detrimental to the species’ ability to maintain fitness and to adapt to environmental change (Soulé, 1980). By contrast, the Northern Continental Divide Ecosystem (NCDE) brown bears have retained relatively high levels of genetic diversity (Waits *et al.*, 1998). Management of these fragmented populations of *U. arctos* of the continental United States would be enhanced by knowledge of specific characteristics of population genetics including population sizes, genetic diversity, gene flow, and differential dispersal patterns between the sexes.

*U. arctos* was designated a threatened species in 1975 in accordance with the U.S. Endangered Species Act (16 U.S.C. 1531-1544), and there is considerable interest in the conservation of *U. arctos* in the NCDE since the majority of the habitat is contained within Glacier National Park. Status as a national park mandates special protection for bears. Moreover, these large mammals attract substantial numbers of tourists annually, who stimulate the general economy of the region.
The Glacier National Park Bear DNA Project is a large, noninvasive sampling scheme designed to elucidate population parameters of *U. arctos* in the NCDE. As one component of this project, I examined mitochondrial DNA (mtDNA) to determine genetic diversity, gene flow and spatial distribution of males and females. Specifically, I sequenced 369 bases pairs of the rapidly evolving control region (Wrischnick *et al.*, 1987) of the mtDNA from samples taken noninvasively from throughout the park.

MtDNA has a high mutation rate (Brown *et al.*, 1979) and is transmitted maternally (Gyllensten *et al.*, 1985), which make it exceptionally informative in detecting female mediated gene flow (Paetkau *et al.*, 1998). Talbot and Shields (1996) described three clades of *U. arctos* in Alaska based on complete sequences three mtDNA genes, and Waits *et al.* (1998) described an additional clade in southern Canada and the Yellowstone and Glacier Park ecosystems in Montana when DNA sequences of the control region were compared.

Noninvasive sampling has become increasingly widespread, and Taberlet *et al.* (1997) discuss the utility of this technique for small populations of mammals. While the limitations of this mode of sampling are currently under debate, its utility in phylogenetic studies has been confirmed (Taberlet *et al.*, 1999).

In an earlier study, Waits *et al.* (1998) described five haplotypes of the mitochondrial control region of *U. arctos* of Glacier National Park but the spatial distributions of these haplotypes was unknown. Dispersal differences for males and females may exist (Waits *et al.*, 1998), as females tend to establish home
ranges that overlap their natal ranges; males, on the other hand, frequently disperse over large distances (Reynolds and Hechtel, 1984). Given this information, I hypothesized that there would be a stronger geographic component to the genetic diversity among female brown bears than that among males of Glacier National Park. Elucidation of differential patterns of genetic diversity and geographic distance for male and female brown bears could allow further predictions about other parameters of the population genetics of U. arctos in Glacier National Park.
Materials and Methods

Sample Collection

This project is part of an ongoing program to determine parameters of population biology of brown bears of the Greater Glacier Park Ecosystem. Beginning in the summer of 1998, field biologists of the Montana Department of Fish, Wildlife and Parks established a collection scheme using non-invasive sampling techniques such as the collection of hair and scat (Waits, pers. comm.). A subset of those samples was used in this study.

All mtDNA was extracted from hair samples that had been collected either from hair traps, or rub trees. The study area for the project consisted of roughly 8000 km$^2$, and contained all of Glacier National Park, as well as surrounding areas. This area was divided into 124 cells by an 8x8 km grid. Beginning with the 1998 field season, one hair trap, consisting of a single piece of barbed wire surrounding a scent lure, was placed in each cell. This trap was then moved to another location within the cell during each of five trapping seasons. Every 14 days, the hair was collected from the wire using sterile forceps and placed into dry storage until extraction (http://nrmsc.usgs.gov/research/beardna.htm).

Rub tree traps were placed along the major trails in Glacier National Park. These trees were selected based on proximity to trail and previous rub activity. A single piece of barbed wire was placed around the tree at a standard height and inspected periodically for bear hair. Sterile forceps were once again used to remove the hair from the wire, and samples were placed in dry storage until
The extraction of the mtDNA was done using the Qiagen DNeasy extraction kit (Qiagen, 2000).

**mtDNA Sequencing**

A 369 base pair segment of the mtDNA control region was sequenced in 138 free-ranging brown bears from a wide distribution of localities throughout Glacier National Park. Individual bears were identified as part of a separate study (Roon, pers. comm.). Originally, bears were selected at random from a compilation of individuals that had been found in both the 1998 and 1999 data sets. After sequence from 100 bears had been obtained, samples were selected by location to ensure good geographic coverage for both sexes.

Two-direction amplification was performed on the extracted samples using 40 cycles of the Polymerase Chain Reaction (PCR) in 30 μL total reaction volumes (1.5mM MgCl₂, 10mM dNTP, 1 U/μL Amplitaq Gold and 10x Amplitaq Gold Buffer (Perkin Elmer), primers with a concentration of 2 μM, 4 μL of template, and dH₂O to reaction volume). Two distinct sets of primers were used. The first, L15774 and H16498, are mammalian primers that were previously described by Shields and Kocher (1991). These primers yielded sequence of up to 600 nucleotides in length. The second set of primers, Upst L (5' - AGGAAGAAGCAACAGCTCC-3') and Dnst II H (5' - CATAGAAACCCCCACATTCC-3'), amplified a smaller fragment of 365 nucleotides (Miller, per. comm.), and yielded higher rate of successful amplification.
The PCR amplification with the 600-nucleotide primers was performed on a Perkin-Elmer thermocycler, with activation of the Amphitaq enzyme at 95°C for ten minutes, denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for two minutes. For the amplification of the 350-nucleotide primers activation of the Amphitaq enzyme was 95°C for ten minutes, with denaturation at 95°C, annealing at 45°C for 30 seconds, and extension at 72°C for one minute.

Electrophoresis and visualization of amplified products was done as described in Shields and Kocher (1991). Sequencing was performed on an ABI Prism 377XL Automated Sequencer (Applied Biosystems), and nucleotides were scored using the Sequencer 3.1 software package for Macintosh (Applied Biosystems, 2001).

**Sequence Analyses**

Sequences were aligned manually using Phylogenetic Analysis Using Parsimony (PAUP, Version 3.1.1, Swofford, 1993), and checked for variation in MacClade (Version 3.0, Maddison and Maddison, 1992). Unique DNA sequences were identified in this manner and are referred to as haplotypes. The spatial distribution of these haplotypes was then examined in both males and females for any correlation. A test for the distribution of mean pairwise nucleotide substitutions was conducted as described in Schneider and Excoffier, 1999. Finally a minimum spanning tree was generated from the sequence data (Schneider et al., 2000).
Results

Comparison of 369 homologous nucleotides of the hypervariable region of the 5' end of the mtDNA control region of 138 brown bears of Glacier National Park revealed the presence of six variable sites corresponding to eight haplotypes (Fig. 1). The majority of sites (5/6) were transition substitutions; no transversions were observed. The remaining variable site was a length mutation consisting of a 3-5 base pair insertion-deletion region of repeated thymine nucleotides. This variable site has been omitted from previous studies of the mitochondrial control region because the number of repeated T nucleotides was often difficult to determine accurately (Shields et al., 1993; Waits et al., 1997). However, using automated sequencing, this site yielded consistent repeatable results and, consequently, was included in the final analyses.

More variation was observed among the 71 males studied (seven haplotypes) than among the 67 females (four haplotypes). By far, haplotype A was the most prevalent haplotype observed, constituting 79% of males and 91% of females. All other haplotypes were observed in less than 8% of the bears sampled (Fig. 2).

Significant population structuring was observed in males with haplotype G being distributed exclusively in northern regions of the park and haplotypes C and D being distributed exclusively in southern regions (Fig. 3). In contrast, less population structuring was observed among female brown bears (Fig. 4). Only eight females were observed to have haplotypes other than haplotype A. Haplotypes C, E, F and G occurred at low frequencies (1, 4, 1 and 6 %,
respectively) in males and were absent in females. Haplotype H occurred in 1% of females and was absent in males.

The distribution of mean pairwise nucleotide substitutions (mismatch distributions, Schneider and Excoffier, 1999) for brown bears of Glacier Park is shown in Figure 5. As suggested by Schneider and Excoffier (1999) this "L" shaped distribution with low mean pairwise differences is characteristic of a recently founded population undergoing expansion.

Evolutionary relationships among haplotypes are shown in a minimum spanning tree in Figure 6 (Schneider et al., 2000). The dominance of haplotype A in this figure is also characteristic of a recently founded population.
<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Site Location</th>
<th>Number of bears</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>T - - T G G A</td>
<td>116</td>
</tr>
<tr>
<td>B</td>
<td>. T . . . .</td>
<td>9</td>
</tr>
<tr>
<td>C</td>
<td>. . . C . . G</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>. T . C . . G</td>
<td>3</td>
</tr>
<tr>
<td>E</td>
<td>C T . C A A G</td>
<td>3</td>
</tr>
<tr>
<td>F</td>
<td>. . . C A A G</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>. T . C A A G</td>
<td>4</td>
</tr>
<tr>
<td>H</td>
<td>. T T C A A G</td>
<td>1</td>
</tr>
</tbody>
</table>

A site number is in reference to sequence in Waits et al. (1997).
B site 153 and 154 constitute two variations in the insertion/deletion variable site.

Figure 1: Compressed dot matrix of variable regions among 138 brown bears of Glacier National Park.
Figure 2: Proportions of haplotypes observed in female and male brown bears.
Figure 3: Phylogeographic distribution of male brown bears.
Figure 4: Phylogeographic distribution of female brown bears.
Figure 5: Mean pairwise distribution of variation.
Figure 6: Minimum spanning tree.
Discussion

The brown bears of Glacier National Park are a subset of clade IV described by Waits et al. (1997). This clade also contains individuals from southern British Columbia, southern Alberta, the state of Idaho, other regions of the state of Montana, and the Yellowstone Ecosystem. Talbot and Shields (1996) described clades I, II, and III of brown bears in Alaska and Leonard et al. (2000) and Barnes et al. (2002) described these three clades from fossil bones ranging in age from 35,000 to 45,000 years old. This indicates that the first three mitochondrial clades of brown bears were present, at least in the interior of Alaska at that time. No fossils of brown bears of that age have been found in Glacier National Park, and therefore, clade IV is believed to be recent (Waits et al., 1997). Waits et al. (1997) also estimates that haplotypes of brown bears of the lower 48 states diverged from Alaskan brown bears over 250,000 years ago; but see Ingman et al. (2000).

No new haplotypes were observed in this study; however, the proportions of observed haplotypes differed substantially from those reported previously for Glacier Park brown bears by Waits et al. (1997). For example, they found that lineage 37 occurred in 50% of bears, while lineage 40 was found in 31%. These lineages correspond to haplotypes A and B (lineage 37) and haplotypes F, G, and H (lineage 40) of the present study, with the differences resulting from the addition of the thymine region of the sequence. In this study, haplotypes A and B together accounted for 91% of the bears, while haplotypes F, G, and H account
for only 4% of the brown bears sampled. With the exception of one lineage, 52, all previously reported haplotypes were observed in this study.

The overwhelming majority of individuals of this study exhibited a single haplotype. This could be explained by the founder effect accompanied by genetic drift reducing haplotypic variability and/or the possibility that the brown bears within the park represent a more recently established population than was previously believed.

I observed phylogeographic patterning among the male haplotypes of this study, but not among those of females and, therefore, I must reject my original hypothesis. Structuring among males but not among females was unexpected since females are known to establish home ranges near their place of birth while males disperse much more widely (Schoen, 1991). At the molecular level these differential patterns of dispersal should result in phylogeographic structure among females but not necessarily among males. This disparity in phylogeographic structure between the sexes may be due to a variety of factors including: sample bias, effects of genetic drift, random lineage extinction or a high rate of male gene flow into and out of the park. A larger study, which included sampling of bears in areas adjacent to the park, may resolve this paradox. There are few barriers to dispersal into or out of Glacier National Park, with major roads and human habitation constituting the most substantial of these obstacles. In addition, a contact zone between clades III and IV may exist in Canada (Waits et al., 1997). Moreover, the populations of bears in coastal regions of southern Alaska and western Canada are not geographically isolated.
Only the population of brown bears in the Yellowstone Ecosystem to the south of Glacier is currently isolated (Waits et al. 1997).

It is worth noting that lineage 38 from Waits et al. (1997), which is the predominant lineage in the Yellowstone Ecosystem to the south and in the East and West slopes of the Canadian Rockies to the north of Glacier, corresponds to haplotypes C and D in this study and is only found in the southern reaches of the park. One possibility is that these haplotypes, which are only separated by a single nucleotide difference, represent a remnant population.

The presence of phylogeographic structure among males, although unanticipated, is interesting. The geographic separation of males into haplotype G in the north and haplotypes C and D in the south is also unanticipated since the major physical barrier in the park is the Continental Divide, which runs north to south and generally separates the park into equal halves. A different physical feature of the landscape, which might discourage male movement form north to south and vice versa, is the presence of major lakes (Bowman, Quartz, Logging, Sherburne, McDonald, St. Mary), which generally run west to east. Water barriers may be a hindrance to gene flow in brown bears of Alaska (Paetkau et al., 1998). Alternatively, brown bears are known to move freely across the continental divide at 9,000 feet in Alaska (Shields, pers. comm.). The only human impediment to movement would be the Going to the Sun highway, which takes a generally east to west route across the park. This road is heavily traveled in the summer, and could potentially hinder movement of males from north to south.
Relatively low haplotypic diversity, preponderance of one major haplotype, lack of transversions, low mean pairwise distribution of haplotypes and a relatively simple minimum spanning tree all suggest that the factors influencing the distribution of brown bears in the Greater Glacier Park Ecosystem have had a short temporal duration. More detailed observations of population characteristics may be obtained from the study of more rapidly evolving molecular markers such as nuclear microsatellites and SINEs (short internal nuclear elements) or LINEs (long internal nuclear elements, Hillis, 1999).
Acknowledgments

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