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Corresponding Author: Dr. Dave Paul Onorato, PhD

Corresponding Author's Institution: Fish and Wildlife Research Institute

First Author: Dave P Onorato, PhD

Order of Authors: Dave P Onorato, PhD; Eric C Hellgren, PhD; Ronald A Van Den Bussche, PhD; Diana L Doan-Crider, PhD; Jack R. Skiles, MS

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Abstract: American black bears (Ursus americanus) have recolonized parts of their former range in the Trans-Pecos region of western Texas after a >40-year absence. Assessment of genetic variation, structuring, gene flow, and dispersal among bear populations along the borderlands of Mexico and Texas is important to gain a better understanding of recolonization by large carnivores. We evaluated aspects of genetic diversity and gene flow for 6 sampling areas of black bears in southwestern North America using genotypic data from 7 microsatellite loci. Our results indicated that genetic diversity generally was high in the metapopulation of black bears in northern Mexico and western Texas. The episodic gene flow occurring via desert corridors between populations in northern Mexico and those in western Texas has permitted the establishment of only moderate levels of genetic structuring. Bayesian clustering analyses and assignment testing depicted the presence of 3 subpopulations among our 6 sampling areas and attested to the generally panmictic nature of bear populations in the borderlands region. The potentially ephemeral nature of the

small populations in western Texas and genotypic characteristics of bears recolonizing these habitats attest to the importance of linkages along this portion of the borderlands of the United States and Mexico to effectively conserve and manage the species in this part of its range. 21 July 2006

Miranda Dijksman Editorial Office *Conservation Genetics*

Miranda:

Attached is the revised manuscript COGE-05-185 "Genetic structure of American black bears in the desert southwest of North America: conservation implications for recolonization". We have implemented most of the editorial comments provided by the reviewers and the associated changes are detailed in a subsequent attachment. We thank the reviewers for their helpful comments, specifically in regards to the suggested analysis of sex-biased dispersal patterns.

Should any additional questions or comments arise, please feel free to contact me.

Sincerely,

Dave Onorato Fish and Wildlife Research Institute Florida Fish and Wildlife Conservation Commission 566 Commercial Boulevard Naples, FL 34104

239-643-4220 Dave.onorato@myfwc.com Click here to download Response to reviewer's comments: COGE05_185 response to reviewers Onorato.doc

18 July 2006

COGE-05-185 Onorato et al. Response to reviewers

Comments from Reviewer #1

- 1. Comment on specifics of Figure 1. We have revised the figure description for figure 1 so that it is now clear as to how Pelton et al's population designations are delineated. We felt that placing sample sizes on the map causes extreme clutter on the figure, therefore we included them in the figure title instead.
- 2. "montane islands" is actually correct and a term that we and others have utilized in the literature before.
- 3. We have added a section that discusses our evaluation of error rates associated with our genotyping. Additionally the P_{(ID)sibs} parameter has now been calculated for the 7 loci that we utilized in this study.
- 4. "Major population" has now been qualified as a population from which we obtained 29 or more samples.
- 5. In reference to our "mixed ancestry" statement, we have now qualified that this ancestry has been <u>inferred</u> by our STRUCTURE assignment test. Reviewer #2 recommended delineating this statement as well.

Comments from Reviewer #2

- We have now included an analysis to assess whether dispersal was biased towards males or females using the methodology described by Goudet et al. (2002: Molecular Ecology 11:1103-1114). We have incorporated this analysis into the methods, results and discussion section and concur with the reviewer that this analysis gives further credence to some of our conclusions that relate to metapopulation dynamics.
- 2. See #5 above
- 3. We concur. We added a sentence in the final paragraph that helps make this point.

1 Genetic structure of American black bears in the desert southwest of North America:

2 conservation implications for recolonization

- 3 RH: Genetic Structure of Desert Black Bears
- 4 Dave P. Onorato^{1,4}*, Eric C. Hellgren^{1,5}, Ronald A. Van Den Bussche¹, Diana L. Doan-
- 5 Crider², J. Raymond Skiles Jr.³
- 6
- 7 ¹Department of Zoology and Oklahoma Cooperative Fish and Wildlife Research Unit, 430
- 8 LSW, Oklahoma State University, Stillwater, OK 74078, USA; ²Caesar Kleburg Wildlife
- 9 Research Institute, MSC 218, Texas A & M University-Kingsville, Kingsville, TX 78363
- 10 USA; ³Science and Resource Management Unit, Big Bend National Park, TX 79834, USA;
- ⁴*Current address: Fish and Wildlife Research Institute, Florida Fish and Wildlife*
- 12 Conservation Commission, Naples, FL 34104, USA; ⁵Current address: Cooperative
- 13 Wildlife Research Laboratory, Southern Illinois University,, Carbondale, IL 62901, USA
- 14 (**Author for correspondence: phone: 239-643-4220; fax: 239-643-0385; email:*
- 15 *dave.onorato@myfwc.com*)
- 16
- 17 Key words: Chihuahuan Desert, dispersal, conservation genetics, recolonization, Ursus
- 18 *americanus*.

1 Abstract

2 American black bears (Ursus americanus) have recolonized parts of their former range in 3 the Trans-Pecos region of western Texas after a >40-year absence. Assessment of genetic 4 variation, structuring, gene flow, and dispersal among bear populations along the 5 borderlands of Mexico and Texas is important to gain a better understanding of 6 recolonization by large carnivores. We evaluated aspects of genetic diversity and gene 7 flow for 6 sampling areas of black bears in southwestern North America using genotypic data from 7 microsatellite loci. Our results indicated that genetic diversity generally was 8 9 high in the metapopulation of black bears in northern Mexico and western Texas. The 10 episodic gene flow occurring via desert corridors between populations in northern Mexico 11 and those in western Texas has permitted the establishment of only moderate levels of 12 genetic structuring. Bayesian clustering analyses and assignment testing depicted the 13 presence of 3 subpopulations among our 6 sampling areas and attested to the generally 14 panmictic nature of bear populations in the borderlands region. The potentially ephemeral 15 nature of the small populations in western Texas and genotypic characteristics of bears 16 recolonizing these habitats attest to the importance of linkages along this portion of the 17 borderlands of the United States and Mexico to effectively conserve and manage the 18 species in this part of its range.

19

20 Introduction

Populations of large carnivores have dramatically declined during the last 100
years. Although extinctions have occurred, range contraction is most notable for species
such as lions (*Panthera leo*), brown bears (*Ursus arctos*), and tigers (*Panthera tigris*)

1	(Woodroffe 2001). In North America, a reversal of this trend has been observed via range
2	expansion and natural recolonization of former range by two large carnivores. Gray wolves
3	(Canis lupus) have reappeared in parts of the northern Rocky Mountains, northern
4	Minnesota, Wisconsin, and Michigan (Forbes & Boyd 1996; Thiel et al. 1997; Boyd-Heger
5	& Pletscher 1999; Beyer Jr et al. 2001). This recolonization process has been facilitated by
6	long-distance dispersal of female wolves (Boyd-Heger and Pletscher 1999) and corridors
7	between Canada and the United States (Mladenoff et al. 1995; Forbes & Boyd 1996). The
8	distribution of American black bears (Ursus americanus) in the continental United States
9	has increased since the 1980's (Pelton & van Manen 1994) in many cases via range
10	expansion. For example, descendents of reintroduced black bears are expanding their
11	geographic range from western Arkansas into southeastern Oklahoma (Smith & Clark
12	1994) facilitated by contiguous bear habitat between the two states.
13	Phylogeography, gene flow and population structure have been assessed across a
14	wide portion of the range of black bears (Cronin et al. 1991; Byun et al. 1997; Wooding &
15	Ward 1997; Warrillow et al. 2001). However, the majority of studies have concentrated on
16	populations in the northern latitudes of North America or the southeastern United States.
17	Notably absent from analyses are populations from southwestern North America. The
18	geographic range of black bears in southwestern North America can be described as
19	discontinuous in comparison to ranges in more northern latitudes (Pelton et al. 1999). The
20	map of Pelton et al. (1999) depicts regions known to contain black bear populations along
21	the Mexico-U.S.A border (Figure 1; shaded area). Range expansion by black bears in this
22	region is slowed because populations of bears are restricted to montane islands of habitat
23	separated by expanses of non-occupied Chihuahuan desert (Onorato & Hellgren 2001;

1	Onorato et al. 2003; Onorato et al. 2004a; Hellgren et al. 2005). Additionally, although
2	male black bears commonly disperse > 30 km from their natal area, females rarely disperse
3	(Rogers 1987; Elowe & Dodge 1989; Schwartz & Franzmann 1992). However, black bears
4	recently (mid 1980's) recolonized parts of their former range in the western Texas segment
5	of the Big Bend Ecosystem (Onorato and Hellgren 2001). Previously, we used
6	demographic and mtDNA data to describe black bears in this region as existing in a
7	mainland-island metapopulation (Onorato et al. 2004a) as defined by Hanski and
8	Simberloff (1997). Island populations are defined as encompassing small, suitable habitat
9	patches and are located within dispersal distance from a very large habitat patch that
10	supports a perennial mainland population (Hanski and Simberloff 1997).
11	We describe genetic relationships of black bears from 6 sampling localities in
12	northern Mexico and the southwestern United States using 7 hypervariable microsatellite
13	loci. Our objectives were to (1) assess levels of genetic variation in populations of black
14	bears within the Mexico-Texas mainland-island metapopulation (Onorato et al. 2004a); and
15	(2) evaluate the degree of genetic similarity between the recently recolonized population of
16	bears in Big Bend National Park, Texas (island population) and large populations in
17	northern Mexico (mainland populations) and southwestern New Mexico using measures of
18	genetic structuring and population assignment tests. We predicted that our findings would
19	be consistent with the life-history paradigm of black bears and other large mammalian
20	carnivores, namely that genetics among populations within this metapopulation would be
21	affected by male-biased dispersal and geographic distance.

1 Methods

2 Sampling Areas

3 Tissue collection was conducted in 6 locations within southwestern North America 4 (underlined locations in Figure 1). We collected samples from black bears in Big Bend 5 National Park (Big Bend NP in Figure 1, n = 32), Texas between September 1998 and July 6 2001. The Park encompasses about 320,000 ha of northern Chihuahuan Desert in the 7 Trans-Pecos region of western Texas. The primary habitat for black bears in the Park is located within a 100-km² area comprising the Chisos Mountains (elevation 1400-2385 m) 8 9 where abundant food sources such as oak (Quercus spp.), juniper (Juniperus spp.), and 10 madrone (Arbutus xalapensis) are found. During the same time period, tissue samples from 11 9 individuals also were obtained from a small population of black bears recolonizing 12 adjacent Black Gap Wildlife Management Area (Black Gap in Figure 1). This region 13 contains suitable habitat at lower elevations (900-1400 m) about 60 km northeast of the 14 Chisos Mountains of Big Bend NP. 15 Tissue samples from 8 individuals were collected throughout the Trans-Pecos 16 region of Texas between 1994 and 2002 (Figure 1). Precise locations for 6 of these samples 17 were provided in Onorato et al. (2004a), and the additional two samples were collected in 18 Val Verde and Webb counties along the border of Texas and northern Mexico. All bears in 19 this region were sampled after vehicle collisions, poaching incidents, or during relocation 20 after nuisance complaints. Reproductively viable populations were not present in habitats 21 where these samples were collected (Taylor 1999). However, we combined these samples, 22 designated them as the Trans-Pecos group for several analyses, and qualify them as 23 dispersing or colonizing animals within historical range in western Texas.

1	Tissue samples also were obtained from two mountain ranges in northern Coahuila,
2	Mexico known to support large populations of black bears: Serranias del Burro between
3	1991-1999 (Burros, $n = 58$; (Doan-Crider & Hellgren 1996) and Sierra del Carmen in 1997
4	(Carmens, $n = 5$). The combination of Big Bend NP, Black Gap, Burros, Carmens, and
5	Trans-Pecos populations will hereafter be referred to as the Mexico-Texas metapopulation.
6	Samples collected from the Mogollon Mountains (Mogollons in Figure 1, $n = 29$) of
7	west-central New Mexico during a long-term study by the Hornocker Wildlife Institute
8	(Costello et al. 2001) were analyzed for comparative purposes to determine genetic
9	differentiation between bears from this region and those found in the Mexico-Texas
10	metapopulation. In terms of available habitat, the Mogollon Mountains are similar to the
11	larger mountain ranges found in northern Mexico. The Mogollons study area is primarily
12	in the Gila National Forest and elevations range from $1750 \text{ m to} > 3000 \text{ m}$.
13	A caveat concerning sample size is necessary when completing analyses on a small
14	number of samples for 3 of the 6 sampling areas. Comprehensive field studies on the black
15	bear population in the Carmens have only recently (2004) been initiated. Subsequently, we
16	had few samples from this range. Additionally, dispersing bears in western Texas that were

17 grouped into the Trans-Pecos group were obtained opportunistically via communication

18 with officials from Texas Parks and Wildlife. In the case of Black Gap, the 9 samples

19 composed what is thought to be > 90% of the bears present in that population during this

20 study. Due to the limitations of these data and the subsequent impact of small sample sizes

21 on certain analyses (specifically estimates of Hardy-Weinberg equilibrium, linkage

22 disequilibrium, F statistics, and Nei's genetic distance), these samples were included

- mainly for the Bayesian analysis of genetic structuring and subsequent assignment testing
 (see below).
- 3 DNA Isolation and Microsatellite PCR

4 Samples either were stored frozen $(-20^{\circ}C)$ or in lysis buffer (Longmire et al. 1997). 5 DNA was extracted using the phenol extraction method described by Longmire et al. 6 (1997). Seven $(GT)_n$ microsatellite loci were amplified using the polymerase chain reaction 7 (PCR) and primers described in Paetkau et al. (1998). Six of these loci (G1D, G10B, 8 G10C, G10H, G10J, G10P) were cloned from an American black bear DNA library 9 (Paetkau & Strobeck 1994; Paetkau et al. 1995), whereas CXX20 was derived from a 10 domestic dog (*Canis familiaris*) library (Ostrander et al. 1993). One primer of each pair 11 was synthesized with a fluorescent dye (FAM, HEX, or TET) to permit detection and sizing 12 of microsatellite repeats on a Perkin-Elmer ABI Prism 377 Automated Sequencer. Genotyping error rate using these same loci on a subset of samples included in this study 13 14 was determined to be 1% via repeated amplifications of random samples and via allelic 15 mismatches between mothers and known offspring (Onorato et al. 2004) 16 Amplifications were performed in 15- μ l reactions using 50–200 ng DNA, 0.17 μ M 17 of each primer, 9 µl True Allele PCR premix (Perkin-Elmer Applied Biosystems, Foster 18 City, CA) and 3.8 µl double deionized water. The following thermal profile was used 19 during amplification: 12 min at 95°C; 10 cycles of 15 s at 94°C, 1 min at 49-55°C 20 (annealing temperatures were specific for different loci), 30 sec at 72°C; 25 cycles of 15 s 21 at 89°C, 1 min at 55°C, 30 s at 72°C; and 30 min at 72°C. All DNA extracts and PCR 22 products were monitored for contamination via the inclusion of negative controls during 23 each DNA extraction and subsequent PCR reactions. Products were diluted and combined

1	based on the size, fluorescent dye and yield. One microliter of PCR dilutions was added to
2	3 μ l of loading buffer containing 0.5 μ l GS-400HD ROX size standard, 0.5 μ l of loading-
3	dye, and 2.5 μ l of formamide. The mixture was denatured at 95°C for 5 min and loaded on
4	a 6% Long-Ranger acrylamide gel in an ABI 377 automated sequencer. Resulting data
5	were analyzed using GENESCAN TM version 2.1 and GENOTYPER TM version 2.5 software
6	packages (Perkin-Elmer Applied Biosystems, Foster City, California).
7	Data Analysis
8	Assessment of observed (H_o) and expected (H_e) heterozygosity within the 6
9	sampling areas were quantified using GENETIX 4.05 (Belkhir et al. 2001). Additionally,
10	the delimiting of allelic richness adjusted for sample size was determined using FSTAT
11	2.9.3.2 (Goudet et al. 2002). Significant deviations from Hardy-Weinberg equilibrium
12	(HWE) at the loci level within each major population (\geq 29 samples) were assessed using
13	the Markov chain method through 1000 iterations (Guo & Thompson 1992) using
14	GENEPOP 3.1 (Raymond & Rousset 1995). We tested for the presence of linkage
15	disequilibrium between pairs of loci for these same 3 populations using the unbiased
16	estimates of Fisher's exact test via the Markov chain method in GENEPOP. Tests that
17	involved multiple comparisons in GENEPOP were corrected for the increased likelihood of
18	making a type I error using a sequential Bonferroni adjustment (Sokal & Rohlf 1995). The
19	probability of identity among siblings ($P_{(ID)sib}$; Evett & Weir 1998; Waits et al. 2001), an
20	unbiased and conservative estimator of the probability of observing the same multilocus
21	genotype for two randomly sampled individuals from a population, was calculated using

the program GIMLET 1.3.3 (Valière 2002)

1	Levels of population differentiation (pairwise F_{ST}) and overall genetic
2	differentiation were calculated via genotypic data collected from Big Bend NP, Burros, and
3	Mogollons using FSTAT 2.9.3.2 (Goudet et al. 2002). Pairwise F_{ST} values were compared
4	with ϕ_{ST} values obtained in a previous study by Onorato et al. (2004a) using mtDNA
5	sequences. Additionally, this program allowed us to test for sex-biased dispersal patterns
6	of bears sampled in these same 3 populations using the mean corrected assignment index
7	(mAIc) and the variance of AIc (vAIc) as described by Goudet et al. (2002). Values of
8	mAIc decrease in individuals that contain genotypes that are on average less likely to occur
9	in the population from which the individual was sampled. Conversely, the vAIc values
10	should be larger for the dispersing sex, since this group will contain both resident and
11	immigrant animals, thereby increasing the variance in AIc. Statistical significance was
12	assessed as a one-tailed test via a randomization approach using 10,000 permutations
13	(Goudet et al. 2002). A one-tailed test was chosen because we acknowledged a priori that
14	males are more likely to disperse than females. Only animals of post-dispersal age (≥ 3
15	years old) were included in this analysis.

We utilized the clustering algorithm in program STRUCTURE described by Pritchard et al. (2000) to infer population delineations among all 6 sampling areas. Estimation of the number of subpopulations (K) was completed using 5 independent runs with K = 1-10 (assuming no prior population delineation information) at 100000 MCMC repetitions combined with a 100000 burn-in period. Burn-in period was selected after performing trial runs to determine when log-likelihood values of K became stationary. The optimal K value was chosen according to the highest estimated log normal probability

values (ln P (X | K)) obtained during Bayesian clustering calculations (Pritchard et al.
 2000).

3	We also assessed the likelihood that an individual's multilocus genotype could be
4	assigned to a given population using the program STRUCTURE (Pritchard et al. 2000).
5	Each individual sample was assigned to a subpopulation (K) derived from the preliminary
6	Bayesian analysis of population structure. Individuals were assigned to requisite
7	subpopulations according to the highest percentage of membership (posterior probabilities,
8	q) values that they were allocated.
9	We calculated Nei's (1972) standard genetic distance (D_S) using GENETIX 4.05 for
10	Big Bend NP, Burros and the Mogollons. This distance statistic has been demonstrated to
11	provide fine-scaled estimates of population structure in bears (Paetkau et al. 1997).
12	Pairwise-genetic-distance values (D_S) were subsequently used to generate a neighbor-
13	joining tree using MEGA version 2.1 (Kumar et al. 2001) to visualize genetic
14	differentiation across these 3 major sampling areas.
15	Results
16	DNA from 141 black bears representing 6 sampling localities was amplified and
17	genotyped at 7 microsatellite loci (Appendix I). All loci were polymorphic with an average
18	of 9.29 alleles/locus and a range of 8 to 12. Observed heterozygosity (H _o) was typically \geq
19	H_e in sampled populations and bears in the Mogollons had the lowest level of H_e (Table 1).
20	Allelic richness values adjusted for sample size indicated that the Mogollons population
21	exhibited lower levels of allelic diversity when compared to populations in the Mexico-
22	Texas metapopulation (Table 1). Richness levels within the metapopulation were similar at

1	\sim 3.5 alleles. Assessment of HWE via an exact test using the Markov chain parameters
2	denoted that the assumptions of HWE were violated only once at locus G1D in the Big
3	Bend NP population. The recent nature of the recolonization event in this study area,
4	partial isolation of the habitat, and the impacts of periodic immigration are the potential
5	causes of this deviation. The test for linkage disequilibrium among the 6 populations and 7
6	loci found no critical departures from equilibrium. The $P_{(ID)sibs}$ for the 7 loci utilized in
7	these analyses was 0.001, denoting that genotypic diversity was sufficient to delineate
8	individuals with these genetic markers.
9	Pairwise comparisons of F_{ST} and ϕ_{ST} (Onorato et al. 2004a) amongst Big Bend NP,
10	the Burros and Mogollons inferred significant levels of structuring between populations in
11	the Mexico-Texas metapopulation and the Mogollon Mountains population (Table 2). The
12	combined results from maternally inherited mtDNA and biparentally inherited nuclear
13	DNA for Big Bend NP and the Burros are indicative of a species that demonstrates male
14	biased dispersal and female philopatry. Furthermore, genotypic data corroborated and
15	reinforced the notion that dispersal in black bears in this region is male biased. The mAIc
16	score was lower for the 34 post-dispersal males as opposed to the 43 adult females (-0.481
17	vs. 0.380, respectively; randomization test, $p = 0.069$), denoting that males were more
18	likely to contain genotypes indicative of dispersing individuals. The variance associated
19	with the assignment index was greater for males as opposed to females (vAIc males = 8.09 ,
20	females = 4.33; randomization test, $p = 0.054$).

Standard genetic distances (*D_S*) calculated between Big Bend NP, the Burros and
Mogollons supported results from *F*-statistics, revealing a high level of genetic distance

1	between populations located in Texas and Mexico and the population of bears from the
2	Mogollons of west-central New Mexico ($D_s = 1.653$ and 1.617 for comparisons between
3	the Mogollons vs. Big Bend NP and Burros respectively). Assessment of mean genetic
4	distances between Big Bend NP and the Burros alludes to much lower levels of
5	differentiation ($D_s = 0.185$). An unrooted neighbor-joining tree of D_s values visually
6	illustrates the level of differentiation between bear populations in the Mexico-Texas
7	metapopulation and bears located in the Mogollons of west-central New Mexico (Figure 2).
8	The Bayesian analysis of population structure revealed that the 6 collection sites
9	could be clustered into 3 populations. The mean likelihood value for 5 independent runs
10	was greatest at $K = 3$. Sampling areas could then be assigned to 1 of 3 groups: 1) Big Bend
11	NP in Texas; 2) Mogollon Mountains of New Mexico; and 3) those bears residing in
12	northern Mexico (Burros and Carmens) and remaining areas of western Texas (Black Gap
13	and the Trans-Pecos). Subsequently, these 3 population designations were used in
14	assignment testing. Mean values of the percentage of membership (q) provided further
15	credence to the elevated level of structuring between the Mogollon population and bears in
16	the Mexico-Texas metapopulation (Table 3 and Figure 3). The elevated number of
17	individuals with inferred mixed ancestry (i.e., individuals with memberships allocated to
18	both groups 1 and 3) in northern Mexican populations, the Trans-Pecos and Black Gap
19	exemplifies the bidirectional gene flow that is occurring between these areas and Big Bend
20	NP.
21	Discussion

The present study, coupled with previous work (Onorato and Hellgren 2001,
Onorato et al. 2003, Onorato et al. 2004a), revealed several important characteristics

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1	concerning metapopulation dynamics of American black bears in the western Texas-
2	Mexico borderland of southwestern North America. Overall levels of H_e for each of the 6
3	southwestern populations ranged from 0.499 to 0.707, which is comparable to values
4	reported by Paetkau and Strobeck(1994) for Canadian black bears (0.360-0.801) and higher
5	than those cited by Warrillow et al. (2001) for disjunct black bear populations in the
6	southeastern United States (0.390-0.560). Interestingly, none of the newly established
7	populations sampled in Texas (Big Bend NP and Black Gap) exhibited He values as low as
8	those found in insular populations (0.360 in Newfoundland; Paetkau and Strobeck 1994) or
9	other areas that may be impacted by low levels of gene flow between populations (0.33 in
10	White River National Wildlife Refuge, Arkansas USA; Warrillow et al. 2001). In several
11	studies conducted with large carnivores, such as brown bears (U. arctos) and wolverines
12	(<i>Gulo gulo</i>), H_e values are typically < 0.60 for populations that are isolated from the effects
13	of migration and dispersal from other populations due to anthropogenic factors or natural
14	barriers (Paetkau et al. 1998; Waits et al. 2000; Kyle & Strobeck 2001). Although the
15	desert ecosystem surrounding populations in Black Gap and Big Bend NP impedes
16	migration or dispersal, it apparently is not a complete barrier to periodic movements from
17	mountains in northern Coahuila to parts of western Texas. Our field observations have
18	attested to bidirectional movement of male and female black bears between the Carmens
19	and Big Bend NP (Hellgren et al. 2005) and this movement is corroborated by mtDNA
20	analyses (Onorato et al. 2004a).
21	Combining our configurate diag of mtDNA (Operate at al. 2004a) with work on

Combining our earlier studies of mtDNA (Onorato et al. 2004a) with work on
biparentally inherited nuclear DNA markers demonstrated that both types of data are
needed for more complete deductions concerning phylogeography, population structure,

1	and dispersal patterns of black bears and other species. For example, our combined data
2	sets provide a more accurate assessment of how populations of bears in the Mexico-Texas
3	metapopulation are linked by gene flow. Previous research using maternally inherited
4	mtDNA demonstrated a high degree of genetic structuring between the populations in the
5	Mogollons and Big Bend NP when compared to other populations in the Mexico-Texas
6	ecosystem (Onorato et al. 2004a). Reanalysis of these sequence data incorporating only
7	data from Big Bend NP and the Burros continued to reveal high levels of differentiation
8	($\phi_{ST} = 0.5636$), yet biparentally inherited microsatellite data showed low levels of genetic
9	structuring between these two populations ($F_{ST} = 0.0580$). The lack of concordance
10	between mtDNA and nuclear microsatellite data has been noted in other populations of
11	large carnivores, including brown bears in Scandinavia (Waits et al. 2000) and wolverines
12	from Canada (Chappell et al. 2004), and is often ascribed to differing patterns of male and
13	female gene flow. Our analyses that assessed the presence of sex-biased dispersal patterns
14	in Big Bend NP, the Burros and the Mogollons revealed that dispersal is biased towards
15	males, although our results only trended towards significance. Male biased dispersal and
16	female philopatry are commonly accepted demographic characteristics within populations
17	of Ursids (Rogers 1987; Taberlet et al. 1995; Woods et al. 1999). Ultimately, it is the
18	periodic dispersal of males between Mexico and Texas (and vice-versa) that results in the
19	genotypic homogenization of the metapopulation. Regardless of the molecular marker used
20	to delineate genetic structuring, our analyses suggested that recolonizing populations in
21	western Texas are intimately linked with populations in the Burros and Carmens.

1	The Bayesian approach to population structure did however elucidate a level of
2	structure between the Big Bend NP population and other sampling areas to warrant
3	subpopulation status for this study area. The differentiation is likely a result of a
4	matriarchal founder effect associated with the recolonization of Big Bend NP (Onorato et
5	al. 2004a; 2004b) and the sporadic nature of successful dispersal and migration of black
6	bears from Mexico to the Park. Similar results have been noted for carnivore species such
7	as wolverines (Gulo gulo) and Asiatic black bears (Ursus thibetanus) that persist in isolated
8	populations resulting from human habitat fragmentation (Saitoh et al. 2001; Cegelski et al.
9	2003).
10	Standard genetic distance values (D_S) exhibited similar trends to the structural
11	statistics in characterizing the difference in genotypes present in the Mogollons versus
12	those in the Mexico-Texas metapopulation. Similar relationships have been observed using
13	D_S and F_{ST} in several species of large carnivores (Kyle & Strobeck 2001; Rueness et al.
14	2003). This analysis again demonstrated the low probability for dispersal linkages for
15	black bears between the Mogollons and western Texas. Conversely, within the Mexico-
16	Texas metapopulation, there were low levels of genetic distance. These data suggest the
17	semi-permeable nature of the Chihuahuan Desert barrier to dispersal by bears. This
18	conclusion is consistent with our knowledge of recolonization of black bears in western
19	Texas and their association with populations in northern Coahuila (Onorato and Hellgren
20	2001; Hellgren et al. 2005).
21	Population assignment testing using the Bayesian analysis in program
22	STRUCTURE depicted the elevated mean percentage of membership of the bears in the

23 Mogollon Mountains of New Mexico (q = 0.982, 96.6% of bears correctly assigned at q >

1	0.9). Once again, this high level of correct assignment is a result of the geographic
2	separation between bears in New Mexico and those in the Mexico-Texas metapopulation.
3	This separation and apparent lack of gene flow has ultimately led to differentiation at both
4	maternally (Onorato et al. 2004a) and biparentally inherited markers. Big Bend NP also
5	exhibited an elevated mean percentage of membership ($q = 0.867, 78.1\%$ correctly
6	assigned), consistent with our scenario that most bears in the Park are descendents of a
7	matriarch female that recolonized the Chisos in the 1980's (Onorato and Hellgren 2001).
8	An analysis of paternity and relatedness of bears in Big Bend NP resulted in a pedigree that
9	corroborates this conclusion (Onorato et al. 2004b).
10	The level of correct population assignment of bears in Big Bend NP was
11	comparable to 78% correctly assigned in a study by Warrillow et al. (2001). Several
12	brown bear studies exhibited higher percentages of correct assignment (92%'; Paetkau et al.
13	1998; 84%'; Waits et al. 2000). A study by Paetkau et al. (1995) on 4 populations of polar
14	bears (U. maritimus) obtained a much lower level of correct assignment (60%) and even
15	lower levels have been described for wolverines (43%`; Kyle & Strobeck 2001; 56%`; Kyle
16	& Strobeck 2002). The lower levels of correct population assignment for polar bears and
17	wolverines may result from the biology of these animals, which requires that they maintain
18	large home ranges and disperse greater distances (Garner et al. 1994; Belikov & Boltunov
19	1998; Kyle & Strobeck 2001), ultimately resulting in the increased probability of gene flow
20	between populations. Although brown and black bears of either sex may disperse distances
21	> 50 km (McLellan & Hovey 2001; Hellgren et al. 2005), movements beyond this distance
22	are uncommon in females.

1 The high percentage of correct population assignment for populations of black bears 2 in the Mogollons is noteworthy. No bears from the Mogollons were assigned to any of the 3 populations within Mexico or Texas. Only 1 bear from the Mexico and Texas samples 4 exhibited a percentage of membership with the Mogollons that was > 0.04 (Bear FH-1, q =5 0.353, 0.631, and 0.016 for the Mogollons, Northern Mexico/Western Texas, and Big Bend 6 NP populations respectively). Previous analyses using mtDNA sequence data noted that 7 this bear contained a haplotype that was only present in bears located in Mogollons. This 8 particular male bear was a road-killed specimen collected at Fort Hancock in extreme 9 western Texas >300 km from the Mogollons. This dispersing individual may be an 10 indication of the potential for gene flow between New Mexico or Chihuahua and the Trans-11 Pecos region of Texas. Further research should involve sampling black bears in the 12 northern and western parts of the Trans-Pecos such as Guadalupe Mountains National Park, 13 the Davis Mountains (GMNP and Davis, respectively in Figure 1), Hudspeth, and El Paso 14 counties to quantify linkages between bear populations in New Mexico and Texas. The 15 Davis and Guadalupe Mountains historically contained black bear populations into the 16 early 1900's (Onorato and Hellgren 2001).

Three bears from Big Bend NP were identified as having a high probability of assignment (q > 0.55) to a Mexican/Western Texas population. These include 2 male bears (BIBE28 and BIBE30 with q = 0.780 and 0.853 respectively) and 1 female (BIBE5, q =0.581). These assignments help to attest the natural recolonization process that is ongoing in Big Bend NP. The synthesis of mitochondrial and nuclear data delineates the prevalence of male mediated gene flow within this metapopulation. Male bears are periodically supplementing genetic variation in the park via dispersal or migrations from adjacent range

1	in Mexico. Female gene flow occurs much less frequently. Although BIBE5 is potentially
2	a first generation migrant/dispersing female, it is also plausible that she resulted via a
3	copulation event between a resident Park female and a migrant/dispersing adult male. Of
4	additional merit is the case of BGWMA8, a male bear captured in the Trans-Pecos whom
5	had a high probability ($q = 0.979$) of originating from Big Bend NP. These examples
6	demonstrate the process of gene flow between sampling areas and its subsequent
7	importance to recolonization and conservation of black bears in the borderlands ecosystem.
8	The differentiation between bears in the Mogollons and those in the Mexico-Texas
9	metapopulation may be of interest from an evolutionary perspective. Whether black bears
10	in the Mogollons represent a different subspecies of black bears in comparison to those
11	inhabiting the Mexico-Texas metapopulation will require further investigation and
12	analyses. The modified distribution of black bear subspecies described by Larivière (2001)
13	denotes the possible presence of U. a. eremicus, U. a. machetes, and U. a. amblyceps
14	within this region.
15	Designating bears from these two ecoregions as separate ESU's (Moritz 1994b,
16	1995) is controversial, given the continual debate over the actual definition of an ESU
17	(Fraser & Bernatchez 2001). Additionally, limitations of our data make conclusions
18	concerning ESU status of these two regions contentious. Regardless of whether these two
19	areas should be considered ESU's, it is undeniable that they should be categorized as
20	distinct management units (MU's) as described by Moritz (1994a). The combination of
21	genetic and field data (Onorato et al. 2003) support the fact that very little, if any gene flow
22	occurs between populations in the Mogollons and those in the Mexico-Texas
23	metapopulation (Onorato et al. 2004a). Any proposed reintroduction of black bears in

1 southwestern North America should consider these data before proceeding. In specific regards to the Mexico-Texas metapopulation, it is apparent that uncommon events (e.g. 2 3 long range dispersal, particularly by females) are often key events in delineating the genetic 4 structure and maintaining variation within a metapopulation. Undoubtedly, efforts to 5 conserve and manage populations of black bears within the Mexico-Texas metapopulation 6 will require a bi-national effort via coordination between private landowners, federal and 7 state agencies and Mexican authorities to insure linkages within this region continue to 8 serve as bi-directional pathways for gene flow.

9

12

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9	
10	

1 Figure Legends

Figure 1. Range map depicting the distribution of American black bears (shaded) in the 2 3 southwestern part of North America (derived from Pelton et al. 1999). The 6 underlined 4 localities encompass areas from which tissue samples were collected for this study. The 5 Mexico-Texas mainland-island metapopulation includes Big Bend NP and Black Gap 6 (islands; n = 32 and n = 9 respectively) in western Texas, U.S.A. and the Carmens and Burros (mainland; n = 5 and n = 58 respectively) in Coahuila, Mexico. Samples (n = 8) 7 8 collected throughout the Trans-Pecos region of western Texas were grouped together for 9 several analyses. Black bears were also sampled in the Mogollon Mountains of west-10 central New Mexico, U.S.A (n = 29) to assess differentiation between this population and 11 the Mexico-Texas metapopulation. The Davis Mountains and Guadalupe Mountain 12 National Park (GMNP) are noted in the discussion and formerly sustained black bear 13 populations. 14 Figure 2. Assessment of genetic distinctiveness for the 3 major populations of American 15 black bears using Nei's genetic distance values (D_s) in an unrooted neighbor-joining tree. 16 The scale of the branches is relative to the differences in D_{S} . 17 Figure 3. Plot of STRUCTURE population assignment results coinciding with initial 18 analyses that designated samples from 6 sampling localities as originating from 3 groups. 19 Rows represent individuals and are grouped by sampling area and bars represent the 20 proportion of ancestry attributed to each of the 3 groupings. Ancestry proportions assigned 21 to Big Bend NP are in light grey, northern Mexico and remaining areas of western Texas in 22 white, and the Mogollons in dark grey.

Burros

Carmens

Trans-Pecos

Mogollons

Overall

46

27

30

36

1	Table 1. Genetic variation of American black bears assessed at 7 microsatellite loci for 6								
2	sampling localities in southwestern North America. Abbreviations include observed and								
3	expected heteroz	ygosity (H _o	and He respectiv	vely), and allelic	richness adjusted	l for sample			
4	size (rg). Sampling areas are depicted geographically in Figure 1.								
						Total no.			
	Population	n	Ho	H _e	rg	alleles			
	Big Bend NP	32	0.722	0.682	3.57	37			
	Black Gap	9	0.768	0.662	3.43	28			

0.707

0.662

0.631

0.499

0.717

0.732

0.657

0.680

0.493

0.641

3.70

3.59

3.43

2.87

58

5

8

29

141

5

1 Table 2. Pairwise comparison of mtDNA genetic structure (ϕ_{ST} from Onorato et al. 2004,

- 2 above the diagonal) and microsatellite structure (F_{ST} , below the diagonal) for the 3 major
- 3 sampling areas of American black bears. Significant differentiation (*) for comparisons
- 4 was determined using the Bonferroni correction for pairwise comparisons (k = 3, ∞ = 0.05 /
- 5 3 = P < 0.017). Sampling area locations as depicted in Figure 1.

Big Bend NP 0.5636* 0.7366* Burros 0.0580* 0.7040* Mogollons 0.3482* 0.3265*	Population	Big Bend NP	Burros	Mogollons
Burros 0.0580* 0.7040* Mogollons 0.3482* 0.3265*	Big Bend NP		0.5636*	0.7366*
Mogollons 0.3482* 0.3265*	Burros	0.0580*		0.7040*
0.5102 0.5205	Mogollons	0.3482*	0.3265*	

1 Table 3. Results of assignment tests using the Bayesian analysis in program

- 2 STRUCTURE. Mean values of the percentage of membership (q) from each sampling area
- 3 and subsequent standard deviations are noted. The number of bears assigned a q < 0.9 is an
- 4 indication of potential migrants/dispersers in the population or the presence of admixture.

		K			
				Northern Mexico/Trans-	-
Sampling Areas	N	Big Bend NP	Mogollons	Pecos/Black Gap	q < 0.9
Big Bend NP	32	0.867 (0.229)	0.005 (0.005)	0.128 (0.226)	7
Carmens	5	0.180 (0.272)	0.006 (0.003)	0.814 (0.270)	2
Black Gap	9	0.456 (0.322)	0.005 (0.003)	0.538 (0.324)	9
Burros	58	0.302 (0.307)	0.006 (0.006)	0.692 (0.307)	38
Trans-Pecos	8	0.395 (0.441)	0.049 (0.123)	0.557 (0.413)	5
Mogollons	29	0.007 (0.006)	0.982 (0.036)	0.011 (0.030)	1

5

1 APPENDIX

- 2 Table A1. Allele frequency distribution for 7 microsatellite loci collected from American
- 3 black bears in 6 localities in southwestern North America.

Locus and allele	Big Bend NP	Black Gap	Trans-Pecos	Carmens	Burros	Mogollons
G1D						
172	0.000	0.000	0.000	0.000	0.103	0.241
174	0.000	0.000	0.000	0.000	0.000	0.017
176	0.188	0.333	0.250	0.200	0.164	0.035
180	0.000	0.000	0.000	0.000	0.000	0.103
182	0.078	0.000	0.000	0.100	0.052	0.000
184	0.469	0.278	0.188	0.100	0.276	0.448
186	0.094	0.389	0.563	0.600	0.397	0.155
188	0.172	0.000	0.000	0.000	0.009	0.000
G10B						
155	0.109	0.167	0.000	0.000	0.000	0.000
157	0.469	0.611	0.563	0.400	0.588	0.000
159	0.000	0.000	0.000	0.000	0.000	0.017
161	0.125	0.111	0.063	0.300	0.184	0.000
163	0.250	0.056	0.000	0.100	0.158	0.138
165	0.047	0.000	0.188	0.100	0.035	0.672
167	0.000	0.056	0.125	0.100	0.035	0.172
175	0.000	0.000	0.063	0.000	0.000	0.000

Locus and allele	Big Bend NP	Black Gap	Trans-Pecos	Carmens	Burros	Mogollons
G10C						
95	0.000	0.000	0.000	0.000	0.000	0.052
97	0.000	0.000	0.063	0.000	0.000	0.828
101	0.000	0.000	0.000	0.000	0.009	0.103
103	0.000	0.000	0.063	0.000	0.000	0.000
105	0.000	0.000	0.063	0.000	0.000	0.000
107	0.141	0.000	0.188	0.400	0.053	0.000
109	0.234	0.167	0.063	0.000	0.158	0.017
111	0.266	0.167	0.188	0.300	0.211	0.000
113	0.359	0.556	0.313	0.300	0.518	0.000
117	0.000	0.000	0.063	0.000	0.018	0.000
121	0.000	0.111	0.000	0.000	0.026	0.000
129	0.000	0.000	0.000	0.000	0.009	0.000
G10P						
163	0.156	0.222	0.071	0.000	0.158	0.000
167	0.109	0.111	0.571	0.500	0.368	0.000
169	0.000	0.000	0.000	0.000	0.000	0.552
171	0.016	0.000	0.000	0.100	0.018	0.035
173	0.188	0.000	0.214	0.000	0.000	0.035
175	0.406	0.444	0.071	0.200	0.290	0.103
177	0.000	0.000	0.000	0.000	0.000	0.224
179	0.125	0.222	0.071	0.200	0.167	0.052

Locus and allele	Big Bend NP	Black Gap	Trans-Pecos	Carmens	Burros	Mogollons
CXX20						
122	0.156	0.333	0.214	0.400	0.103	0.035
124	0.000	0.000	0.000	0.100	0.052	0.000
126	0.031	0.000	0.000	0.000	0.000	0.000
128	0.078	0.000	0.000	0.000	0.095	0.000
130	0.000	0.000	0.000	0.000	0.000	0.828
132	0.000	0.000	0.000	0.000	0.000	0.052
136	0.000	0.167	0.000	0.000	0.017	0.017
138	0.266	0.278	0.429	0.300	0.440	0.017
140	0.297	0.222	0.071	0.200	0.181	0.000
142	0.172	0.000	0.286	0.000	0.069	0.000
144	0.000	0.000	0.000	0.000	0.000	0.052
148	0.000	0.000	0.000	0.000	0.043	0.000
G10H						
233	0.033	0.250	0.000	0.375	0.116	0.804
235	0.150	0.188	0.300	0.250	0.071	0.071
237	0.733	0.500	0.500	0.375	0.384	0.000
239	0.05	0.000	0.200	0.000	0.321	0.000
241	0.000	0.000	0.000	0.000	0.018	0.125
243	0.000	0.000	0.000	0.000	0.036	0.000
245	0.017	0.000	0.000	0.000	0.027	0.000

Locus and allele	Big Bend NP	Black Gap	Trans-Pecos	Carmens	Burros	Mogollons
G10H (cont')						
247	0.017	0.063	0.000	0.000	0.027	0.000
243	0.000	0.000	0.000	0.000	0.036	0.000
245	0.017	0.000	0.000	0.000	0.027	0.000
247	0.017	0.063	0.000	0.000	0.027	0.000
G10J						
83	0.233	0.000	0.400	0.100	0.118	0.000
85	0.417	0.389	0.600	0.400	0.300	0.018
87	0.050	0.333	0.000	0.000	0.273	0.018
93	0.000	0.000	0.000	0.000	0.000	0.232
95	0.000	0.000	0.000	0.000	0.000	0.054
101	0.267	0.167	0.000	0.100	0.200	0.446
103	0.033	0.111	0.000	0.400	0.100	0.107
105	0.000	0.000	0.000	0.000	0.000	0.125
113	0.000	0.000	0.000	0.000	0.009	0.000

Figure 1 powerpoint file Click here to download attachment to manuscript: Onorato fig 1 Con Gen.ppt



Figure 2 powerpoint file Click here to download attachment to manuscript: Onorato Fig 2 Con Gen.ppt



Fig 3.doc (color used for grey scale effect when printing) Click here to download attachment to manuscript: Cong Gen Fig3 Onorato.doc

