

Genetic consequences of habitat fragmentation and loss: the case of the Florida black bear (*Ursus americanus floridanus*)

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Abstract Habitat loss and fragmentation can influence the genetic structure of biological populations. We studied the genetic consequences of habitat fragmentation in Florida black bear (*Ursus americanus floridanus*) populations. Genetic samples were collected from 339 bears, representing nine populations. Bears were genotyped for 12 microsatellite loci to estimate genetic variation and to characterize genetic structure. None of the nine study populations deviated from Hardy–Weinberg equilibrium. Genetic variation, quantified by mean expected heterozygosity (H_E), ranged from 0.27 to 0.71 and was substantially lower in smaller and less connected populations. High levels of genetic differentiation among populations (global $F_{ST} = 0.224$; global $R_{ST} = 0.245$) suggest that fragmentation of once contiguous habitat has resulted in

genetically distinct populations. There was no isolation-by-distance relationship among Florida black bear populations, likely because of barriers to gene flow created by habitat fragmentation and other anthropogenic disturbances. These factors resulted in genetic differentiation among populations, even those that were geographically close. Population assignment tests indicated that most individuals were genetically assigned to the population where they were sampled. Habitat fragmentation and anthropogenic barriers to movement appear to have limited the dispersal capabilities of the Florida black bear, thereby reducing gene flow among populations. Regional corridors or translocation of bears may be needed to restore historical levels of genetic variation. Our results suggest that management actions to mitigate genetic consequences of habitat fragmentation are needed to ensure long-term persistence of the Florida black bear.

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Introduction

The fragmentation and loss of habitat is one of the most serious problems facing the conservation of biodiversity worldwide (Harris 1984; Meffe and Carroll 1997). Habitat fragmentation can increase mortality rates (Jules 1998), reduce abundance (Flather and Bevers 2002), alter movement patterns (Brooker and Brooker 2002), disrupt the social structure of populations (Ims and Andreassen 1999; Cale 2003), and may reduce population viability (Harrison and Bruna 1999;

Davies et al. 2001). Additionally, habitat fragmentation can lead to isolation of populations and reduction in population size which may cause a decrease in genetic variation (Frankham 1996). The loss of genetic variation may reduce the ability of individuals to adapt to a changing environment, cause inbreeding depression (Ebert et al. 2002), reduce survival and reproduction (Frankham 1995; Reed and Frankham 2003) and increase the probability of extinction (Saccheri et al. 1998; Westemeier et al. 1998).

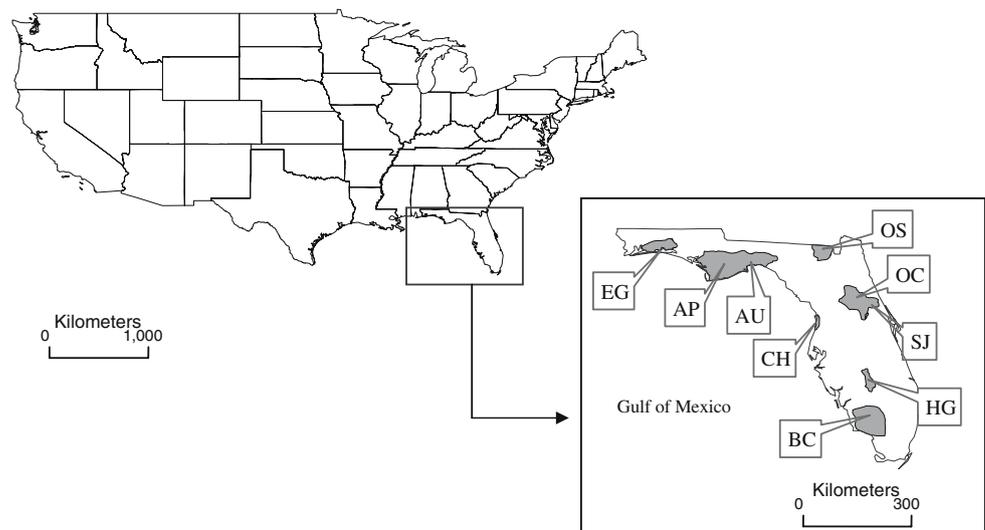
Populations that occur within contiguous habitats are expected to follow an isolation-by-distance model, where the distance between populations is the overriding factor contributing to genetic differentiation (Slatkin 1993). However, the process of habitat fragmentation can create dispersal barriers, which can deter gene flow and lead to isolation of populations (Hitchings and Beebee 1997; Gerlach and Musolf 2000).

Large mammalian carnivores are particularly vulnerable to habitat loss and fragmentation because of their relatively low numbers, large home ranges, and interactions with humans (Noss et al. 1996; Crooks 2002). The plight of the Florida panther (*Puma concolor coryi*) and the giant panda (*Ailuropoda melanoleuca*) are examples of large carnivores that have been reduced to small numbers due largely to the impacts of habitat fragmentation and loss. As a result of the low fecundity and long generation times, large carnivores tend to have reduced levels of genetic variation (Roelke et al. 1993; Lu et al. 2001). Another large carnivore that has been negatively impacted by habitat fragmentation is the Florida black bear (*Ursus americanus floridanus*) (Hellgren and Maehr 1993).

The Florida black bear historically roamed throughout the peninsula of Florida and southern portions of Georgia, Alabama and Mississippi (Brady and Maehr 1985). From the 1800s to the 1970s, numbers of Florida black bears were significantly reduced due to loss and fragmentation of habitat and unregulated hunting (Cory 1896; Hendry et al. 1982). Only an estimated 300–500 bears remained in the state of Florida in the 1970s (McDaniel 1974; Brady and Maehr 1985). Consequently, the Florida Game and Freshwater Fish Commission classified the Florida black bear as a threatened species in most Florida counties in 1974 (Wooding 1993). Destruction and fragmentation of once contiguous habitat has reduced the distribution of Florida black bears to nine disjunct populations: Eglin (EG), Apalachicola (AP), Aucilla (AU), Osceola (OS), Ocala (OC), St. Johns (SJ), Chassahowitzka (CH), Highlands/Glades (HG) and Big Cypress (BC) (Fig. 1).

Fragmentation of populations can reduce genetic variation (Sherwin and Moritz 2000), gene flow (Vos et al. 2001) and increase the probability of extinction (Saccheri et al. 1998; Westemeier et al. 1998), but the genetic consequences of habitat fragmentation on Florida black bear populations are unknown. Using microsatellite analyses, our objectives were to estimate within-population genetic variation, and to investigate the level of genetic differentiation among populations. Theory predicts a positive correlation between genetic variation and population size (Frankham 1996), and between genetic differentiation and geographic distance among populations (Slatkin 1993). Thus, we tested these predictions by examining the relationship between measures of genetic variation and recent

Fig. 1 Geographic distribution of the Florida black bear populations in Florida. Populations are: Eglin (EG), Apalachicola (AP), Aucilla (AU), Osceola (OS), Ocala (OC), St. Johns (SJ), Chassahowitzka (CH), Highlands/Glades (HG), and Big Cypress (BC). The distribution map was compiled by the Florida Fish and Wildlife Conservation Commission



estimates of population size, and between measures of genetic differentiation and geographic distances among populations. Finally, we used population assignment tests to identify potential dispersers among our study populations.

Methods

Hair and tissue samples from individual bears were collected from Florida black bear populations during 1989–2004. The majority of samples were collected from field studies, some using non-invasive techniques (Woods et al. 1999), but samples also were collected from translocated animals and from bears killed on roadways. Hair and tissue samples were sent to Wildlife Genetics International (<http://www.wildlifegenetics.ca/>) for microsatellite analysis. DNA was extracted using QIAGEN's DNeasy Tissue kits, as per QIAGEN's instructions (<http://www.qiagen.com/literature/genomlit.asp>), and microsatellite loci were amplified using polymerase chain reaction (PCR). Each individual was genotyped for 12 microsatellite loci G1A, G10B, G1D, G10L, G10C, G10M, G10P, G10X (Paetkau et al. 1995) G10H, G10J (Paetkau et al. 1998) MU50, and MU59 (Taberlet et al. 1997). Laboratory methods used in this study are described in detail by Paetkau and Strobeck (1994), Paetkau et al. (1998), and Paetkau (2003). Individual genotypes were reanalyzed when genotypes matched at all but one or two of the 12 markers. Error checking and quality control were performed according to the methods described in Paetkau (2003).

Departures from Hardy–Weinberg equilibrium (HWE) were tested using the HWE probability test in Genepop 3.4 (Raymond and Rousset 1995). Exact *P*-values were computed using the complete enumeration method for loci with fewer than four alleles (Louis and Dempster 1987) and the Markov chain method (dememorization 1000; batches 100; iterations per batch 1000) for loci with more than four alleles (Guo and Thompson 1992). Using this same program, linkage disequilibrium tests were used to test for non-random associations between alleles of different loci using the Markov chain method. The *P*-values were adjusted using a Bonferroni sequential correction for multiple comparisons (Rice 1989).

Within each bear population, genetic variation was measured as the observed average heterozygosity (H_O), expected average heterozygosity (H_E), and the average number of alleles per locus (*A*). Spearman's rank correlation was used to test for the correlation between genetic variation and estimated population size.

Genetic differentiation was estimated using Genepop 3.4 (Raymond and Rousset 1995) with global F_{ST} (across all populations), pairwise F_{ST} (Weir and Cockerham 1984) and pairwise R_{ST} (Michalakis and Excoffier 1996). The significance of population differentiation was tested using the genic differentiation test in Genepop 3.4, and *P*-values were adjusted for multiple comparisons using a Bonferroni sequential correction (Rice 1989). The likelihood ratio genetic distance, D_{LR} (Paetkau et al. 1995) was estimated for each pair of populations using the Doh assignment calculator (<http://www2.biology.ualberta.ca/jbrzusto/Doh.php>). This genetic distance is based on the ratio of genotype likelihoods between pairs of populations. The software program Phylip 3.5c (Felsenstein 1993) and the subprogram FITCH (Fitch and Margolia 1967) were used to generate an unrooted phylogenetic tree, with branch lengths corresponding to D_{LR} values.

Geographic distances among populations were estimated as the shortest land distance between population centroids using least cost path analysis in ArcGIS 8.1.2 (McCoy and Johnston 2000). Centroids were estimated as the harmonic mean of the sample collection locations in each study site. The subprogram ISOLDE in Genepop 3.4 (Raymond and Rousset 1995) was used to test for a relationship between geographic distances, and F_{ST} , R_{ST} , and D_{LR} values. Statistical significance of these relationships was tested using a Mantel test (Mantel 1967) with 10,000 permutations.

We used the population assignment test implemented in program STRUCTURE to assign bears to a cluster or population based on their genotypes without regard to where samples were collected (Pritchard et al. 2000). Allele frequencies were assumed independent and analyses were conducted with 100,000 iterations and 100,000 repetitions of Markov chain Monte Carlo. We used the no admixture model, which assumes that each individual comes purely from one of *K* clusters. Analyses were conducted with the number of clusters (*K*) set from 1 to 15 to determine the likely number of clusters representative of the data (Pritchard et al. 2000).

Results

A total of 339 bears from nine populations were genotyped for 12 microsatellite loci (Table 1). When all but one or two of the loci were the same, we checked for genotyping errors by reexamining these results. The outcomes were replicated and the results were strong, clear, and consistent in every case.

Table 1 Measures of genetic variation^a at 12 microsatellite loci in nine Florida black bear populations

Population ^b	H_O	H_E	A
Apalachicola (40)	0.690 ± 0.036	0.708 ± 0.032	5.92 ± 0.358
Aucilla (40)	0.566 ± 0.046	0.590 ± 0.044	5.00 ± 0.369
Big Cypress (41)	0.642 ± 0.036	0.650 ± 0.026	5.50 ± 0.435
Chassahowitzka (29)	0.287 ± 0.058	0.271 ± 0.054	2.25 ± 0.179
Eglin (40)	0.613 ± 0.071	0.537 ± 0.062	4.08 ± 0.379
Highlands/Glades (28)	0.327 ± 0.049	0.384 ± 0.051	2.75 ± 0.250
Ocala (40)	0.579 ± 0.045	0.610 ± 0.045	4.75 ± 0.305
Osceola (41)	0.705 ± 0.030	0.713 ± 0.027	6.67 ± 0.225
St. Johns (40)	0.650 ± 0.048	0.663 ± 0.041	5.75 ± 0.494

^aMeasures of genetic variation (mean ± 1SE) are: observed average heterozygosity (H_O), expected average heterozygosity (H_E), and mean alleles per locus (A)

^bSample sizes are in parentheses

There were no significant departures from HWE for any locus or population ($P > 0.05$). The linkage disequilibrium test indicated that only 6% of loci pairings had significant non-random associations ($P < 0.05$). Loci used in this analysis have been found to be independent (D. Paetkau, pers. comm.). Thus, any significant linkage observed among loci pairs may be a result of non-random mating, sampling bias, recent admixture, or genetic drift (Frankham et al. 2002). We speculate that the majority of these non-random associations are primarily due to random genetic drift in these highly subdivided populations.

The population with the highest level of genetic variation was Osceola (mean ± 1SE; $H_E = 0.713 ± 0.027$). Chassahowitzka had the lowest level of genetic variation ($H_E = 0.271 ± 0.054$) (Table 1). Estimated population sizes ranged from 20 in Chassahowitzka (note that sample size for genetic analysis is higher than estimated population size because genetic samples were collected over several years) to 830 (including the adjacent Okefenokee population) in Osceola (Maehr et al. 2001; Dobey et al. 2005; Simek et al. 2005).

Although population size was positively related to all measures of genetic variation, the relationship was nonlinear (A : $r_s = 0.683$, $P = 0.042$; H_O : $r_s = 0.567$, $P = 0.112$; H_E : $r_s = 0.633$, $P = 0.067$ (Fig. 2). All measures of genetic variation increased initially as the population size increased, but this relationship approached an asymptote when population size reached about 200 bears (Fig. 2).

Global F_{ST} , the measure of population subdivision across all populations, was 0.224 (± 0.037). Estimates of F_{ST} ranged from 0.010 to 0.572 and R_{ST} ranged from 0.009 to 0.628 (Table 2). Pairwise F_{ST} indicated that gene flow was highest between Ocala and St. Johns, whereas Highlands/Glades and Chassahowitzka had the lowest levels of gene flow (Table 2). All tests of

genetic differentiation among populations were highly significant ($P < 0.001$). An unrooted phylogenetic tree based on D_{LR} values suggested that the Ocala and St. Johns populations were closely related, whereas Chassahowitzka, Highlands/Glades, and Eglin were the

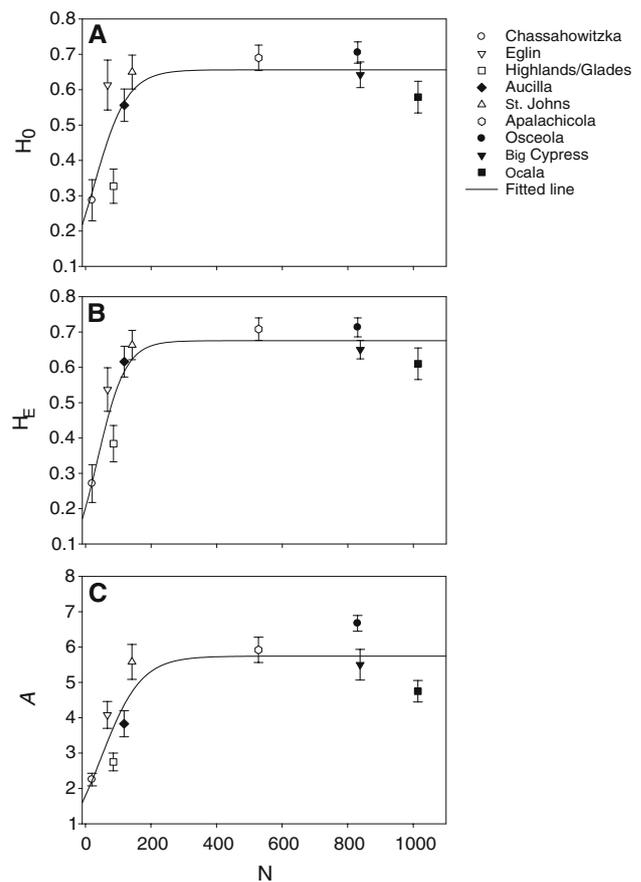


Fig. 2 Relationship between estimated population size (N) and (a) H_O (Observed average heterozygosity), (b) H_E (Expected average heterozygosity), and (c) A (Average alleles per locus). Estimates of population size are from most recent studies on each area

Table 2 Pairwise estimates of F_{ST} (below diagonal) and R_{ST} (above diagonal) for nine Florida black bear populations^a. Standard errors are in parentheses

	AP	AU	BC	CH	EG	HG	OC	OS	SJ
AP	0.1316 (±0.019)								
AU	0.0751 (±0.027)	0.1357 (±0.034)							
BC	0.1389 (±0.026)	0.2114 (±0.047)	0.1357 (±0.034)						
CH	0.3572 (±0.040)	0.1934 (±0.021)	0.3457 (±0.067)	0.3457 (±0.067)					
EG	0.1638 (±0.029)	0.3993 (±0.052)	0.3748 (±0.046)	0.4846 (±0.065)	0.1562 (±0.062)				
HG	0.2964 (±0.038)	0.3229 (±0.056)	0.2348 (±0.032)	0.5724 (±0.064)	0.0976 (±0.029)	0.4180 (±0.045)			
OC	0.1614 (±0.030)	0.1915 (±0.032)	0.1360 (±0.029)	0.3906 (±0.067)	0.3026 (±0.073)	0.4349 (±0.094)	0.2012 (±0.049)		
OS	0.1160 (±0.023)	0.1694 (±0.025)	0.1277 (±0.032)	0.3483 (±0.049)	0.5472 (±0.087)	0.2440 (±0.063)	0.2139 (±0.050)	0.0719 (±0.043)	
SJ	0.1414 (±0.033)	0.1814 (±0.035)	0.1211 (±0.018)	0.3586 (±0.052)	0.3986 (±0.067)	0.6275 (±0.074)	0.2847 (±0.071)	0.1192 (±0.034)	0.2225 (±0.049)
					0.2239 (±0.056)	0.5141 (±0.088)	0.1062 (±0.029)	0.1422 (±0.051)	0.2493 (±0.065)
					0.2243 (±0.035)	0.2713 (±0.035)	0.0100 (±0.004)	0.3443 (±0.078)	0.0849 (±0.037)
						0.3062 (±0.044)		0.1477 (±0.055)	0.3429 (±0.062)
						0.2232 (±0.036)		0.3787 (±0.050)	0.3195 (±0.074)
								0.0842 (±0.014)	0.1571 (±0.049)
									0.0095 (±0.029)
									0.1344 (±0.042)

^aPopulations are: Apalachicola (AP), Aucilla (AU), Big Cypress (BC), Chassahowitzka (CH), Eglin (EG), Highlands/Glades (HG), Ocala (OC), Osceola (OS), and St. Johns (SJ). See Fig. 1 for geographic locations of these populations

most divergent of all populations (Fig. 3). There was no significant relationship between geographic distance and F_{ST} ($P = 0.253$), R_{ST} ($P = 0.089$), or D_{LR} ($P = 0.104$).

Population assignment tests indicated that bears sampled from the nine populations had the highest probability of assignment into eight clusters according to the estimated log probability of data [$\ln P(D)$]. At $K = 7$, $\ln P(D)$ was -9852 . At $K = 8$, $\ln P(D)$ plateaus at -9481 . At $K = 9$, $\ln P(D)$ slightly decreases to -9410 , clearly making $K = 8$ the choice for the most logical distribution of populations according to Pritchard et al. (2000). These results suggest that Ocala and St. Johns populations are genetically identical, and should be grouped into the same population. The majority of individuals were assigned to the population where they were sampled. The Apalachicola, Aucilla, Big Cypress, Highlands/Glades, and Osceola populations had immigrants that had originated from neighboring populations (Table 3). Two individuals sampled from Osceola did not meet the minimum criteria to be assigned to a single cluster, suggesting that these two individuals may be offspring of parents from two different populations (Dixon et al. 2006).

Discussion

Habitat fragmentation can reduce genetic variation, which can adversely influence fitness [e.g. the Florida panther (Roelke et al. 1993)], increase susceptibility to disease [e.g. cheetah (*Acinonyx jubatus*) (O'Brien 1994)], and decrease population viability (Sherwin and Moritz 2000). Habitat fragmentation and hunting are thought to be responsible for the loss of genetic variation in wolverines (*Gulo gulo*) (Kyle and Strobeck 2001), lynx (*Lynx lynx*) (Spong and Hellborg 2002), mountain lions (*Puma concolor*) (Ernest et al. 2003), Ethiopian wolves (*Canis simensis*) (Gottelli et al. 1994) and brown bears (*Ursus arctos*) (Miller and Waits 2003). Large carnivores may be much more susceptible than other taxa to losses in genetic variation due to habitat fragmentation because of their large home ranges, low population densities, and long generation times (Paetkau and Strobeck 1994; Johnson et al. 2001).

The genetic variation in most Florida black bear populations was within the range reported for other black bear populations in North America using > 5 of the same microsatellite loci (Paetkau and Strobeck 1994; Warrillow et al. 2001; Marshall and Ritland 2002; Boerson et al. 2003; Csiki et al. 2003). However,

Fig. 3 An unrooted phylogenetic tree depicting the genetic relationships among Florida black bear populations. Branch lengths correspond to the likelihood ratio genetic distance, D_{LR} . D_{LR} values from some populations to the first node are displayed

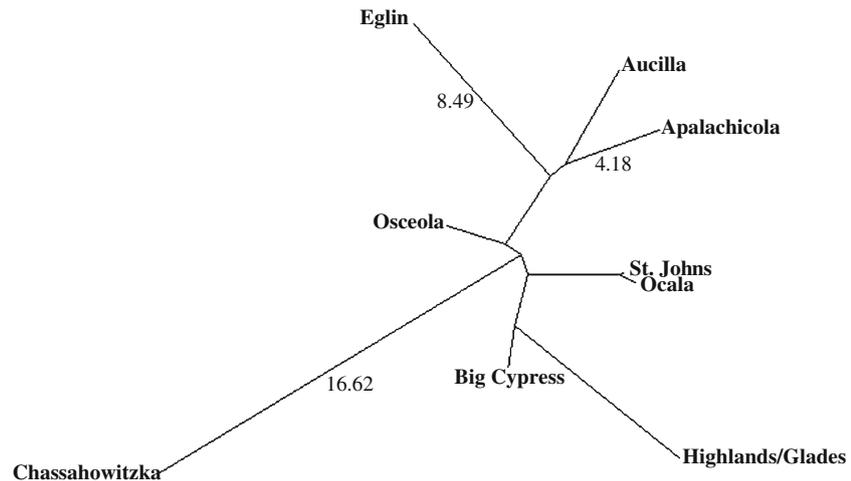


Table 3 Number of individuals assigned to eight clusters based on multilocus genotypes. Sample sizes are given in parentheses

Population	Clusters							
	1	2	3	4	5	6	7	8
Apalachicola (40)	38	2						
Aucilla (40)	3	36						
Big Cypress (41)			40			1		1
Chassahowitzka (29)				29				
Eglin (40)					40			
Highlands/Glades (28)			1			27		
Ocala ^a (40)								40
Osceola (41) ^b								2
St. Johns ^a (40)								40

^aIndividuals from the Ocala and St. Johns populations were assigned to the same cluster

^bTwo individuals sampled in the Osceola population did not meet the minimum values to be assigned to a single cluster

genetic variation in Chassahowitzka was the lowest reported for any black bear population. Chassahowitzka was characterized by a small population size, and accordingly, this population had the lowest level of genetic diversity. Osceola was characterized by a large population size because of its connection with the Okefenokee population and had the highest levels of genetic diversity.

The Chassahowitzka and Highlands/Glades populations are thought to have remained isolated from other Florida black bear populations for a longer period than any other Florida black bear population. The relatively low genetic variation in both the Chassahowitzka and Highlands/Glades populations is remarkable because these populations have supposedly been isolated from other populations for fewer than 100 years. Given what is believed to be historically small population sizes and our observation of low levels of genetic variation, we hypothesize that inbreeding depression might have reduced the fitness and persistence of these populations (Reed and

Frankham 2003). Symptoms of inbreeding depression, such as cryptorchidism, have been observed in other Florida black bears found in the western panhandle of Florida (Dunbar et al. 1996) and southern Alabama (Kasbohm and Bentzien 1998).

Most contiguous populations of black bears have high levels of genetic variation (Marshall and Ritland 2002). Thus, efforts should be made to restore historic levels of genetic variation within Florida black bear populations, using these figures as a baseline. This may be achieved by translocation of bears among populations, and by increasing local abundance. It has been suggested that a minimum of 50 effective breeders is needed to prevent inbreeding depression and population levels in the hundreds or thousands to maintain evolutionary potential (Franklin 1980; Lande 1995). Our results indicate that maintaining bear population size at ≥ 200 individuals will preserve most of the genetic variation. When population size falls below this level and there is little connectivity among populations, demographic and genetic stochastic events may lead to

further loss of genetic variation. In the Chassahowitzka population and possibly the Highlands/Glades population, local abundance may be area-limited and it is unlikely that these populations could approach the desired size of 200, thereby making genetic nourishment by other means critical.

The measures of genetic differentiation indicated that there was an extensive differentiation among Florida black bear populations, with Chassahowitzka, Highlands/Glades, and Eglin populations being most divergent. R_{ST} values were generally higher than the F_{ST} values suggesting that mutation rather than migration and drift are responsible for the differentiation among the populations (Frankham et al. 2002). The level of genetic differentiation among Florida black bear populations ($F_{ST} = 0.01$ – 0.57 , $R_{ST} = 0.01$ – 0.63) was substantially greater than those between other large carnivore populations (e.g. mountain lions ($F_{ST} = 0.01$ – 0.37 , $R_{ST} = 0.01$ – 0.42); (Ernest et al. 2003) wolverines ($F_{ST} = 0.01$ – 0.21 ; (Kyle and Strobeck 2001; Walker et al. 2001) lynx ($F_{ST} = 0.01$ – 0.29 ; (Hellborg et al. 2002; Schwartz et al. 2002).

The genetic differentiation among Florida black bear populations was substantial, although the average distance between nearest neighboring populations (134 km) is within the dispersal capabilities of black bears (Rogers 1987; Maehr et al. 1988). The global estimate of F_{ST} , the measure of population subdivision across all populations, was 0.224. This degree of subdivision is expected if there are on average 0.87 successful migrants entering each population per generation (approximately 8 years for black bears) assuming an island model of migration (Frankham et al. 2002). Therefore, on average across all Florida black bear populations, there is one successful migrant every 10 years, a relatively low level of gene flow. Dispersal of bears is sex-biased, and males typically disperse farther than females. Females tend to establish home ranges near their mother's home range (Rogers 1987; Schwartz and Franzmann 1992; Moyer et al. 2006). It has been suggested that dispersing black bears may be able to maintain connectivity among populations even when populations are fragmented (Noss et al. 1996; Maehr et al. 2001; Hellgren et al. 2005). These travels may be limited when there are significant barriers to movement preventing bears from moving among populations. High-volume roads and other anthropogenic barriers may have been responsible for the lack of isolation-by-distance relationship, which has been reported for other bear populations occupying contiguous habitats (Paetkau et al. 1997). Habitat fragmentation and other anthropogenic disturbances can create barriers to gene flow even among

populations that are geographically close, and this can lead to extensive genetic differentiation among populations as observed in our study.

Anthropogenic barriers such as roads or other human development, can limit species distribution and gene flow (Mader 1984). Although bears are able to cross some highways successfully (McCown et al. 2004), the impact of highways can be detrimental. From 2000 to 2005, at least 711 bears were documented as killed on roads in Florida. The majority of these were young males that may have been attempting dispersal or migration to distant populations (FWC, unpublished data). Additionally, highways and development can act as partial or complete barriers. Some bears may avoid interstate highways (Brody and Pelton 1989; Proctor et al. 2002), and other forms of human development may alter movement patterns (Maehr et al. 2003), further decreasing the probability of movement of bears among populations. Population fragmentation by roads has been reported to reduce the level of gene flow in several natural populations (e.g., moor frog *Rana arvalis*, (Vos et al. 2001) ground beetle *Carabus violaceus*, (Keller and Largiader 2003) and bank vole *Clethrionomys glareolus*, (Gerlach and Musolf 2000). Although large carnivores are thought to be highly vagile (Paetkau et al. 1999; Schwartz et al. 2002), our results and those of some other studies suggest that anthropogenic barriers can substantially reduce the gene flow among bear populations (Kyle and Strobeck 2001; Walker et al. 2001; Ernest et al. 2003).

Population assignment tests indicated that most bears had originated from the populations where they were sampled, and that the St. Johns and Ocala populations could potentially be considered a single population. We also found evidence of the presence of immigrants in five populations. However, it is not known if these immigrants were translocated nuisance bears, or dispersers from the source populations. Nonetheless, there exists the strong possibility that some adjacent populations (i.e., Apalachicola and Aucilla, and Ocala and Osceola) may be connected via dispersal.

These dispersal events and the resulting gene flow among bear populations is needed to restore and maintain genetic variation (Waits 1999). A minimum of one and a maximum of 10 successful migrants per generation has been suggested as a rule of thumb to maintain levels of genetic variation (Mills and Allendorf 1996). We suggest that Florida black bear populations should be managed as a metapopulation so that gene flow can occur among populations connected with conservation corridors (Craighead and Vyse 1996; Maehr et al. 2001; Larkin et al. 2004). However, the

effectiveness of corridors in maintaining gene flow among populations of carnivores is not well understood (Beier and Noss 1998). A recent study has shown that the corridor between the Ocala and Osceola populations is functional and facilitates gene flow between these populations (Dixon et al. 2006); similar studies are needed to evaluate the functionality of potential corridors. Additionally, wildlife crossing structures may be needed to allow safe passage for bears across roadways that pose significant barriers to bear movements (Foster and Humphrey 1995). In situations where population connection via corridors is impractical, artificial translocation of animals should be considered (Griffith et al. 1989). Translocation of animals has been successful in curbing some effects of inbreeding depression and increasing levels of genetic variation in some animal populations (Mansfield and Land 2002). Conservation biologists should be cognizant that the effects of translocated animals on population structure and hierarchy have not been fully understood.

We conclude that the loss and fragmentation of once contiguous habitat has caused the loss of genetic variation in the Florida black bear, and that genetic variation in smaller populations is among the lowest reported for all black bears. This substantial loss of genetic variation has contributed to extensive genetic differentiation among populations. Given that Florida black bear populations have been reduced in size, gene flow among bear populations is needed to restore and maintain genetic variation (Waits 1999). Finally, further reduction or fragmentation of habitat will likely have a detrimental impact on the demographic and genetic health of the Florida black bear populations, and efforts to conserve remaining habitat cannot be overemphasized.

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References

- Beier P, Noss RF (1998) Do habitat corridors provide connectivity? *Conserv Biol* 12:1241–1252
- Boerson MR, Clark JD, King TL (2003) Estimating black bear population density and genetic diversity at Tensas River, Louisiana using microsatellite DNA markers. *Wildl Soc Bull* 31:197–207
- Brady JR, Maehr DS (1985) Distribution of black bears in Florida. *Fla Field Nat* 13:1–7
- Brody AJ, Pelton MR (1989) Effects of roads on black bear movements in western North Carolina. *Wildl Soc Bull* 17:5–10
- Brooker L, Brooker M (2002) Dispersal and population dynamics of the blue-breasted fairy-wren, *Malurus pulcherrimus*, in fragmented habitat in the Western Australian wheatbelt. *Wildl Res* 29:225–233
- Cale PG (2003) The influence of social behaviour, dispersal and landscape fragmentation on population structure in a sedentary bird. *Biol Conserv* 109:237–248
- Cory CB (1896) Hunting and fishing in Florida. Estes and Lauriet Publishing, Boston
- Craighead FL, Vyse ER (1996) Brown/grizzly bear metapopulations. In: McCullough DR (ed) *Metapopulations and wildlife conservation*. Island Press, Washington D.C., pp. 325–351
- Crooks KR (2002) Relative sensitivities of mammalian carnivores to habitat fragmentation. *Conserv Biol* 16:488–502
- Csiki I, Lam C, Key A, Coulter E, Clark JD, Pace RM, Smith KG, Rhoads DD (2003) Genetic variation in black bears in Arkansas and Louisiana using microsatellite DNA markers. *J Mammal* 84:691–701
- Davies KF, Gascon C, Margules CR (2001) Habitat fragmentation: consequences, management, and future research priorities. In: Soule ME, Orians GH (eds) *Conservation biology: research priorities for the next decade*. Island Press, Washington, pp. 81–97
- Dixon JD, Oli MK, Wooten MC, Eason TH, McCown JW, Paetkau D (2006) Effectiveness of a regional corridor in connecting two Florida black bear populations. *Conserv Biol* 20:155–162
- Dobey S, Masters DV, Scheick BK, Clark JD, Pelton MR, Sunquist ME (2005) Population ecology of Florida black bears in the Okefenokee-Osceola ecosystem. University of Tennessee, Knoxville
- Dunbar MR, Cunningham MW, Wooding JB, Roth RP (1996) Cryptorchidism and delayed testicular descent in Florida black bears. *J Wildl Dis* 32:661–664
- Ebert D, Haag C, Kirkpatrick M, Riek M, Hottinger JW, Pajunen VI (2002) A selective advantage to immigrant genes in a *Daphnia* metapopulation. *Science* 295:485–488
- Ernest HB, Boyce WM, Bleich VC, May B, Stiver SJ, Torres SG (2003) Genetic structure of mountain lion (*Puma concolor*) populations in California. *Conserv Genet* 4:353–366
- Felsenstein J (1993) PHYLIP (Phylogeny Inference Package). Department of Genetics, University of Washington, Seattle
- Fitch WM, Margolia E (1967) Construction of phylogenetic trees. *Science* 155:279–284
- Flather CH, Bevers M (2002) Patchy reaction-diffusion and population abundance: the relative importance of habitat amount and arrangement. *Am Nat* 159:40–56
- Foster ML, Humphrey SR (1995) Use of highway underpasses by Florida panthers and other wildlife. *Wildl Soc Bull* 23:95–100
- Frankham R (1995) Inbreeding and extinction: a threshold effect. *Conserv Biol* 9:792–799
- Frankham R (1996) Relationship of genetic variation to population size in wildlife. *Conserv Biol* 10:1500–1508
- Frankham R, Ballou J, Briscoe D (2002) *Introduction to conservation genetics*. Cambridge University Press, New York

- Franklin IR (1980) Evolutionary change in small populations. In: Soule ME, Wilcox BA (eds) Conservation biology: an evolutionary – ecological perspective. Sinauer, Sunderland, Massachusetts, pp. 135–150
- Gerlach G, Musolf K (2000) Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conserv Biol* 14:1066–1074
- Gottelli D, Sillerozubiri C, Applebaum GD, Roy MS, Girman DJ, Garciamoreno J, Ostrander EA, Wayne RK (1994) Molecular genetics of the most endangered canid: the Ethiopian wolf (*Canis simensis*). *Mol Ecol* 3:301–312
- Griffith B, Scott J, Carpenter J, Reed C (1989) Translocation as a species conservation tool: status and strategy. *Science* 245:477–480
- Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics* 48:361–372
- Harris LD (1984) The fragmented forest: Island biogeography theory and the preservation of biotic diversity. The University of Chicago Press, Chicago
- Harrison S, Bruna E (1999) Habitat fragmentation and large-scale conservation: what do we know for sure? *Ecography* 22:225–232
- Hellborg L, Walker CW, Ruess EK, Stacy JE, Kojola I, Valdmann H, Vila C, Zimmermann B, Jakobsen KS, Ellegren H (2002) Differentiation and levels of genetic variation in northern European lynx (*Lynx lynx*) populations revealed by microsatellites and mitochondrial DNA analysis. *Conserv Genet* 3:97–111
- Hellgren EC, Maehr DS (1993) Habitat fragmentation and black bears in the eastern United States. In: Orff EP (ed) Eastern black bear workshop for research and management. Waterville Valley, New Hampshire, pp. 154–165
- Hellgren EC, Onorato DP, Skiles JR (2005) Dynamics of a black bear population within a desert metapopulation. *Biol Conserv* 122:131–140
- Hendry LA, Goodwin TM, Labisky RF (1982) Florida's vanishing wildlife. Florida Cooperative Extension Service, Gainesville
- Hitchings SP, Beebe TJC (1997) Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: implications for biodiversity conservation. *Heredity* 79:117–127
- Ims RA, Andreassen HP (1999) Effects of experimental habitat fragmentation and connectivity on root vole demography. *J Anim Ecol* 68:839–852
- Johnson WE, Eizirik E, Roelke-Parker M, O'Brien SJ (2001) Applications of genetic concepts and molecular methods to carnivore conservation. In: Gittleman JL, Funk SM, Macdonald D, Wayne RK (eds) Carnivore conservation. Cambridge University Press, New York, pp. 335–358
- Jules ES (1998) Habitat fragmentation and demographic change for a common plant: trillium in old-growth forest. *Ecology* 79:1645–1656
- Kasbohm JW, Bentzien MM (1998) The status of the Florida black bear. United States Fish and Wildlife Service, Jacksonville, Florida
- Keller I, Largiader CR (2003) Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. *Proc R Soc Lond Ser B-Biol Sci* 270:417–423
- Kyle CJ, Strobeck C (2001) Genetic structure of North American wolverine (*Gulo gulo*) populations. *Mol Ecol* 10:337–347
- Lande R (1995) Mutation and conservation. *Conserv Biol* 9:782–791
- Larkin JL, Maehr DS, Hoctor TS, Orlando MA, Whitney K (2004) Landscape linkages and conservation planning for the black bear in west-central Florida. *Anim Conserv* 7:1–12
- Louis EJ, Dempster ER (1987) An exact test for Hardy–Weinberg and multiple alleles. *Biometrics* 43:805–811
- Lu Z, Johnson WE, Menotti-Raymond M, Yuhki N, Martenson JS, Mainka S, Shi-Qiang H, Zhihe Z, Li GH, Pan WS, Mao XR, O'Brien SJ (2001) Patterns of genetic diversity in remaining giant panda populations. *Conserv Biol* 15:1596–1607
- Mader HJ (1984) Animal habitat isolation by roads and agricultural fields. *Biol Conserv* 29:81–96
- Maehr DS, Layne JE, Land ED, McCown JW, Roof J (1988) Long distance movements of a Florida black bear. *Fla Field Nat* 16:1–6
- Maehr DS, Hoctor TS, Quinn LJ, Smith JS (2001) Black bear habitat management guidelines for Florida. Florida Fish and Wildlife Conservation Commission, Tallahassee
- Maehr DS, Smith JS, Cunningham MW, Barnwell ME, Larkin JL, Orlando MA (2003) Spatial characteristics of an isolated Florida black bear population. *Southeast Nat* 2:433–446
- Mansfield KG, Land ED (2002) Cryptorchidism in Florida panthers: prevalence, features, and influence of genetic restoration. *J Wildl Dis* 38:693–698
- Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res* 27:209–220
- Marshall HD, Ritland K (2002) Genetic diversity and differentiation of Kermode bear populations. *Mol Ecol* 11:685–697
- McCown JW, Kubitlis PS, Eason TH, Scheick BK (2004) Black bear movements and habitat use relative to roads in Ocala national forest. Florida Fish and Wildlife Conservation Commission, Tallahassee
- McCoy J, Johnston K (2000) Using ArcGIS spatial analyst. ESRI Publishing, Redlands, California
- McDaniel J (1974) Florida report on black bear management and research. In: Pelton MR, Conley D (eds) Proceedings of the second eastern workshop on black bear management and research. Gatlinburg, Tennessee, pp. 157–162
- Meffe GK, Carroll CR (1997) Principles of conservation biology. Sinauer, Sunderland, Massachusetts
- Michalakis Y, Excoffier L (1996) A generic estimation of population subdivision using distances between alleles with special interest to microsatellite loci. *Genetics* 142:1061–1064
- Miller CR, Waits LP (2003) The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implications for conservation. *Proc Natl Acad Sci USA* 100:4334–4339
- Mills LS, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and management. *Conserv Biol* 10:1509–1518
- Moyer MA, McCown JW, Eason TH, Oli MK (2006) Does genetic relatedness influence space use pattern? A test on Florida black bears. *J Mammal* 87:255–261
- Noss RF, Quigley HB, Hornocker MG, Merrill T, Paquet PC (1996) Conservation biology and carnivore conservation in the Rocky mountains. *Conserv Biol* 10:949–963
- O'Brien SJ (1994) A role for molecular genetics in biological conservation. *Proc Natl Acad Sci USA* 91:5748–5755
- Paetkau D (2003) An empirical exploration of data quality in DNA-based population inventories. *Mol Ecol* 6:1375–1387
- Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Mol Ecol* 3:489–495
- Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Mol Ecol* 4:347–354

- Paetkau D, Waits LP, Clarkson PL, Craighead L, Strobeck C (1997) An empirical evaluation of genetic distance statistics using microsatellite data from bear (*Ursidae*) populations. *Genetics* 147:1943–1957
- Paetkau D, Shields GF, Strobeck C (1998) Gene flow between insular, coastal and interior populations of brown bears in Alaska. *Mol Ecol* 7:1283–1292
- Paetkau D, Amstrup SC, Born EW, Calvert W, Derocher AE, Garner GW, Messier F, Stirling I, Taylor MK, Wiig O, Strobeck C (1999) Genetic structure of the world's polar bear populations. *Mol Ecol* 8:1571–1584
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959
- Proctor MF, McLellan BN, Strobeck C (2002) Population fragmentation of grizzly bears in southeastern British Columbia, Canada. *Ursus* 13:153–160
- Raymond M, Rousset F (1995) Genepop (Version-1.2) population genetics software for exact tests and ecumenicism. *J Hered* 86:248–249
- Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conserv Biol* 17:230–237
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225
- Roelke ME, Martenson JS, O'Brien SJ (1993) The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Curr Biol* 3:340–350
- Rogers LL (1987) Factors influencing dispersal in the black bear. In: Chepko-Sade BD, Halpin ZT (eds) *Mammalian dispersal patterns: the effects of social structure on population genetics*. University of Chicago Press, Chicago, pp. 75–84
- Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature* 392:491–494
- Schwartz CC, Franzmann AW (1992) Dispersal and survival of subadult black bears from the Kenai Peninsula, Alaska. *J Wildl Manage* 56:426–431
- Schwartz MK, Mills LS, McKelvey KS, Ruggiero LF, Allendorf FW (2002) DNA reveals high dispersal synchronizing the population dynamics of Canada lynx. *Nature* 415:520–522
- Sherwin WB, Moritz C (2000) Managing and monitoring genetic erosion. In: Young AG, Clarke GM (eds) *Genetics, demography, and viability of fragmented populations*. Cambridge University Press, New York, pp. 9–34
- Simek SL, Jonker SA, Scheick BK, Endries MJ, Eason TH (2005) Statewide assessment of road impacts on bears in six study areas in Florida. Florida Fish and Wildlife Conservation Commission, Tallahassee
- Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47:264–279
- Spong G, Hellborg L (2002) A near-extinction event in lynx: do microsatellite data tell the tale? *Conserv Ecol*, 6, Art. No. 15
- Taberlet P, Camarra JJ, Griffin S, Uhres E, Hanotte O, Waits LP, DuboisPaganon C, Burke T, Bouvet J (1997) Noninvasive genetic tracking of the endangered Pyrenean brown bear population. *Mol Ecol* 6:869–876
- Vos CC, Antonisse-De Jong AG, Goedhart PW, Smulders MJM (2001) Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). *Heredity* 86:598–608
- Waits LP (1999) Molecular genetic applications for bear research. *Ursus* 11:253–260
- Walker CW, Vila C, Landa A, Linden M, Ellegren H (2001) Genetic variation and population structure in Scandinavian wolverine (*Gulo gulo*) populations. *Mol Ecol* 10:53–63
- Warrillow J, Culver M, Hallerman E, Vaughan M (2001) Sub-specific affinity of black bears in the White River National Wildlife Refuge. *J Hered* 92:226–233
- Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38:1358–1370
- Westemeier RL, Brawn JD, Simpson SA, Esker TL, Jansen RW, Walk JW, Kershner EL, Bouzat JL, Paige KN (1998) Tracking the long-term decline and recovery of an isolated population. *Science* 282:1695–1698
- Wooding JB (1993) Management of the black bear in Florida, a staff report to the commissioners. Florida Game and Fresh Water Fish Commission, Tallahassee
- Woods JG, Paetkau D, Lewis D, McLellan BN, Proctor M, Strobeck C (1999) Genetic tagging of free-ranging black and brown bears. *Wildl Soc Bull* 27:616–627