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GENETIC STRUCTURE OF COUGAR POPULATIONS ACROSS THE WYOMING BASIN: METAPOPULATION OR MEGAPOPULATION

CHARLES R. ANDERSON, JR.,* FEDERICK G. LINDZEY, AND DAVID B. MCDONALD

Zoology and Physiology Department, University of Wyoming, Box 3166, University Station, Laramie, WY 82071, USA (CRA)
United States Geological Survey, Wyoming Cooperative Fish and Wildlife Research Unit, Box 3166, University Station, Laramie, WY 82071, USA (FGL)
Zoology and Physiology Department, University of Wyoming, Bioscience Room 413, University Station, Laramie, WY 82071, USA (DBM)
Present address of CRA: Trophy Game Section, Wyoming Game & Fish Department, 260 Buena Vista, Lander, WY 82520, USA

We examined the genetic structure of 5 Wyoming cougar (*Puma concolor*) populations surrounding the Wyoming Basin, as well as a population from southwestern Colorado. When using 9 microsatellite DNA loci, observed heterozygosity was similar among populations ($H_O = 0.49$–0.59) and intermediate to that of other large carnivores. Estimates of genetic structure ($F_{ST} = 0.028$, $R_{ST} = 0.029$) and number of migrants per generation (*Nm*) suggested high gene flow. *Nm* was lowest between distant populations and highest among adjacent populations. Examination of these data, plus Mantel test results of genetic versus geographic distance ($P \leq 0.01$), suggested both isolation by distance and an effect of habitat matrix. Bayesian assignment to population based on individual genotypes showed that cougars in this region were best described as a single panmictic population. Total effective population size for cougars in this region ranged from 1,797 to 4,532 depending on mutation model and analytical method used. Based on measures of gene flow, extinction risk in the near future appears low. We found no support for the existence of metapopulation structure among cougars in this region.

Key words: central Rocky Mountains, cougar, gene flow, genetic structure, metapopulation, microsatellite DNA, panmixia, *Puma concolor*

Cougars are solitary carnivores exhibiting a polygynous breeding strategy where dominant males typically breed with females that reside within their home range (Murphy 1998). Resident males aggressively defend their territories against male intruders, whereas females allow more overlap with conspecifics, but express mutual avoidance (Logan and Sweanor 2001; Ross and Jalkotzy 1992). Size of female home ranges tends to be large enough to provide sufficient prey for themselves and their young, whereas male home ranges tend to be larger, overlapping those of several females, apparently to maximize their reproductive success (Murphy et al. 1998). Female recruits commonly express philopatric behavior upon independence, but males typically disperse from their natal range (Anderson et al. 1992; Lindzey et al. 1994; Ross and Jalkotzy 1992); movements of >450 km have been documented for subadult males (1998–1999 harvest records, Wyoming Game and Fish Department, Rock Springs—Logan and Sweanor 2001). The purpose of this paper was to assess connectivity among cougar populations by using microsatellite DNA markers.

Conflicting evidence currently exists for whether cougars in North America are panmictic or whether local populations occur in a less connected metapopulation structure. A metapopulation is a population distributed in subpopulations across a set of suitable habitat patches typically isolated in a matrix of unsuitable habitat, in which each subpopulation in each patch has a nontrivial probability of extinction (Gilpin and Hanski 1991). Suitable habitat patches for cougar populations in the western United States typically occur in mountainous regions with some form of overstory canopy, whereas unsuitable habitat consists of open shrub and/or grassland basins separating mountain ranges (e.g., Laing 1988; Logan and Irwin 1985; Williams et al. 1995). Other factors, such as heavy exploitation of the population or human development, may inhibit or alter gene flow, enhancing the potential for metapopulation structure

* *Correspondent: charles.anderson@wgf.state.wy.us*

of cougar populations. Beier (1996) convincingly demonstrated cougar metapopulation structure from telemetry studies in California, where increased development created small, isolated pockets of occupied cougar habitat. Sweanor et al. (2000), without genetic data, proposed cougar metapopulation structure in New Mexico from estimates of dispersal, emigration, and immigration by using radiocollared cougars, but they also suspected gene flow might be high enough to limit risk of extinction in the near future. Culver et al. (2000) and Sinclair et al. (2001) examined genetic structure of cougar populations in the Western Hemisphere and Utah, respectively. Culver et al. (2000) concluded that North American cougars were a single genetic subpopulation and Sinclair et al. (2001) reported high gene flow across Utah. However, both studies used only small regional samples, which limited insight into whether cougars over large areas exhibit a metapopulation structure.

Wyoming offers an excellent opportunity to assess the existence of metapopulation structure of forest-dwelling species because the Wyoming Basin, running diagonally through the center of the state, separates several terminal mountain ranges dominated by conifer forests with open, basin habitats (Fig. 1) and may be a natural barrier to gene flow among cougar populations. Genetic studies support the Wyoming Basin as a barrier to gene flow in other species, including long-tailed voles (*Microtus longicaudus*—Conroy and Cook 2000), pikas (*Ochotona princeps*—Hafner and Sullivan 1995), and black bears (*Ursus americanus*—D. B. McDonald, University of Wyoming; http://www.uwyo.edu/dbmcd/molmark/lect09/lect9.html). Our objective was to assess genetic structure and gene flow among 5 geographically distinct cougar populations terminating in Wyoming and 1 distant population in southwestern Colorado and to determine whether the structure is consistent with metapopulation dynamics.

Materials and Methods

The Wyoming Game and Fish Department provided tissue samples from 234 cougars harvested in Wyoming during 1996–1998. Fecske (2003) provided 8 cougar blood samples from the Black Hills, South Dakota, collected during 2000–2001; Koloski (2002) provided 15 cougar blood samples collected from southwestern Colorado during 2000–2001; and we collected blood samples from 55 cougars in the Snowy Range in southeastern Wyoming (Fig. 1) during 1997–2001. Cougar capture procedures from the Snowy Range are described in Anderson (2003). Capture protocols were reviewed and approved under the University of Wyoming Animal Care and Use Committee, form A-3216-01, by following the American Society of Mammalogists guidelines (http://www.mammalogy.org/committees/indes.asp).

We genotyped cougars by using microsatellite DNA primers from the domestic cat (Menotti-Raymond and O'Brien 1995; Menotti-Raymond et al. 1999) at 10 loci (FCA008, FCA035, FCA043, FCA057, FCA077, FCA081, FCA082, FCA098, FCA132, and FCA149). Using conditions suggested by Li-Cor, Inc. (2000; Lincoln, Nebraska), an MJ PTC-200 and MJ tetrad Peltier thermal cycler (M. J. Research, Inc., Waltham, Massachusetts) performed 10-µl polymerase chain reactions (PCRs) on 60 ng of template DNA. We included 2 fluorescent primers complementary to a 19- and 20-base-pair extension on the 5′ end of the forward primer in the PCR reaction; the fluorescent primer binds to the amplifying product during the annealing stage of the PCR reaction. We used a Li-Cor 4200-S automated DNA sequencer running 25-cm polyacrylamide gels to visualize PCR amplicons detected by infrared laser fluorescence. Analog gel images were viewed by using GeneImagIR (version 3.0, Li-Cor, Inc.) and SAGAGen2 (version 2.1, Li-Cor, Inc.). To validate allele scores, 30% of our DNA samples were genotyped at least twice; we found no evidence of allelic dropout.

Data analyses.—We examined genetic variability (expected heterozygosity [H_E] or gene diversity—Nei 1987) and structure (θ, the F_{ST} analog of Weir and Cockerham [1984] and R_{ST} following Goodman [1997]) by using program FSTAT (version 2.9.3, Université de Lausanne, Dorigny, Switzerland; http://www.unil.ch/izea/softwares/fstat.html; Goudet 2001). We approximated number of migrants per generation (Nm) by following Slatkin (1995), where N is the effective population size, m is the proportion of migrants per generation, and $Nm = (1/F_{ST} - 1)/4$. Potential departures from Hardy–Weinberg equilibrium were examined by using GENEPOP (version 3.3, Center of Ecology and Functional Evolution, Montpellier, France; http://www.cefe.cnrs-mop.fr/GENEPOP; Raymond and Rousset 1995). Nine of the 10 loci occurred on different chromosomes or different linkage groups on the same chromosome (Menotti-Raymond et al. 1999), and were thus considered independent markers. The 10th locus (FCA098) was not genetically mapped by Menotti-Raymond et al. (1999), and we therefore tested pairwise genotypic linkage disequilibrium between FCA098 and the other 9 loci by using GENEPOP. The alpha levels for all statistical comparisons were adjusted by using a Bonferroni correction for number of populations and/or number of loci, where $P < 0.005$ and $P < 0.0008$ were deemed significant for tests within (10 comparisons) and among (60 comparisons) populations, respectively. Loci that were not in Hardy–Weinberg equilibrium and therefore might be linked to other loci were not included in subsequent analyses (1 of 10 loci).

Because dispersal behavior differs between cougar sexes, we examined potential differences between the sexes in genetic structure and relatedness and examined male-biased dispersal. We applied the model-based clustering method of Pritchard et al. (2000) to infer population structure from individual genotypes for all cougars, female cougars, and male cougars by using program STRUCTURE (version 2.0, University of Chicago, Chicago, Illinois; http://pritch.bsd.uchicago.edu). This approach represents a Bayesian, model-based clustering method that accounts for the presence of Hardy–Weinberg or linkage disequilibrium by introducing population structure and attempts to find the optimal number of clusters (K) that best fits Hardy–Weinberg equilibrium. We assumed individuals may have mixed ancestry (admixture model) and used only genetic information (excluding information on sampling location) to infer population structure. We examined $K = 1$–6 for all cougars and $K = 1$–3 for males and females. We selected a burn-in period of 30,000 iterations and increased the number of independent runs of the Gibbs sampler by 100,000 for each increase in K; this procedure was repeated 3 or 4 times for each K to enhance consistency of estimates. To examine potential differences in relatedness between males and females, we estimated pairwise relatedness (r_{xy}) by applying the method of Lynch and Ritland (1999) with program IDENTIX (version 4.03, Université Montpellier II, Montpellier, France; http://www.univ-montp2.fr/%7Egenetix/identix_ms.pdf.; Belkhir et al. 2002) and approximated 95% confidence intervals applying $SE = SD/\sqrt{n}$. We compared relatedness of female cougars among populations and relatedness between males and females within populations. Comparisons were limited to populations with sample sizes > 10. We also tested for male-biased dispersal by using the assignment t-test described by Goudet et al. (2002) by using program FSTAT.

We used program MISAT (version 1.0, University of California, Berkeley, California; http://mw511.biol.berkeley,edu/software.html;

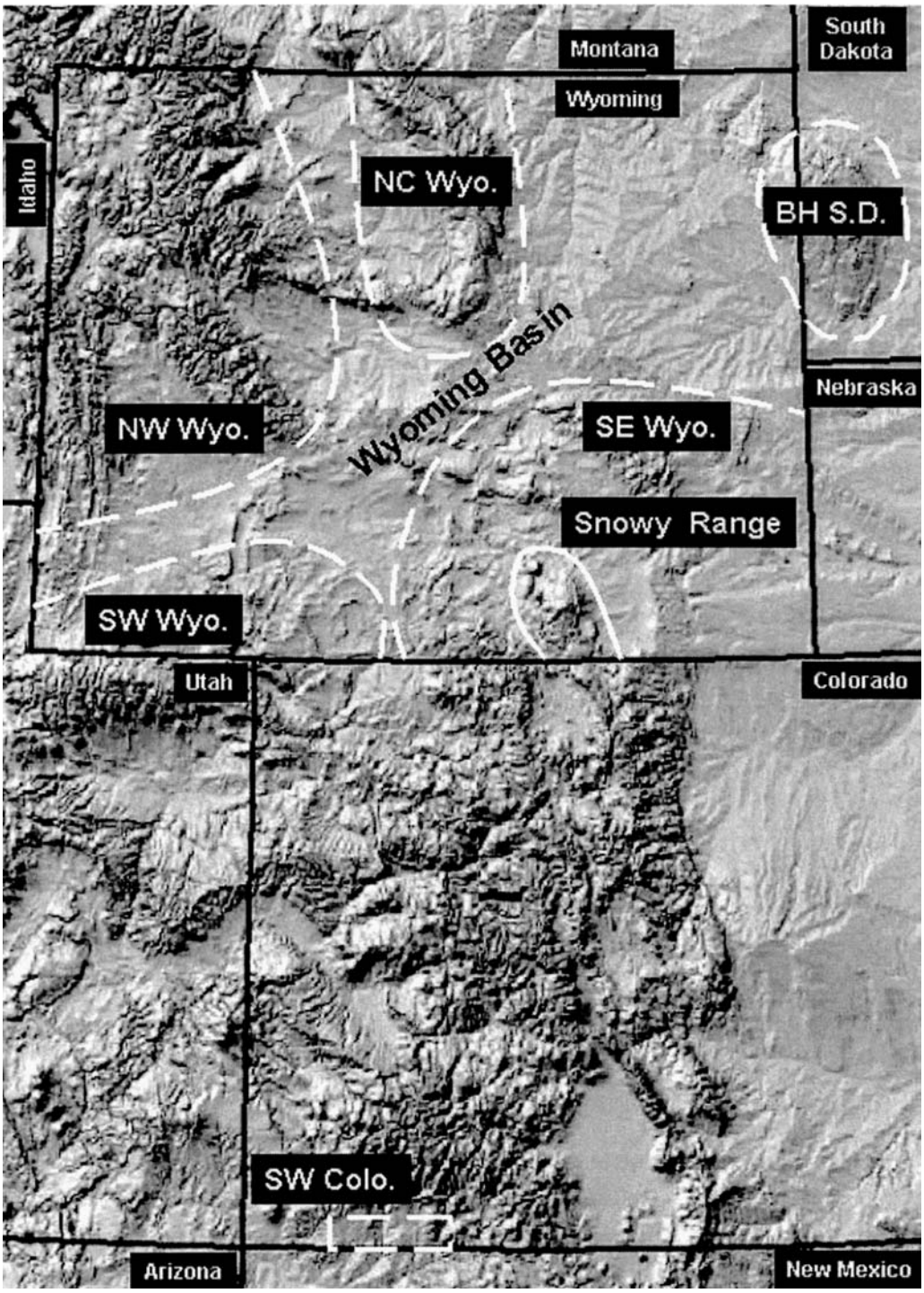

FIG. 1.—Six geographic regions in Wyoming, South Dakota, and Colorado providing cougar DNA samples (dashed lines), and the Snowy Range study site (solid line) in southeastern Wyoming. The Wyoming Basin represents a nonforested region separating mountainous cougar habitats. Coniferous forests dominate mountain ranges (within dashed lines) and sagebrush grasslands characterize basins at lower elevations. BH = Black Hills, NC = north-central, NW = northwest, SE = southeast, and SW = southwest.

Nielsen 1997) to estimate relative effective population size and mutation rate for 5 populations at 9 loci; we excluded the Black Hills population because of small sample size. The program provides a separate maximum likelihood estimate of $4N_e\mu$ (4 times effective population size times mutation rate) for each population–locus combination. To reconcile estimates across loci and populations, we log-transformed the estimates and then used multivariate linear regression to calculate coefficients and estimate relative effective population sizes (assuming constant mutation rate) across populations and loci; we used coefficient standard errors to evaluate differences between populations with 95% confidence intervals. We also reported mean values of $4N_e\mu$ across 9 loci for each population.

Table 1.—Allele size range (base pair [bp] length), number of alleles, and heterozygosities of 312 cougars sampled from Colorado, Wyoming, and South Dakota at 10 microsatellite loci.

Locus	Allele size range (bp)	No. alleles	Observed heterozygosity	Expected heterozygosity
FCA008	148–160	2	0.426	0.448
FCA035	122–136	3	0.571	0.512
FCA043	123–137	5	0.581	0.624
FCA057	146–158	5	0.744	0.679
FCA077	129–133	2	0.222	0.218
FCA081[a]	120–128	4	0.565	0.633
FCA082	239–251	6	0.655	0.691
FCA098	103–119	5	0.738	0.723
FCA132	159–179	5	0.625	0.688
FCA149	112–128	3	0.221	0.227
Mean	—	4	0.535	0.544

[a] Deviated from Hardy–Weinberg equilibrium and was therefore excluded from further analyses.

We estimated effective population size (N_e) for each locus as $N_e = [1/(1 - H_E)^2 - 1]/(8\mu)$ for the model assuming a stepwise mutation process and as $N_e = H_E/4\mu(1 - H_E)$ for the model assuming an infinite alleles mutation process (Lehmann et al. 1998; Nei 1987), where μ is the mutation rate. The stepwise mutation model assumes mutation is a stronger force than genetic drift, whereas the infinite alleles model assumes genetic drift is the dominant force. We also used program MISAT to estimate $4N_e\mu$ across all populations at each locus and then solved for N_e. We estimated N_e by using the average mutation rate from 3 other mammal studies ($\mu = 2.05 \times 10^{-4}$—Rooney et al. 1999).

We examined isolation by distance by comparing pairwise genetic distances and F_{ST} estimates with geographic distances by using the Mantel test (Manly 1991). We also assessed regional phylogenies of 5 cougar populations (excluding the Black Hills where $n = 8$) by constructing neighbor-joining trees from bootstrapped gene frequency data ($\beta = 1{,}000$) by using the SeqBoot, GenDist, Neighbor, and Consense routines in PHYLIP (version 3.5c, University of Washington, Seattle; http://evolution.genetics.washington.edu/phylip.html; Felsenstein 1995). Genetic distances were estimated by using Cavalli–Sforza chord distance, which has been shown to perform well with microsatellite data (Kalinowski 2002) and requires no biological assumptions.

Results

We genotyped 312 cougars from Colorado ($n = 15$), South Dakota ($n = 8$), and Wyoming ($n = 289$) at 10 microsatellite loci. Number of alleles per locus ranged from 2 to 6 and observed heterozygosity varied from 0.221 to 0.744 (overall heterozygosity = 0.535; Table 1). Within-population gene diversity was comparable among populations, ranging from 0.491 to 0.588 (Table 2). Within populations, we found significant deviations from Hardy–Weinberg equilibrium at FCA047 from the southeastern Wyoming population, at FCA081 from the southwestern Colorado population, and at FCA098 from the northwestern Wyoming population ($P < 0.005$). When we examined all populations collectively, we noted that only FCA081 deviated significantly from Hardy–Weinberg equilibrium ($P < 0.0008$). Tests of pairwise genotypic disequilibrium suggested FCA081 and FCA098 were linked ($P < 0.0008$). Because FCA081 deviated from Hardy–Weinberg equilibrium and appeared linked to FCA098, we excluded this locus from further analyses.

Overall F_{ST} and R_{ST} were 0.028 and 0.029, respectively. Pairwise F_{ST} and Nm estimates suggested high gene flow, where effective number of migrants per generation ranged from 2.9 to 30.2 (Table 2). Number of migrants per generation was lowest between the southwestern Colorado cougar population and cougar populations north of the Wyoming Basin ($Nm = 2.9$–3.0) and highest from adjacent cougar populations (e.g., northwestern and north-central Wyoming; $Nm = 10.2$–30.2; Table 2). Inferred population structure from individual genotypes when using program STRUCTURE suggested a single cougar population. Support for a single population was consistent whether the sample included all cougars, only females, or only males (Table 3). Accordingly, relatedness of males and females was similar within populations and did not differ from 0 ($P < 0.05$; Fig. 2), and assignment test results did not support male-biased dispersal ($P = 0.820$). The only hint of female philopatry came from the observation that female cougars from the northwestern and north-central Wyoming populations were less related to cougars in the Snowy Range population than they were to each other (Fig. 2). A neighbor-joining tree based on Cavalli–Sforza distances among the 5 major populations had 98% bootstrap support for a node separating the southeastern and southwestern Wyoming populations, plus the southwestern Colorado population, from the north-central and northwestern Wyoming populations. The 3 southern populations are separated from the 2 northern populations by the treeless expanse of the Wyoming Basin, traditionally considered the dividing line

Table 2.—Pairwise F_{ST} estimates above the diagonal, estimated number of migrants/generation (Nm) between populations below the diagonal, and estimated within-population gene diversity (H_E) along the diagonal from 6 cougar populations sampled at 9 microsatellite loci.

		Cougar population					
Cougar population	n	Northwestern Wyoming	North-central Wyoming	Black Hills South Dakota	Southeastern Wyoming	Southwestern Wyoming	Southwestern Colorado
Northwestern Wyoming	59	0.54	0.008	0.040	0.022	0.017	0.077
North-central Wyoming	59	30.2	0.51	0.024	0.029	0.038	0.076
Black Hills South Dakota	8	6.0	10.3	0.49	0.021	0.051	0.079
Southeastern Wyoming	154	11.1	8.3	11.5	0.55	0.024	0.036
Southwestern Wyoming	17	14.4	6.4	4.6	10.2	0.59	0.048
Southwestern Colorado	15	3.0	3.0	2.9	6.8	4.9	0.53

TABLE 3.—Inferred number of populations[a] (K) when using 9 microsatellite loci from 6 geographically distinct cougar populations for all cougars ($n = 312$), female cougars ($n = 148$), and male cougars ($n = 164$).

	All cougars		Females		Males	
K	$\ln P(X \mid K)$	$P(K \mid X)$	$\ln P(X \mid K)$	$P(K \mid X)$	$\ln P(X \mid K)$	$P(K \mid X)$
1	−5,417	1.000	−2,593	1.000	−2,887	1.000
2	−5,528	0.000	−2,619	0.000	−2,983	0.000
3	−5,612	0.000	−2,758	0.000	−3,189	0.000
4	−5,936	0.000				
5	−5,992	0.000				
6	−6,117	0.000				

[a] The inferred number of populations was derived from the estimated ln probability of the data [$\ln P(X \mid K)$] and the estimated posterior probability of the number of populations [$P(K \mid X)$], where $P(K \mid X) = \exp_{K=1}{}^{\ln P(X \mid K)} / [\exp_{K=1}{}^{\ln P(X \mid K)} + \exp_{K=2}{}^{\ln P(X \mid K)} + \exp_{K=3}{}^{\ln P(X \mid K)} + \cdots]$ (Pritchard et al. 2000).

between the southern and central Rocky Mountains. We also found a significant relationship between pairwise genetic and geographic distances ($r = 0.61$, $P = 0.011$; Table 4) and an even stronger relationship between pairwise F_{ST} estimates and geographic distances ($r = 0.95$, $P < 0.001$) supporting an effect of isolation by distance.

Southwestern Wyoming cougars exhibited the largest relative effective population size (N_e), but the confidence interval overlapped those from other populations (Table 5); estimated relative effective population sizes were smallest from the 2 less contiguous populations from terminal mountain ranges in north-central Wyoming and the Snowy Range (Fig. 1). We estimated an effective population size for the central Rockies (applying our estimates of expected heterozygosity from Table 1) of 1,797 when assuming the infinite alleles model and 3,547 when assuming the stepwise mutation model. Solving for effective population size from $4N_e\mu$ averaged over the 9 loci resulted in an estimate of 4,532.

DISCUSSION

Genetic variability of cougars we examined ($H_O = 0.54$) was comparable to that found in other cougar studies in the western United States and intermediate among other large felids and other large carnivores sampled by using microsatellite DNA analyses. Murphy (1998) reported genetic variability of 0.56 from northern Yellowstone cougars, Sinclair et al. (2001) reported 0.47 from Utah cougars, and Culver et al. (2000) reported 0.42–0.52 for cougars sampled from the western United States. Genetic variability of other large felids was estimated to be 0.39 in cheetahs (*Acinonyx jubatus*), 0.66 in African lions (*Panthera leo*—Menotti-Raymond and O'Brien 1995), and 0.77 in leopards (*P. pardus*—Spong et al. 2000). Genetic variation was estimated at 0.30 in Kodiak Island brown bears (*U. arctos*—Paetkau et al. 1998), 0.54 in gray wolves (*Canis lupus*—Roy et al. 1994), and 0.80 in black bears (Paetkau and Strobeck 1994). As suggested by Culver et al. (2000), the moderate level of genetic variability found in western North American cougars may reflect recolonization events following the most recent Pleistocene glaciation.

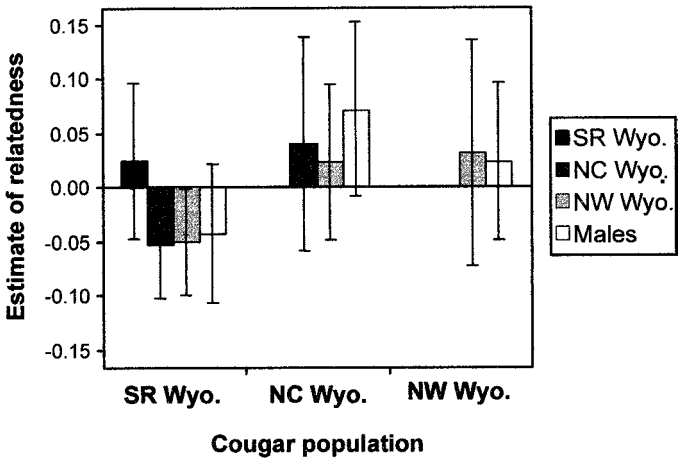

FIG. 2.—Estimated pairwise relatedness (r_{xy}—Lynch and Ritland 1999) of male cougars (white bars) within and female cougars (shaded bars) within and among 3 Wyoming cougar populations (SR = Snowy Range, southeastern Wyoming, NC = north-central, NW = northwest, Wyo. = Wyoming). Error bars represent 95% *CI*. Note similarities between the sexes within populations and slightly negative relatedness between females from the Snowy Range when compared to females from northwestern and north-central Wyoming.

Our findings are similar to those of Sinclair et al. (2001), who reported high gene flow across Utah, and Culver et al. (2000), who suggested North American cougars be reclassified as a single subspecies (*P. concolor couguar*) due to lack of genetic structure. Our low structure and high migration estimates (Table 2) suggest cougar movements are not greatly inhibited by inhospitable habitat (i.e., the Wyoming Basin) or recent development within the Colorado Rocky Mountains; expanses of open habitat across the Wyoming Basin represent distances of about 80–200 km between unconnected, adjacent mountain ranges. These results also were supported by Bayesian simulation methods assigning individuals to a single population regardless of the input pool (e.g., males, females, or both; Table 3). Further, relatedness values were similar within and among cougar populations we surveyed (Fig. 2), and we were unable to detect male-biased dispersal. The only real hint of female philopatry comes from the slightly negative relatedness among females from the Snowy Range compared to elsewhere. However, this difference was not statistically significant (based on overlapping confidence intervals; Fig. 2). We were somewhat surprised to find lack of genetic structure in female cougars and to find that relatedness among females was similar to that among males, despite field-based evidence of a tendency for females to express philopatric behavior and for males to disperse from their natal population (Anderson et al. 1992; Lindzey et al. 1994; Ross and Jalkotzy 1992). This suggests that either high genetic contribution from male immigration is swamping genetic patterns in differential dispersal behavior, or that female dispersal is sufficiently high to preserve genetic cohesiveness, or both. However, the method we used to assess male-biased dispersal provides limited power unless dispersal bias is extreme (80:20—Goudet et al. 2002). One additional factor to consider is postglacial colonization of the area, which

TABLE 4.—Estimated pairwise Cavalli–Sforza chord distances above the diagonal and pairwise geographic distances (km) below the diagonal. Mantel test results ($P = 0.011$) support an effect of isolation by distance and suggest that cougar populations exhibit an equilibrium between migration and genetic drift.

	Population					
Population	Northwestern Wyoming	North-central Wyoming	Black Hills South Dakota	Southeastern Wyoming	Southwestern Wyoming	Southwestern Colorado
Northwestern Wyoming		0.034	0.155	0.060	0.073	0.109
North-central Wyoming	190		0.137	0.070	0.098	0.127
Black Hills South Dakota	550	370		0.113	0.198	0.185
Southeastern Wyoming	450	340	330		0.048	0.074
Southwestern Wyoming	340	360	540	240		0.089
Southwestern Colorado	820	810	820	510	480	

would have an homogenizing effect. In other words, the dominant factor is historical homogeneity, with more recent philopatry not yet evident in genetic data. Also, because isolation by distance is demonstrated, there may be greater geographical structure at a larger geographical scale.

One migrant per generation has been proposed as a necessary minimum to obscure any disruptive effects of genetic drift (Spieth 1974). Mills and Allendorf (1996) investigated this issue further and suggested that more than 1 migrant per generation may be necessary in some cases. Of the 7 criteria they listed (Mills and Allendorf 1996:1516), 2 likely apply to cougar populations, including cases where migrants are closely related to each other or to the local population (Fig. 2) and cases where effective population size is much lower than total population size. Spong et al. (2000) approximated an N_e:N ratio of 0.40 when using cougar data from other studies (Dueck 1990; Harris and Allendorf 1989). As a rule of thumb, Mills and Allendorf (1996) concluded that 1–10 migrants per generation should be sufficient to maintain adequate connectivity while minimizing concerns of local adaptation and outbreeding depression in cases where populations are isolated. The level of gene flow and lack of isolation we observed in the central Rocky Mountains (Table 2) is likely adequate to maintain viable and well-connected cougar populations at the present time.

An effective population size of 500 has been proposed as a minimum to enhance long-term population viability (Franklin 1980). Our estimates of effective population size from cougars in the central Rocky Mountains were well above this minimum and ranged from 1,797 to 4,532, depending on the method used and the assumed mutation model. Genetic drift is inversely proportional to N_e, so cougar populations may be similarly driven by both drift and mutation, which was supported by our Mantel test results showing isolation by distance, thereby suggesting that cougars in the central Rocky Mountains exhibit equilibrium between migration and drift. We therefore suggest a provisional estimate of N_e of approximately 2,500, which represents the approximate midpoint of our estimates. Sinclair et al. (2001) reported a much lower effective population size from Utah cougars ($N_e = 571$). When we applied the equations we used assuming the infinite alleles model and the stepwise mutation model (Lehmann et al. 1998; Nei 1987) and using their estimates of expected heterozygosity (Sinclair et al. 2001: table 2, page 261), we calculated N_es of 2,583 when assuming the infinite alleles model and 5,732 when assuming the stepwise mutation model. Although their method was not clearly explained, it was obviously more conservative than ours. However, both studies applied a mutation rate estimated from other mammal species (i.e., Rooney et al. 1999), suggesting these estimates of cougar effective population size be used cautiously until microsatellite mutation rates in cougars are quantified.

Although cougars appear to exhibit metapopulation dynamics in highly developed regions of California (Beier 1996) and possibly in New Mexico (Sweanor et al. 2000), our findings for the central Rocky Mountains are more consistent with a large panmictic cougar population exhibiting rapid and reasonably thorough interchange among subpopulations. The most isolated region we sampled was the Black Hills, which represents the most easterly extension of the Rocky Mountains and is surrounded by grasslands, with the nearest viable cougar populations occurring in the Big Horn Mountains (200 km distant) and the Laramie Mountains (160 km distant) of Wyoming. Historic records suggest the Black Hills population once became greatly reduced or possibly extirpated by the early 1900s (Fecske 2003). Although sample size warrants caution, our findings suggest that extirpation, if it occurred, was brief and genetic cohesiveness was maintained, evidenced by similar estimates of gene flow, heterozygosity, and structure relative to north-central and southeastern Wyoming cougar populations (Table 2). We suspect isolated conifer islands and riparian

TABLE 5.—Maximum likelihood estimates of $4N_e\mu$ (4 times effective population size times mutation rate) averaged across 9 microsatellite loci and relative effective population size (N_e ratio) and 95% confidence intervals (*CI*) from 4 Wyoming cougar populations and 1 Colorado cougar population.

Population	$4N_e\mu$	N_e ratio[a]	95% *CI*
Southwestern Wyoming	4.54	1.00	0.78−1.22
Southwestern Colorado	4.49	0.96	0.75−1.22
Northwestern Wyoming	3.98	0.85	0.66−1.08
Snowy Range Wyoming	3.66	0.73	0.57−0.93
North-central Wyoming	3.43	0.73	0.57−0.93

[a] Ratio relative to largest effective population subjectively set at 1.00 (southwestern Wyoming).

corridors may have provided movement pathways for immigrants from these areas, which are largely undeveloped and may warrant some protection to maintain connectivity in the future.

Although our results support high gene flow, hints of genetic structure were evident in the slightly negative relatedness of females between cougar populations separated by the Wyoming Basin (Fig. 2). The neighbor-joining tree's split between the southern and northern Wyoming populations coincides with a biogeographic divide between the southern and central Rocky Mountains. Findley and Anderson (1956) pointed out that the Wyoming Basin marks the boundary for morphologically based subspecific breaks in at least 6 mammalian species. Conroy and Cook (2000) found an estimated 350,000-year break in the mitochondrial DNA of the long-tailed vole across this same divide. Our results, although regional in geographic coverage, therefore have implications for a wider region of the North American west. The region we examined represents an area of low human density (among the lowest in the western United States) that could be impacted by future development (e.g., Beier 1996). Thus, periodic monitoring of cougar genetics throughout the western United States to identify changes seems prudent, as does combining results of genetic studies from other regions to determine if cougars are structured at larger geographic scales.

Conclusions

Cougars in the central Rocky Mountains exhibit high gene flow and low structure, presumably because high male dispersal suffices to maintain connectivity between subpopulations. Positive associations between genetic and geographic distances suggest an equilibrium between migration and genetic drift in the historic range of cougars and a lack of significant barriers to gene flow. These attributes are not consistent with metapopulation structure, which requires that subpopulations experience periodic extinctions. Rather, cougars in this region are best considered a large panmictic population. Management and conservation efforts will benefit from periodic monitoring of cougar population structure that will allow detection of fragmentation due to future human development or excess mortality (e.g., disease and exploitation) and determination of whether cougar populations are structured at larger spatial scales. However, because genetic studies will mostly provide insight into past events, periodically assessing status of cougar subpopulations and maintaining habitat corridors sufficient to maintain connectivity will be important to maintain long-term viability.

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