Genetic structure of mountain lion (*Puma concolor*) populations in California

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Abstract

Analysis of 12 microsatellite loci from 431 mountain lions (*Puma concolor*) revealed distinct genetic subdivision that was associated with geographic barriers and isolation by distance in California. Levels of genetic variation differed among geographic regions, and mountain lions that inhabited coastal areas exhibited less heterozygosity than those sampled inland. The San Francisco Bay and Sacramento-San Joaquin River Delta, the Central Valley, and the Los Angeles Basin appeared to be substantial barriers to gene flow, and allele frequencies of populations separated by those features differed substantially. A partial barrier to gene flow appeared to exist along the crest of the Sierra Nevada. Estimated gene flow was high among mountain lions inhabiting the Modoc Plateau, the western Sierra Nevada, and northern section of the eastern Sierra Nevada. Southern California mountain lion populations may function as a metapopulation; however, human developments threaten to eliminate habitat and movement corridors. While north-south gene flow along the western Sierra Nevada was estimated to be very high, projected loss and fragmentation of foothill habitat may reduce gene flow and subdivide populations. Preservation of existing movement corridors among regions could prevent population declines and loss of genetic variation. This study shows that mountain lion management and conservation efforts should be individualized according to region and incorporate landscape-level considerations to protect habitat connectivity.

Introduction

Mountain lions in western North America are assumed to not be in danger of extinction. However, that assumption has been based not on specific biological evidence, but, instead, on landscape-level inferences from smaller-scale demographic studies or mountain lion sightings and reports of conflicts with people and domestic animals. Accurate census data at the landscape level are not available. As with individual mountain lions, their population structure is cryptic and poorly described. Analyses by Culver et al. (2000) indicated that North American mountain lions had less genetic diversity than those in South America. However, data indicating whether any groups of mountain lions in western North America have genetic

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or biological differences that warrant specific conservation actions are lacking.

Mountain lions are an indicator species for habitat connectivity (Penrod 2000) and an important umbrella species (Beier 1993; Logan and Sweanor 2001). In the western United States, and especially California, human populations are growing at a rapid rate and expanding into lands inhabited by mountain lions (Heim 2000). This has exacerbated habitat loss and fragmentation (Beier 1996), losses of domestic animals by predation (Torres et al. 1996), and public safety incidents (Beier 1991). Additionally, mountain lion predation has been identified as an important source of mortality among certain endangered populations of bighorn sheep (*Ovis canadensis*; Wehausen 1996; Hayes et al. 2000; Schaefer et al. 2000) and may threaten population persistence (Ernest et al. 2002; US Fish and Wildlife Service 2000). Because mountain lions play a major role in ecosystem processes and require large tracts of wild land to persist, and because of the increasing probability of future conflicts with a burgeoning human population, accurate information on these large carnivores is essential for their conservation.

Demographic studies suggest that some mountain lion populations may be structured as metapopulations (Beier 1996; Sweanor et al. 2000). In contrast, long distance movements (Pierce et al. 1999) and rapid expansion of range (Riley and Malecki 2001) should facilitate gene flow and reduce genetic substructure. One genetic study of mountain lions (Walker et al. 2000) indicated that substructure may be present between west and south Texas; however, Sinclair et al. (2001) did not find genetic differentiation among 10 sites sampled in Utah. Knowledge of mountain lion demography and genetic structure is vital to determine levels of risk to regional persistence of mountain lion populations. Decreased levels of genetic variation might signal that gene flow has been disrupted, and could lead to reduced evolutionary potential to adapt to changes in the environment (Lande and Barrowclough 1987) and increased risk of extinction. Indeed, reduction in genetic diversity have been associated with declines in disease immunity and reproduction in several felid species, including mountain lions (Wildt et al. 1987; Heeney et al. 1990; Barone et al. 1994).

Our aim was to apply molecular genetic techniques in a hierarchical approach to describe the genetic structure of mountain lion populations in California. Multilocus microsatellite genotype data were examined for 431 mountain lions from California

and contiguous regions in northwestern Nevada. We first tested for panmixis and the presence of genetic clusters using a Bayesian model that did not incorporate available geographic information. Using information from the model output and knowledge of mountain lion habitat as a foundation, we next delineated geographic regions to test for population structure. We hypothesized that expanses of poor mountain lion habitat (Central Valley, San Francisco Bay and Sacramento-San Joaquin River Delta ['San Francisco Bay and Delta'], and Los Angeles Basin) would reduce gene flow and subdivide populations. Furthermore, gene flow was expected to be high within mountain ranges that contained contiguous habitat. The data were tested to determine how genetic variation and substructure differed among geographic areas, and to identify major barriers to gene flow.

Methods

We obtained opportunistic samples for analysis of nuclear DNA from mountain lions in California (n = 412) and western Nevada within 50 km of California (n = 19) during 1988–1999 (Figure 1). DNA was extracted from tissue, blood, saliva, hair, and fecal samples of mountain lions that had been captured for telemetry studies or killed for public safety reasons, for livestock depredation, on roads, or by hunters (Nevada only). When fecal samples were used, only genotypes that qualified as unique individuals by match probabilities (Ernest et al. 2000; Ernest et al. 2002) were included. Known relatives of represented individuals were excluded. Samples were stored at −20 °C until DNA was extracted.

Chelex (Walsh et al. 1991), salting out (Miller et al. 1988), or the QIAamp® Blood and Tissue Kit (Qiagen) were used to extract DNA from muscle, hair, or skin, and a chloroform-phenol protocol was used for fecal samples (Ernest et al. 2000). The twelve microsatellite primers (FCA008, FCA023, FCA026, FCA035, FCA043, FCA045, FCA077, FCA078, FCA090, FCA096, FCA126, and FCA132) used in this study were developed by Menotti-Raymond et al. (1999). Polymerase chain reaction and electrophoretic conditions were described by Ernest et al. (2000). Electrophoresis and digital measurement of length polymorphisms were carried out on an ABI 373 (Perkin-Elmer) using the program STRand (Veterinary Genetics Laboratory, University of California, Davis, CA).
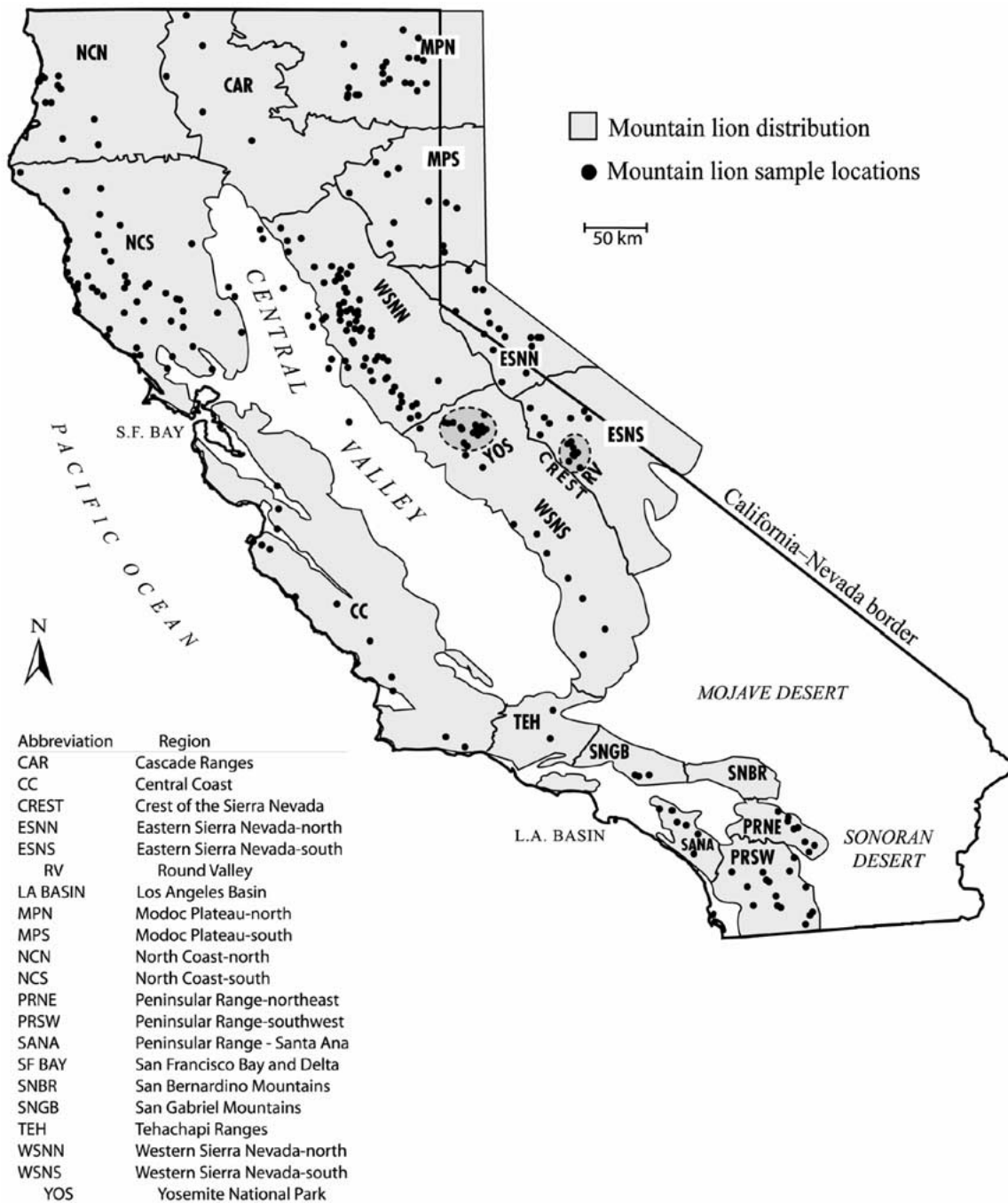
Figure 1. Map of sample locations and regions used in genetic analysis of mountain lion samples from California and western Nevada collected during 1988–1999. More than one sample may be represented by each dot. Mountain lion habitat distribution based on Torres et al. 1996.

We analyzed 421 genotypes using a model-based clustering method (STRUCTURE software; Pritchard et al. 2000) without regard to geographic location in order to assess population structure from a purely genetic perspective (genetic clusters). Because the Central Valley is not mountain lion habitat (Torres et al. 1996) and mountain lions sampled there could have traveled from one of several regions, those samples (n = 10) were excluded. This method applied a Bayesian approach to assign individuals probabilistically to populations based on their genotypes.

Using the genetic clusters as an initial foundation for population structure, we then added information on the distribution of mountain lions, geography, and regional ecology of California to further define regions for additional genetic analysis. The California Geographic Subdivisions ('Jepson regions'), as described in Hickman (1993), incorporate natural landscape features including vegetative, geologic, topographic, and climatic variation. Based on Hickman (1993) and the California Department of Fish and Game mountain lion habitat relation model (Torres et al. 1996), we defined the following 'major regions' of mountain lion habitat in California and adjacent northwestern Nevada (Figure 1): North Coast (NC; n = 106), Cascade Range (CAR; n = 5), western Sierra Nevada (WSN; n = 141), Modoc Plateau region in the Great Basin including adjacent regions of Nevada (MP; n = 45), region east of the Sierra Nevada crest in the Great Basin including Mono and Inyo counties and regions of adjacent Nevada ('eastern Sierra Nevada' [ESN]; n = 49), Central Coast from south of San Francisco Bay and Delta to, and including, Santa Barbara County (CC; n = 18), and Southwestern California including the San Gabriel and San Bernardino Mountains and Peninsular Ranges (SW; n = 51).

We further subdivided the state to examine genetic patterns at a finer scale. To establish the minimum areas demonstrating substructure, we subdivided long, linear areas for which we had sufficient sample size (North Coast and western Sierra Nevada) into 'subunits' of approximately 2000 square km in area (4 subunits in NC and 7 subunits in WSN). Subunits that were not genetically differentiated were recombined forming north and south 'units', with each unit comprising approximately half of the area in each major region. The Modoc Plateau and eastern Sierra Nevada were each arbitrarily divided to form north and south units, with each unit comprising approximately half of each major region (MPN, MPS, ESNN, and ESNS). Subdivision of the Central Coast was

precluded by sample size; therefore, it was treated as a single unit. Because of low sample size, the Cascade Range was excluded from F_{ST}, R_{ST}, and assignment analyses. Tehachapi Mountains (n = 2), San Gabriel Mountains (n = 4), and San Bernardino Mountains (n = 0) had low sample sizes or lacked samples; therefore, these units were excluded from genetic differentiation, F_{ST}, R_{ST}, and assignment analyses. Central Valley was excluded from F_{ST} and R_{ST} analyses.

Because of other research (Ernest et al. 2000; Pierce et al. 2000a, b) in Yosemite National Park in the southern unit of the western Sierra Nevada and Round Valley in the southern unit of the Eastern Sierra Nevada (Figure 1), these areas had been intensively sampled. Since they were close in proximity, Round Valley (n = 22) and Yosemite National Park (n = 28) were compared to test gene flow across the crest of the Sierra Nevada. Coastal regions were compared with inland regions to assess differences in heterozygosity and numbers of alleles. F_{ST} analyses were conducted to determine whether river drainages and major highways acted as major barriers to gene flow. Heterozygosity levels and numbers of alleles were evaluated to determine whether proximity to large urban centers (within 100 km of San Francisco, Sacramento, Los Angeles, and San Diego) was correlated with changes in genetic variation.

The validity of geographic boundaries of populations was tested a number of ways. Major regions were tested for Hardy-Weinberg equilibrium using a modified Fisher's exact test (Guo and Thompson 1992) performed in ARLEQUIN (version 2.0 with 2001 update; Schneider et al. 2000). F_{IS} (inbreeding coefficient) was estimated for major regions using GENEPOP (version 3.3; Raymond and Rousset 1995) and used to test for the presence of excess homozygotes. Log-likelihood probabilities for each individual multilocus genotype, calculated in ARLEQUIN (Paetkou et al. 1995) were used to test assignment to region. Genic and genotypic differentiation among major regions, units, and subunits were tested using the log-likelihood (G) based exact test of Goudet et al. (1996) using GENEPOP. Genetic distance was measured by pair wise F_{ST} and R_{ST} and tested for statistical significance using an ARLEQUIN permutation test (10,000 permutations per comparison). The data were also tested for linkage disequilibrium using GENEPOP. The sequential Bonferroni technique was used to correct for multiple applications of the same test (Weir 1996).

MSTools3 (Stephen Park, Genetics Dept, TCD, Ireland) was used to create input files and to calculate allele frequencies and diversity statistics, including observed heterozygosity (mean, standard deviation, and 95% confidence interval). Isolation by distance was evaluated using the subprogram ISOLDE in GENEPOP, which computed a regression of F_{ST} on geographic distance (Rousset 1997) and applied Mantel's tests with 10,000 permutations per comparison (Mantel 1967). Geographic distances were measured as the shortest distance between unit centroids and did not traverse major areas lacking suitable habitat for mountain lions (Central Valley, San Francisco Bay and Delta, Mojave Desert, and Los Angeles Basin). To evaluate the effect of differences in sample size, 15 samples from each unit were randomly chosen and analyzed for genetic differentiation and genetic distance (F_{ST}). Units with < 15 samples were included in their entirety. We also tested whether males differed from females in genetic differentiation and genetic distances among major regions and units. The threshold for significance of all statistical tests was $P = 0.05$.

The programs TOPO! California (Wildflower Productions, San Francisco, CA) and All Topo Maps Nevada (iGage, Salt Lake City, UT) were used to estimate geographic coordinates for samples. We used ArcView 3.2a (Environmental Systems Research Institute, Inc.) to group samples within major regions and units and to display geographic patterns of variation (private alleles and differences in allele frequencies).

Results

All 12 microsatellite loci amplified in samples from California and Nevada. One locus (FCA 045) was monomorphic; the 11 polymorphic loci displayed between 2 and 10 alleles. Across all samples and polymorphic loci, the average observed heterozygosity (H_O) was 44%, average expected heterozygosity (H_E) was 50%, and average number of alleles per locus was 4.4 (Table 1). The 11 polymorphic loci did not deviate from Hardy-Weinberg and linkage equilibria in major regions, except as noted below. Analyses with 15 random samples per unit, samples from only males, and samples from only females mirrored the pattern of the full data set for genic and genotypic differentiation and F_{ST} and R_{ST} estimates. Therefore, differences in male:female proportions and sample sizes did not

appear to affect genetic analyses and we combined the sexes for subsequent analyses.

Model-based clustering (STRUCTURE) without geographic information incorporated demonstrated that two clusters assorted distinctly from the rest of the data. Eighty-seven percent of the genotypes from Southwestern California assorted in one cluster and 82% of the genotypes from the North Coast assorted in another cluster. With the exception of the Central Coast, ≤ 7% of genotypes from the other geographic regions assorted with either the Southwestern or North Coast cluster. Twenty-eight percent of the Central Coast genotypes clustered with Southwestern California and none with the North Coast. When the STRUCTURE model was run without North Coast and Southwestern California data, 89% of the genotypes from the Central Coast assorted in a cluster that was distinct from the Cascade Range, Modoc Plateau, Western Sierra Nevada, and Eastern Sierra Nevada. Less than 7% of genotypes from those geographic regions assorted with the Central Coast cluster. The STRUCTURE model provided some evidence of additional population substructure in the Sierra Nevada Mountains. Fifty-five percent of the Eastern Sierra Nevada genotypes assorted in a cluster that also included 27% of the Modoc Plateau, 12% of the western Sierra Nevada, and 6% of the Central Coast genotypes.

Population structure was also demonstrated through geographic analysis of the genetic data. The major regions were highly differentiated from each other (pairwise genic and genotypic differentiation, $P < 0.0001$), except for two comparisons: Cascade Range with Modoc Plateau and western Sierra Nevada (CAR-MP genic differentiation $P = 0.49$ and genetic differentiation $P = 0.61$; CAR-WSN genic differentiation $P = 0.27$ and genetic differentiation $P = 0.50$). Samples from the Southwest and Central Coast regions exhibited private alleles (alleles not observed in other areas), lacked alleles found in other areas, and displayed remarkably different allele frequencies for certain loci (Table 2). Within the Southwest region, the Peninsular Ranges (Santa Ana Mountains, Peninsular Ranges-NE, and Peninsular Ranges-SW) lacked five alleles that were observed in all other units: FCA008:164, FCA078:190, FCA090:107, FCA096:209, and FCA132:162. All of these alleles, except FCA096:209, were observed in samples from the San Gabriel Mountains. The Central Coast lacked two alleles that were observed in all other major regions: FCA126:131 and FCA132:178. The Central

Table 1. Genetic diversity of mountain lions across regional units in California and western Nevada for 11 polymorphic microsatellite loci. Cascade Ranges (n = 5), Tehachapi Mountains (n = 2), and San Gabriel Mountains (n = 4) were included only in the 'All samples' analysis due to low sample sizes. Central Valley (n = 10) was included only in the 'All samples' analysis because it is not mountain lion habitat (Torres et al. 1996) and mountain lions sampled there could have traveled from one of several regions

Region	Sample Size # individuals	Average % H_O (95% CI)[a]	Total # of alleles	Average # alleles per locus (SD)[b]
All samples[c]	431	44 (42–45)	48	4.4 (2.4)
North Coast-south	91	32 (29–35)	36	3.3 (1.6)
North Coast-north	15	44 (37–52)	31	2.8 (1.3)
Modoc Plateau-north	29	45 (39–50)	37	3.4 (1.4)
Modoc Plateau-south	16	56 (49–64)	34	3.1 (1.5)
Western Sierra Nevada-north	60	48 (44–52)	37	3.4 (1.7)
Western Sierra Nevada-south	81	49 (46–52)	39	3.5 (1.8)
Eastern Sierra Nevada-north	27	52 (47–58)	34	3.1 (1.2)
Eastern Sierra Nevada-south	22	52 (46–58)	35	3.2 (1.7)
Central Coast	18	36 (29–43)	33	3.0 (1.2)
Peninsular Ranges – Santa Ana	14	34 (26–41)	25	2.3 (1.1)
Peninsular Ranges-northeast	14	42 (34–51)	28	2.5 (0.8)
Peninsular Ranges-southwest	23	43 (37–49)	28	2.5 (1.3)

[a] Average percent observed heterozygosity across all loci and 95 percent confidence interval.
[b] Average number of alleles per locus and standard deviation.
[c] Including Cascade Ranges, Tehachapi Mountains, San Gabriel Mountains, and Central Valley.

Coast and Peninsular Ranges together lacked two alleles observed in all other regions: FCA043:136 and FCA126:143. Two alleles were seen only in the Central Coast and San Gabriel samples: FCA035:133 and FCA090:109. North Coast and western Sierra Nevada each displayed one private allele (Table 2). In addition, allele 152 of locus FCA026 was found only in samples from an east-west band across the North Coast and Western Sierra Nevada from 39.0 to 39.9 degrees latitude. Samples from coastal areas with peninsular geography (Figure 1; Santa Ana unit of Southwest, Central Coast, and peninsular southern section of the North Coast) had significantly lower average observed heterozygosity (32–36%) than inland units of western and eastern Sierra Nevada and Modoc Plateau (44–56%). However, average numbers of alleles per locus were not significantly different among regions (Table 1). Proximity to large urban centers (San Francisco, Sacramento, Los Angeles, and San Diego) was not significantly correlated with lower observed heterozygosities or numbers of alleles (95% confidence intervals overlapped).

Population substructure was also revealed within major regions. The southernmost three of the four North Coast subunits were not differentiated from each other and, therefore, were combined into a single unit (NCS) for further analysis. NCS (the peninsular section of the North Coast bounded by the Pacific Ocean and Central Valley) was differentiated from the northern section of the North Coast (NCN) and was more genetically differentiated from other units in the state than was NCN by an average of 0.09 F_{ST} units (Table 3). NCN exhibited high gene flow ($F_{ST} = 0.04$–0.05) with Modoc Plateau-north, eastern Sierra Nevada-north, western Sierra Nevada-north, and western Sierra Nevada-south. The north and south units of Modoc Plateau were differentiated from each other (G test, $P = 0.01$), however gene flow was high ($F_{ST} = 0.02$). Data suggested very high gene flow between Modoc Plateau-north and western Sierra Nevada units ($F_{ST} = 0.01$) and eastern Sierra Nevada-north ($F_{ST} = 0.02$). Data from Modoc Plateau-south indicated very high genetic interchange with western Sierra Nevada-north (F_{ST} not significantly different from zero, $P = 0.06$). North and south units of eastern Sierra Nevada were differentiated from each other and all other subunits (G test, $P < 0.0001$ for all comparisons). The eastern Sierra Nevada-south displayed somewhat more isolation from north and south units of western Sierra Nevada ($F_{ST} = 0.06$ and 0.07; $R_{ST} = 0.13$ and 0.19, respectively) than south Modoc Plateau ($F_{ST} = 0.04$; $R_{ST} = 0.08$) and

Table 2. Allele frequency distributions in percentages among major regions in California for 12 microsatellite loci in mountain lions. Allele sizes noted in base pairs. Major region abbreviations: NC = North Coast (n = 106), MP = North Great Basin-Modoc Plateau (n = 45), WSN = western Sierra Nevada (n = 141), ESN = South Great Basin-east of the Sierra Nevada crest (n = 49), CC = Central Coast (n = 18), and SW = Southwest (n = 51). Private alleles are noted by *

Locus	Alleles	Major Regions					
		NC	MP	WSN	ESN	CC	SW
FCA008	152	92	71	70	59	92	98
	164	8	29	30	41	8	2
FCA023	138	0	2	1	2	42	11
	142	100	98	99	98	58	89
FCA026	140	76	56	60	65	91	38
	142	2	23	12	24	3	2
	144	21	21	25	10	6	21
	150*	0	0	0	0	0	39
	152	1	0	3	0	0	0
FCA035	123	65	36	43	58	47	8
	133	0	0	0	0	3	1
	135	35	64	57	42	50	91
FCA043	124	3	33	17	31	31	28
	134	82	47	55	41	69	59
	136	15	20	28	29	0	2
	138*	0	0	0	0	0	11
FCA045	127	100	100	100	100	100	100
FCA077	129	27	34	35	43	59	64
	133	73	66	65	57	41	36
FCA078	186	14	20	14	22	6	37
	188	81	78	79	62	88	62
	190	6	2	6	15	6	1
FCA090	105	75	33	30	23	8	35
	107	13	22	16	4	3	0
	109	0	0	0	0	11	2
	113	13	22	27	44	47	57
	117	0	4	3	2	28	2
	119	0.5	18	23	27	3	4
FCA096	191	13	17	31	15	47	7
	201	83	66	54	57	36	87
	203*	2	0	0	0	0	0
	205	0	9	5	12	0	7
	209	2	8	11	15	17	0
FCA126	131	61	18	23	34	0	6
	135*	0	1	0	0	0	0
	137	12	54	46	38	44	66
	139	20	24	23	28	50	27
	141*	0	0	0	0	6	0
	143	7	2	8	0	0	1

Table 2. Continued

Locus	Alleles	Major Regions					
		NC	MP	WSN	ESN	CC	SW
FCA132	162	13	14	32	6	6	4
	172*	0	0	0.4	0	0	0
	174	58	54	48	41	69	29
	176	1	0	0.4	1	0	0
	178	6	10	11	13	0	11
	180	0	8	1	5	0	16
	182	13	6	3	21	0	1
	184	1	3	0	0	0	0
	186	1	4	4	12	3	11
	188	8	0	0.4	0	22	27

eastern Sierra Nevada-north (F_{ST} = 0.04; R_{ST} = 0.06).

Within the Sierra Nevada, but separated by a direct distance of only 50 km and the Sierra Nevada crest, Yosemite National Park (a subset of western Sierra Nevada-south) and Round Valley (a subset of eastern Sierra Nevada-south) were genetically differentiated from each other (G test, $P < 0.0001$; F_{ST} = 0.07 and R_{ST} = 0.16). Sample subsets bordered by major river drainages in the western Sierra Nevada (North and South Forks of the American River, and Merced River) and major highways (I-80 and I-50) were not differentiated.

Central Coast samples were genetically differentiated from all other units, and were most similar to the western and eastern Sierra Nevada and Modoc Plateau units (F_{ST} = 0.09–0.11; Table 3). North and Central Coast units exhibited very low genetic interchange. The closest units, North Coast-south and Central Coast, separated by only 100 km, had a pair wise F_{ST} = 0.26, while more distant pairings had lower genetic distances (North Coast-north and Central Coast F_{ST} = 0.18; Modoc Plateau-north and Central Coast F_{ST} = 0.11). The F_{IS} estimate for the Central Coast (0.20) was much higher than the other major regions (North Coast 0.05, Modoc Plateau 0.03, Western Sierra Nevada 0.05, Eastern Sierra Nevada 0.04, Southwest 0.06), indicating a higher level of inbreeding. However low sample size (n = 18) may contribute to the differences. The Southwest was the only major region demonstrating Hardy Weinberg disequilibrium, due to low gene flow between the Santa Ana Mountains and the rest of the Peninsular Ranges. When analyzed as separate units, data

Table 3. F_{ST} (below diagonal) and R_{ST} (above diagonal) estimates for 11 polymorphic microsatellite loci examined from mountain lions in California and western Nevada (ARLEQUIN; Schneider et al. 2000). Statistical significance tested using a permutation test (10,000 permutations per comparison). All values were significantly different from zero ($P < 0.05$) except those denoted with *

Region[a]	NCS	NCN	MPN	MPS	WSNN	WSNS	ESNN	ESNS	CC	SANA	PRNE	PRSW
NCS	–	0.05	0.15	0.21	0.14	0.16	0.12	0.33	0.26	0.42	0.36	0.34
NCN	0.07	–	0.05	0.06	0.06	0.09	0.09	0.22	0.15	0.27	0.26	0.25
MPN	0.13	0.04	–	0.01*	0.02	0.04	0.03	0.13	0.06	0.17	0.19	0.22
MPS	0.18	0.07	0.02	–	0.02*	0.08	0.03	0.08	0.08	0.19	0.18	0.22
WSNN	0.11	0.04	0.01	0.01*	–	0.02	0.04	0.13	0.06	0.23	0.18	0.23
WSNS	0.11	0.05	0.01	0.03	0.01	–	0.09	0.19	0.12	0.27	0.25	0.29
ESNN	0.09	0.05	0.02	0.03	0.02	0.02	–	0.06	0.07	0.22	0.18	0.22
ESNS	0.23	0.11	0.08	0.04	0.06	0.07	0.04	–	0.12	0.29	0.23	0.31
CC	0.26	0.18	0.11	0.12	0.10	0.10	0.09	0.15	–	0.21	0.18	0.22
SANA	0.37	0.22	0.13	0.17	0.18	0.18	0.17	0.21	0.21	–	0.12	0.14
PRNE	0.28	0.16	0.12	0.13	0.13	0.12	0.12	0.17	0.19	0.08	–	–0.01*
PRSW	0.25	0.13	0.11	0.15	0.14	0.13	0.13	0.20	0.18	0.08	0.01*	–

[a] Due to low sample sizes, the Cascade (n = 5), Tehachapi (n = 2), and San Gabriel (n = 4) units are not included. Abbreviations: NCS = North Coast-south, NCN = North Coast-north, MPN = Modoc Plateau-north, MPS = Modoc Plateau-south, WSNN = Western Sierra Nevada-north, WSNS = Western Sierra Nevada-south, ESNN = Eastern Sierra Nevada-north, ESNS = Eastern Sierra Nevada-south, CC = Central Coast, SANA = Santa Ana Mountains, PRNE = Peninsular Ranges-northeast, PRSW = Peninsular Ranges-southwest.

from Santa Ana, PRNE, and PRSW were in equilibrium. The Santa Ana unit in the Southwest showed the greatest isolation in the state (Table 3). While the Southwest units of PRNE and PRSW showed genetic differentiation from each other ($P = 0.03$), F_{ST} values were not significantly different from zero ($P = 0.16$), indicating very high gene flow between them.

Gene flow was diagrammed (Figure 2) using F_{ST} estimates (Table 3). With some exceptions (for example, Central Coast comparisons with western and eastern Sierra Nevada and Modoc Plateau units), R_{ST} reflected F_{ST} patterns. However, R_{ST} was often smaller than F_{ST} for F_{ST} values < 0.10, and greater than F_{ST} for F_{ST} values > 0.10. Isolation by distance was highly significant ($P < 0.0001$; $R^2 = 0.56$) for distances measured between regional units excluding those that crossed expanses of non-suitable habitat (Central Valley, San Francisco Bay and Delta, Mojave Desert, and Los Angeles Basin).

Individual genotypes from the most isolated regions were most likely to assign (log-likelihood probabilities; ARLEQUIN) to the region from which they were sampled (North Coast 86%, Modoc Plateau 76%, western Sierra Nevada 71% [with 64% for Yosemite], and eastern Sierra Nevada 67% [with 82% for Round Valley], Central Coast 94%, and Southwest 96%). Of Yosemite genotypes that did not assign to the western Sierra Nevada (10 out of 28, 36%), one assigned to the Central Coast, one

to the North Coast, and four each to Modoc Plateau and eastern Sierra Nevada. Of Round Valley genotypes that did not assign to the eastern Sierra Nevada using log likelihood analysis (4 out of 22, 18%), three assigned to the Modoc Plateau and one to the western Sierra Nevada. Eighty percent (8 of 10) of genotypes that were collected from the Central Valley assigned to the closest adjacent region. One mountain lion sampled near Oakdale, California, in the Central Valley (expected assignment with western Sierra Nevada) assigned to the North Coast and one near Williams, California, (expected assignment with North Coast) assigned to the western Sierra Nevada.

Discussion

Although mountain lions are distributed widely and have the ability to travel over great distances, our findings indicate that California populations are genetically structured. Analysis of genetic data without regard to geographic location (model-based clustering method) provided evidence that mountain lions in the north Coast Ranges and southwestern California are genetically distinct from those inhabiting other regions of the state. Genetic analysis (genetic distance and genetic differentiation tests) of mountain lions grouped into geographic regions demonstrated both structure among, and substructure within, regions.
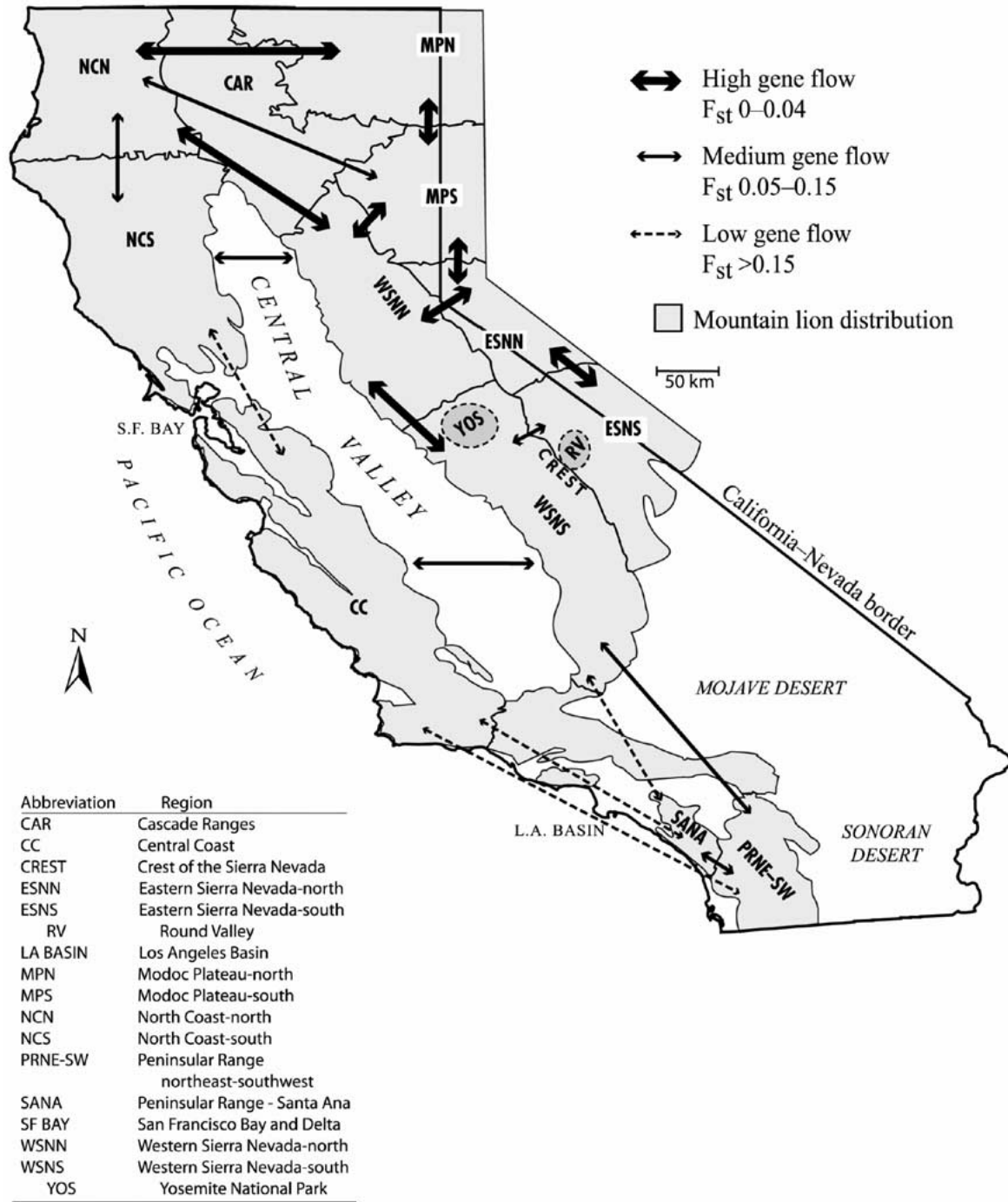
Abbreviation	Region
CAR	Cascade Ranges
CC	Central Coast
CREST	Crest of the Sierra Nevada
ESNN	Eastern Sierra Nevada-north
ESNS	Eastern Sierra Nevada-south
RV	Round Valley
LA BASIN	Los Angeles Basin
MPN	Modoc Plateau-north
MPS	Modoc Plateau-south
NCN	North Coast-north
NCS	North Coast-south
PRNE-SW	Peninsular Range northeast-southwest
SANA	Peninsular Range - Santa Ana
SF BAY	San Francisco Bay and Delta
WSNN	Western Sierra Nevada-north
WSNS	Western Sierra Nevada-south
YOS	Yosemite National Park

Figure 2. Map of gene flow among mountain lion populations in California as determined from F_{ST} estimates calculated from microsatellite data from 410 California and western Nevada samples collected during 1988–1999 (Table 3). Mountain lion habitat distribution based on Torres et al. (1996, Figure 7). Cascade Range (n = 5), Tehachapi Mountains (n = 2), San Gabriel Mountains (n = 4), and San Bernardino Mountains (n = 0) had low sample sizes or lacked samples. Central Valley (n = 10) is not mountain lion habitat (Torres et al. 1996) and mountain lions sampled there could have traveled from one of several regions. Therefore, these units were excluded from F_{ST} analyses. Peninsular Ranges-NE was combined with Peninsular Ranges-SW (combined units noted as PRNE-SW) to simplify gene flow depictions because F_{ST} estimates were very similar for the two units.

Genetic assignment testing (log-likelihood probabilities) also indicated that the regions identified in this study were valid for grouping mountain lions.

The landscape of California provides obstacles to genetic interchange. Major barriers include the Central Valley, San Francisco Bay and Delta, and the Los Angeles Basin (Figure 2). The low, flat, and highly agricultural Central Valley separates two long, linear mountain chains (Coast Ranges and Sierra Nevada), and the San Francisco Bay and Delta divide the Coast Ranges. Mountain lion habitat in southwestern California is fragmented by the metropolitan Los Angeles Basin, Mojave and Sonoran Deserts. Mountain lions inhabiting regions (particularly coastal) bounded by these barriers had greater genetic distances from other populations and had lower heterozygosities than mountain lions from other areas (Tables 1–3; Figure 2). The North Coast-south and Central Coast units, separated by the San Francisco Bay and Delta, were the most differentiated (F_{ST} = 0.26; Table 3) of all unit pairs north of the Los Angeles Basin. The Tehachapi Mountains and Transverse Ranges (San Gabriel and San Bernardino Mountains) appear to function as a movement corridor for gene flow between regions to the north and south of the Los Angeles Basin. Central Coast and Santa Ana units, separated by the Los Angeles Basin, were more differentiated than pairings of the Central Coast with more distant units (e.g. Central Coast-Modoc Plateau-north). To a lesser extent, partial barriers also appear to exist along the crest of the Sierra Nevada and possibly in the northern Coast Ranges.

The diverse ecological communities that occupied the Central Valley prior to the mid-1800's (Farquhar 1930; Longhurst et al. 1952; Jackson and Spence 1980) are nearly gone, replaced primarily by agriculture and cities. Mountain lions occasionally venture into the Central Valley, but crossings between the Sierra Nevada on the east and the coastal regions on the west are likely to be uncommon (F_{ST} = 0.11; Table 3). One allele that was present only in an east-west band across the North Coast and central Sierra Nevada may be indicative of a small level of gene flow occurring across the Central Valley; alternatively, it may be a historical remnant.

The Tehachapi Mountains are the only likely corridor for movement among the western Sierra Nevada, Central Coast and Southwest regions. The only likely movement corridor between the Tehachapi Mountains and the Southwest region are the Transverse Ranges (San Gabriel and San Bernardino Moun-

tains), both of which support populations of mountain lions. Movement through this corridor, however, requires that lions cross the San Gorgonio Pass and I-10, a busy interstate highway. Habitat in the Santa Ana Mountains is completely surrounded by urbanization and heavily used highways (I-15, I-5, and S-74; Beier 1995) and habitat in the other sections of the Peninsular Ranges is becoming increasingly fragmented. Further, the Peninsular Ranges are not likely to receive many migrants from the low elevation Sonoran Desert (poor mountain lion habitat) to the east (Germaine et al. 2000), but there could be immigration of mountain lions from Mexico. Consequently, mountain lion populations in southwestern California exist in a matrix of semi-isolated 'patches' surrounded by unsuitable habitat (Table 3; Figure 2; Beier 1996; Hanski and Simberloff 1997).

Beier (1995, 1996) demonstrated that mountain lions in Santa Ana Mountains occupied habitat patches that were semi-isolated as a result of anthropogenic changes to the environment, consistent with metapopulation structure. Moreover, Logan and Sweanor (2001) provided some evidence for metapopulation structure for desert-dwelling mountain lions in New Mexico. Our data (genetic differentiation and distance indices for the Southwest; Table 3; Figure 2) demonstrated genetic structure and gene flow among habitat patches, consistent with one aspect of metapopulation function. However, other fundamental characteristics of metapopulation function have not yet been demonstrated from a landscape perspective including: 1) largely independent dynamics among patches; 2) natural extirpations; and 3) natural recolonizations of extirpated populations (Harrison and Taylor 1997). Although the term metapopulation has been used to describe any subdivided population, habitat fragmentation has created numerous situations where once-continuous distributions of species now resemble metapopulations, but may not function as such. Hanski and Gilpin (1991) have cautioned that the dynamics of such fragmented populations are poorly understood, and must be further explored before proper conservation prescriptions can be implemented.

Ecological, geographic, and anthropogenic factors may also affect population substructure at finer scales. The crest of the Sierra Nevada separates genetically subdivided groups of mountain lions (Figure 2; Table 3). While the direct-line distance is as close as 50 km, mountain lions sampled from Yosemite National Park in the western Sierra Nevada, and Round Valley on the eastern scarp, were geneti-

cally differentiated ($F_{ST} = 0.07$). The reasons for this subdivision are not clear since seasonal movements of mountain lions across the crest have been documented. In Round Valley, most mountain lions remain as residents year-round, but some migrate west across the crest of the Sierra Nevada during the spring, and return in the autumn (Pierce et al. 1999). Conversely, dispersing juvenile mountain lions do not have a predictable directional component and apparently move in random directions (Ruth et al. 1998). Telemetry data have shown long distance mountain lion movements in all directions from Round Valley, including an adult female that emigrated 170 km to the north and dispersing males that moved 120 km to the east and 200 km to the southwest, respectively (unpublished data). In contrast, 5 of 7 adult mountain lions captured near Yosemite Valley did not exhibit strong seasonal migration patterns and instead remained in the foothills, west of Yosemite National Park (L. Chow, pers. comm.). The remaining two mountain lions displayed seasonal movements between higher and lower elevations.

The southern three-quarters of the North Coast were genetically differentiated from the northern part of the region despite apparently contiguous habitat. While the small sample size for the north unit could have contributed to observed differences, the difference remained following the trial using 15 random samples from each unit. It is unlikely that specific geographical features, such as rivers or mountain crests, in the North Coast greatly inhibit gene flow because elevations do not exceed 2400 meters and rivers are seasonally traversable.

Mountain lion movements, and therefore gene flow, among regions may be influenced by seasonal availabilities in alternative prey. Wild horse (*Equus caballus*) foals in the Montgomery Pass Wild Horse Territory along the California-Nevada border in the eastern Sierra Nevada (Turner and Morrison 2001), Roosevelt elk (*Cervus elaphus roosevelti*) in Humboldt and Del Norte counties in the North Coast-north unit (J. Ellingson pers. comm.), and bighorn sheep in the Sierra Nevada (Wehausen 1996), Transverse Ranges (Schaefer et al. 2000), and Peninsular Ranges (Hayes et al. 2000) provide alternative food sources that may allow persistence of mountain lions when primary prey are not abundant. Sex and age of mountain lions are also factors that affect migration and dispersal (Anderson et al. 1992; Pierce et al. 1999; Sweanor et al. 2000). Juvenile males are more likely than females to disperse long distances out of their natal range, thereby facilitating nuclear gene flow. Mountain lion mortality may also differ regionally and according to sex and age. Finally, observed genetic patterns may also be influenced by translocation of mountain lions by humans for management reasons or by the illegal release of captive animals. We concluded that the former was an unlikely influence since very few management translocations have been recorded, and those few animals were released into the same general area from which they were captured (unpublished California Department of Fish and Game [CDFG] data).

Conclusions and conservation implications

Culver et al. (2000) found much lower genetic variation among North American than South American mountain lions. North American mountain lions displayed about 50% fewer alleles than those from South America for the loci that Culver et al. (2000) analyzed in common with our study. California mountain lions (this study) displayed 73% fewer alleles than those in South America (Culver et al. 2000). These findings of genetic diversity, structure and function of mountain lion populations and metapopulations are valuable in a conservation context because accelerating anthropogenic changes are likely to decrease gene flow among populations, increase the risk of extirpations, and decrease the chance of recolonizations. Because heterozygosities of mountain lions inhabiting California's coastal regions was lower than that of inland regions (Table 1; but note the average number of alleles per locus), and coastal populations have fewer routes for gene flow, continued habitat fragmentation and destruction are likely to further reduce genetic diversity.

Mountain lions in southwestern California exhibited substructure (this study) and movement patterns (Beier 1995, 1996) consistent with some aspects of metapopulation function, and may be a result of anthropogenic fragmentation of habitat. Maintenance of corridors that provide opportunities for movement between discontinuous populations will become increasingly important. The potential metapopulation of mountain lions existing in southwestern California is likely to become increasingly fragmented as human developments further eliminate habitat and populations become more isolated (Beier 1996).

Additionally, the human population in the western Sierra Nevada foothills is projected to more than triple

in size over the next 40 years (Centers for Water and Wildland Resources, Sierra Nevada Ecosystem Project 1996). While north-south gene flow along the western Sierra Nevada was estimated to be very high, fragmentation and loss of habitat due to human population growth will reduce gene flow and result in subdivided mountain lion populations. Preservation of contiguous tracts of habitat of sufficient size to allow movements by mountain lions will be necessary to preserve population structure, function, and genetic diversity, particularly in southwestern California, coastal regions, and the Sierra Nevada.

Conversely, specific types of habitat alterations may lead to region-specific increases in the distribution and abundance of mountain lions. For example, mule deer and mountain lions may have been rare in the western Great Basin, including eastern Sierra Nevada and Modoc Plateau, prior to the 20th century. Berger and Wehausen (1991) hypothesized that increases in mule deer (and, therefore, mountain lions) in that area were correlated with habitat changes induced by heavy grazing of domestic animals. If that was the case, then gene flow within the Modoc Plateau, and between the Modoc Plateau and the Sierra Nevada, may have been facilitated by those changes.

Mountain lions are oblivious to political boundaries. Arizona, Nevada, Oregon, and Mexico permit hunting of mountain lions; California permits killing of mountain lions only to protect livestock, pets, and people. Interstate and international differences in conservation strategies and natural and human-made differences in habitat may play important roles in source-sink dynamics and metapopulation function in mountain lions (Logan and Sweanor 2001). This study confirms that mountain lions in California do not exist as a single population, therefore management should be individualized according to region. Management and conservation efforts should not be constrained by political boundaries but, instead, should incorporate ecosystem considerations for predator and prey habitat, protect connectivity of regions, and prevent further degradation of regions that already have been severely fragmented. Adequate planning and cooperation among agencies are in the best interest of conservation (Bleich et al. 1996, Salwasser et al. 1987), and are necessary to ensure the persistence of mountain lions as humans further dominate the landscape of California.

References

Anderson AE, Bowden DC, Kattner DM (1992) The puma on Uncompahgre plateau, Colorado. *Col. Div. Wildl. Tech. Pub.*, **40**, 1–116.

Barone MA, Roelke ME, Howard JG, Brown JL, Anderson AE, Wildt DE (1994) Reproductive characteristics of male Florida panthers: Comparative studies from Florida, Texas, Colorado, Latin America, and North American zoos. *J. Mammal.*, **75**, 150–162.

Beier P (1991) Cougar attacks on humans in the United States and Canada. *Wildl. Soc. Bull.*, **19**, 403–412.

Beier P (1993) Determining minimum habitat areas and habitat corridors for cougars. Conserv. *Biol.*, **7**, 94–108.

Beier P (1995) Dispersal of juvenile cougars in fragmented habitat. *J. Wildl. Manage.*, **59**, 228–237.

Beier P (1996) Metapopulation models, tenacious tracking, and cougar conservation. In: *Metapopulations and Wildlife Conser-*

vation (ed. McCullough DR), pp. 293–323. Island Press, Washington, DC.

Berger J, Wehausen JD (1991) Consequences of a mammalian predator-prey disequilibrium in the Great Basin Desert. *Conserv. Biol.*, **5**, 244–248.

Bleich VC, Wehausen JD, Ramey RR, II, Rechel JL (1996) Metapopulation theory and mountain sheep: Implications for conservation. In: *Metapopulations and Wildlife Conservation* (ed. McCullough DR), pp. 353–373. Island Press, Washington, DC.

Centers for Water and Wildland Resources, Sierra Nevada Ecosystem Project (1996) *Status Of The Sierra Nevada, Final Report To Congress: Summary*. Wildland Resources Center Report Number 39, Centers for Water and Wildland Resources, 1323 Academic Surge, One Shields Ave, University of California, Davis. See also <http://ceres.ca.gov/snep/pubs/> (visited Nov. 19, 2001).

Culver M, Johnson WE, Pecon-Slattery J, O'Brien SJ (2000) Genomic ancestry of the American puma (*Puma concolor*). *J. Hered.*, **91**, 186–197.

Ernest HB, Rubin ES, Boyce WM (2002) Fecal DNA analysis and risk assessment of mountain lion predation of bighorn sheep. *J. Wildl. Manage.*, **66**, 75–85.

Ernest HB, Penedo MCT, May BP, Syvanen M, Boyce WM (2000) Molecular tracking of mountain lions in the Yosemite Valley region in California: Genetic analysis using microsatellites and faecal DNA. *Mol. Ecol.*, **9**, 433–441.

Farquhar FP (1930) *Up and down California in 1860–1864*, The Journal of William H. Brewer, Professor of Agriculture in the Sheffield Scientific School from 1864 to 1903. Yale University Press, New Haven, CT.

Germaine SS, Bristow KD, Haynes LA (2000) Distribution and population status of mountain lions in southwestern Arizona. *Southwestern Nat.*, **45**, 333–338.

Goudet J, Raymond M, De Meeüs T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1933–1940.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics*, **48**, 361–372.

Hanski I, Gilpin M (1991) Metapopulation dynamics: Brief history and conceptual domain. *Biol. J. Linn. Soc.*, **42**, 3–16.

Hanski I, Simberloff D (1997) The metapopulation approach. In: *Metapopulation Biology: Ecology, Genetics, and Evolution* (eds. Hanski IA, Gilpin ME), pp. 5–26. Academic Press, Inc. San Diego, CA.

Harrison S, Taylor AD (1997) Empirical evidence for metapopulation dynamics. In: *Metapopulation Biology: Ecology, Genetics, and Evolution* (eds. Hanski IA, Gilpin ME), pp. 27–42. Academic Press, Inc. San Diego, CA.

Hayes C, Rubin ES, Jorgensen MC, Botta RA, Boyce WM (2000) Mountain lion predation on bighorn sheep in the Peninsular Ranges, California. *J. Wildl. Manage.*, **64**, 954–959.

Heeney JL, Evermann JF, McKeirnan AJ, Marker-Kraus L, Roelke ME, Bush M, Wildt DE, Meltzer DG, Colly L, Luca J, Manton VJ, Caro T, O'Brien SJ (1990) Prevalence and implications of feline coronavirus infections of captive and free-ranging cheetahs (*Acinonyx jubatus*). *J. Virol.*, **64**, 1964–1972.

Heim M (2000) State predicts high growth for agricultural counties. *Calif. Agric.*, **54**, 3.

Hickman JC (1993) Geographic subdivisions of California. In: *The Jepson Manual: Higher Plants of California* (ed. Hickman JC), pp. 37–48. University of California Press, Berkeley.

Jackson D, Spence ML (1980) *The Expeditions of John Charles Frémont*. University of Illinois Press, Urbana.

Lande R, Barrowclough GF (1987) Effective population size, genetic variation, and their use in population management. In: *Viable Populations for Conservation* (ed. Soulé ME), pp 87–123. Cambridge University Press, New York, NY.

Logan KA, Sweanor LL (2001) *Desert Puma: Evolutionary Ecology and Conservation of an Enduring Carnivore*. Island Press, Washington, DC.

Longhurst WM, Leopold AS, Dasmann RF (1952) A survey of California deer herds: Their ranges and management problems. *Calif. Department of Fish and Game, Game Bull.*, **6**, 1–135.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res.*, **27**, 209–220.

Menotti-Raymond M, David VA, Lyons LA, Shäffer AA, Tomlin JF, Hutton MK, O'Brien SJ (1999) A genetic linkage map of microsatellites of the domestic cat (*Felis catus*). *Genomics*, **57**, 9–23.

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.*, **16**, 1215.

Paetkou D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.*, **4**, 347–354.

Penrod K (2000) *Missing Linkages: Restoring Connectivity to the California Landscape*. Proceedings of the Conference of November 2. San Diego Zoo, CA.

Pierce BM, Bleich VC, Bowyer RT (2000a) Selection of mule deer by mountain lions and coyotes: Eeffects of hunting style, body size, and reproductive status. *J. Mammal.*, **81**, 462–472.

Pierce BM, Bleich VC, Bowyer RT (2000b) Social organization of mountain lions: Does a land-tenure system regulate population size? *Ecology*, **81**, 1533–1543.

Pierce BM, Bleich VC, Wehausen JD, Bowyer RT (1999) Migratory patterns of mountain lions: Implications for social regulation and conservation. *J. Mammal.*, **80**, 986–992.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Raymond M, Rousset F (1995) GENEPOP (version 1.2) – population genetics software for exact tests and ecumenicism. *J. Hered.*, **86**, 248–249.

Riley SJ, Malecki RA (2001) A landscape analysis of cougar distribution and abundance in Montana, USA. *Environ. Manage.*, **28**, 317–323.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Ruth RK, Logan KA, Sweanor LL, Hornocker Mg, Temple LJ (1998) Evaluating cougar translocation in New Mexico. *J. Wildl. Manage.*, **62**, 1264–1275.

Salwasser H, Schonewald-Cox C, Baker R (1987) The role of interagency cooperation in managing for viable populations. In: *Viable Populations for Conservation* (ed. Soulé), pp. 159–173. Cambridge University Press, Cambridge, MA.

Schaefer RJ, Torres SG, Bleich VC (2000) Survivorship and cause-specific mortality in sympatric populations of mountain sheep and mule deer. *Calif. Fish and Game*, **86**, 127–135.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN ver. 2.000 A Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sinclair EA, Swenson EL, Wolfe ML, Choate DC, Bates B, Crandall KA (2001) Gene flow estimates in Utah's cougars imply management beyond Utah. *Anim. Conserv.*, **4**(PT3), 257–264.

Sweanor LL, Logan KA, Hornocker MG (2000) Cougar dispersal patterns, metapopulation dynamics, and conservation. *Conserv. Biol.*, **14**, 798–808.

Torres SG, Mansfield TM, Foley JE, Lupo T, Brinkhaus A (1996) Mountain lion and human activity in California: Testing speculations. *Wildl. Soc. Bull.*, **24**, 451–460.

Turner JW Jr, Morrison ML (2001) Influence of predation by mountain lions on numbers and survivorship of a feral horse population. *Southwestern Nat.*, **46**, 183–190.

US Fish and Wildlife Service (2000) *Recovery plan for bighorn sheep in the Peninsular Ranges, California*. US Fish and Wildlife Service, Portland, Oregon, USA.

Walker CW, Harveson LA, Pittman MT, Tewes ME, Honeycutt RL (2000) Microsatellite variation in two populations of mountain lions (*Puma concolor*) in Texas. *Southwestern Nat.*, **45**, 196–203.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*, **10**, 506–513.

Wehausen JD (1996) Effects of mountain lion predation on bighorn sheep in the Sierra Nevada and Granite Mountains of California. *Wildl. Soc. Bull.*, **24**, 471–479.

Weir BS (1996) *Genetic Data Analysis II*. Sinauer Associates, Inc. Publishers, Sunderland, MA.

Wildt DE, Bush KL, Goodrowe KL, Packer C, Pusey AE, Brown JL, Joslin P, O'Brien SJ (1987) Reproductive and genetic consequences of founding isolated lion populations. *Nature*, **329**, 328–331.