

FECAL DNA ANALYSIS AND RISK ASSESSMENT OF MOUNTAIN LION PREDATION OF BIGHORN SHEEP

HOLLY B. ERNEST, Department of Veterinary Pathology, Microbiology, and Immunology, University of California, One Shields Avenue, Davis, CA 95616, USA, and Wildlife Health Center, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA 95616, USA

ESTHER S. RUBIN,¹ Department of Veterinary Pathology, Microbiology, and Immunology, University of California, One Shields Avenue, Davis, CA 95616, USA, and California Department of Fish and Game, 1416 Ninth Street, Sacramento, CA 95814, USA

WALTER M. BOYCE,² Department of Veterinary Pathology, Microbiology, and Immunology, University of California, One Shields Avenue, Davis, CA 95616, USA

Abstract: We analyzed fecal DNA to identify individual mountain lions (*Puma concolor*) associated with kills of federally listed endangered bighorn sheep (*Ovis canadensis*) in the Peninsular Ranges of California from 1993–1999. We identified 18 different mountain lions at 26 bighorn sheep kill sites, as well as 5 mountain lions not associated with bighorn sheep mortalities. Thirteen genotypes were each identified at only 1 kill site, while 2 genotypes were unambiguously detected at multiple kill sites. We developed a Monte Carlo simulation model incorporating the DNA data to evaluate the benefits (bighorn sheep saved and reduction in extinction risk) and costs (mountain lions removed) of mountain lion removal under 2 management strategies for a 5-year period. One strategy removed individual mountain lions only after they killed 1 bighorn sheep (kill-site removal), while the other strategy removed mountain lions found anywhere in bighorn sheep habitat (habitat removal). The habitat removal strategy was equal or superior to the kill-site removal in terms of reducing extinction risk for all sizes of ewe populations. However, the kill-site strategy more efficiently targeted bighorn sheep predators and resulted in the removal of fewer non-bighorn sheep-killing mountain lions than the less selective habitat removal strategy. Removal of 1–2 mountain lions per year by either strategy effectively decreased extinction risk for populations consisting of 15–30 ewes, while more intensive removal (3–4 mountain lions per year) was necessary to reduce the risk for smaller populations containing <15 ewes. Removal of mountain lions for a short period of time may be the best option available for bighorn sheep populations in immediate danger of extinction due to mountain lion predation. Given that site-specific information and several assumptions were incorporated in our model, we strongly recommend that individualized and updated assessments be performed on the potential costs and benefits of predator control actions so that the rescue of 1 species or population does not jeopardize another.

JOURNAL OF WILDLIFE MANAGEMENT 66(1):75–85

Key words: California, fecal DNA, microsatellites, Monte Carlo simulation, mountain lion, mountain sheep, *Ovis canadensis*, Peninsular Ranges, predator control, *Puma concolor*.

Bighorn sheep inhabiting the Peninsular Ranges of California have declined in numbers over the past 30 years (Rubin et al. 1998), resulting in state listing as rare in 1971 (California Department of Fish and Game 1992) and federal listing as endangered in 1998 (Federal Register 1998). Factors implicated in this decline include disease, drought, habitat loss, and predation by mountain lions. During 1992–1998, 42 of 61 (69%) radiocollared bighorn sheep that died in the Peninsular Ranges were killed by mountain lions (Hayes et al. 2000), resulting in an average annual adult survivorship of bighorn sheep of only 0.79. In this study, we analyzed microsatellite DNA from mountain lion fecal (scat) sam-

ples collected at bighorn sheep kill sites to identify individual mountain lions. Sloughed intestinal cells provide a source of DNA for microsatellite analysis of scats (Kohn and Wayne 1997). Microsatellites are highly variable segments of nuclear DNA that have been used successfully to identify individuals in certain species, including mountain lions (Goldstein and Schlötterer 1999, Ernest et al. 2000). We estimated the minimum number of mountain lions that killed bighorn sheep and identified mountain lions that killed bighorn sheep repeatedly. We then incorporated these data in a Monte Carlo simulation model to evaluate management strategies that have been proposed to reduce the impact of predation by mountain lions on bighorn sheep. In the model, we compared the relative differences in bighorn sheep savings, mountain lion losses, and population extinction risks under 2 lion removal strategies.

¹ Present address: Center for Reproduction of Endangered Species, Zoological Society of San Diego, P.O. Box 120551, San Diego, CA 92112, USA.

² E-mail: wmboyce@ucdavis.edu

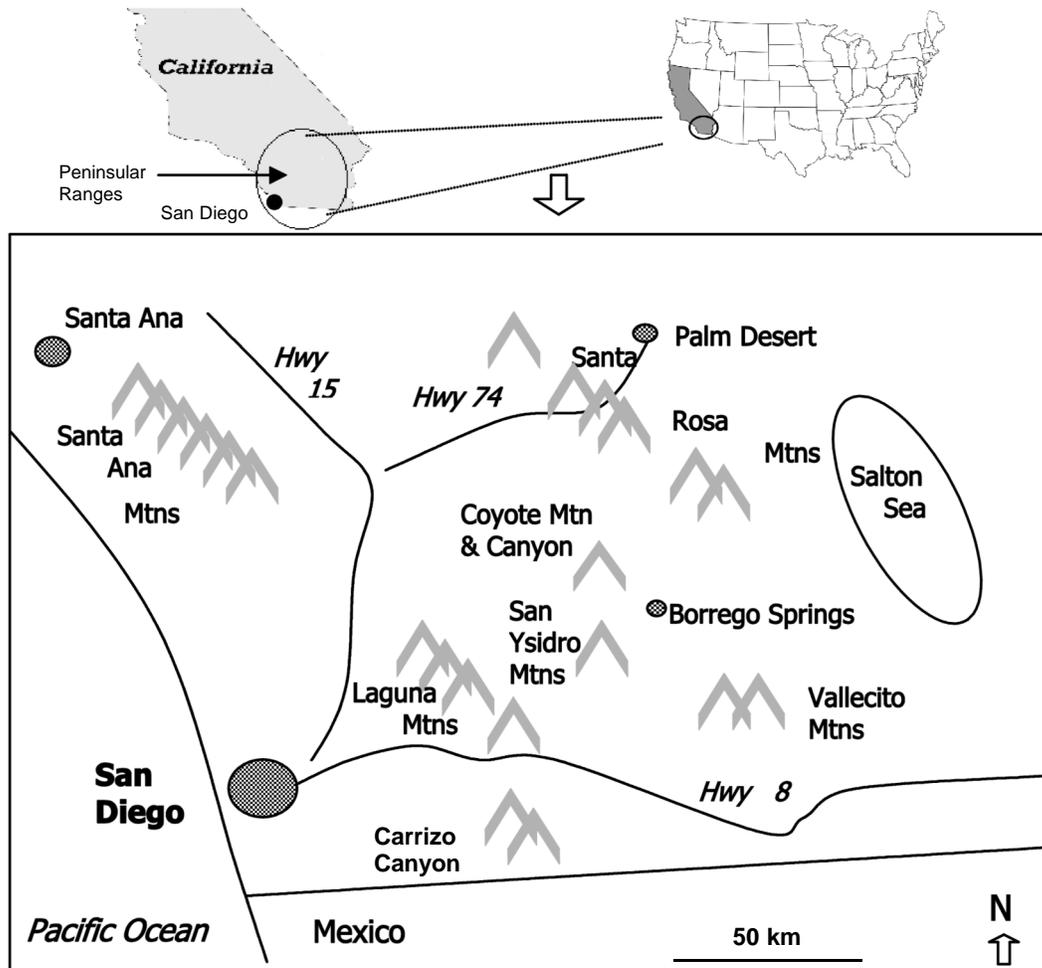


Fig. 1. Map of study site. Peninsular Mountains of southern California.

STUDY AREA

The Peninsular Ranges north of Baja California, Mexico, extend for approximately 225 km on a north-south line between the Pacific Coast and the Imperial and Coachella valleys of southern California (Fig. 1). In the Peninsular Ranges, bighorn sheep habitat is bordered on the west by densely vegetated, chamise (*Adenostoma fasciculatum*)-dominated chaparral, and on the east by lowland valleys within the lower Colorado Desert subdivision of the Sonoran desert scrub community (Brown and Lowe 1980). Bighorn sheep in these ranges are found below elevations of 1,400 m (Jorgensen and Turner 1975, Rubin et al. 1998), typically below the pinyon pine (*Pinus monophylla*)-juniper (*Juniperus californica*) associa-

tion. In these areas, temperatures ranged from below freezing to above 45 °C, and precipitation averaged approximately 17 cm per year (National Oceanic and Atmospheric Administration 1992–1998). Our study spanned 7 subpopulations of bighorn sheep within the Peninsular Ranges, corresponding to 7 of the 8 subpopulations delineated by Rubin et al. (1998). These groups, from south to north, were located in Carrizo Canyon, Vallecito Mountains, south San Ysidro Mountains, north San Ysidro Mountains, Coyote Canyon, Santa Rosa Mountains southwest of Highway 74, and Santa Rosa Mountains northwest of Highway 74. Bighorn sheep habitat in our study area covered about 3,000 km² (U.S. Fish and Wildlife Service 2000).

METHODS

This study was conducted between February 1993 and March 1999 in association with the bighorn sheep mortality investigations described by Hayes et al. (2000), and by the Bighorn Institute (Palm Desert, California, USA; DeForge et al. 1997, U.S. Fish and Wildlife Service 2000). Adult bighorn sheep were captured by helicopter netgun and fitted with radiocollars with mortality sensors (Telonics, Mesa, Arizona, USA) in 1992, 1993, and 1997 (Rubin et al. 1998, Hayes et al. 2000). Monitoring began immediately following capture, and animals whose radiocollars were transmitting a mortality signal were located as soon as possible. We investigated mortality sites as described by Hayes et al. (2000) to determine cause of mortality. A kill site was defined as the area at which at least 1 bighorn sheep was killed by a mountain lion. Kill sites where 68 radiocollared bighorn sheep and 9 uncollared bighorn sheep (discovered opportunistically) had been killed by mountain lions were searched for scats that were likely from mountain lions. Although scavenging by other mountain lions may have occurred, we assumed that the mountain lions that killed the bighorn sheep had deposited the scats that were collected at the sites. We also collected scats opportunistically whenever they were encountered to identify mountain lions present in bighorn sheep habitat that may not have been detected at bighorn sheep kills.

We also analyzed DNA from 38 mountain lions (reference group) killed, found dead, or captured in the Peninsular Ranges within 60 km of current bighorn sheep distribution as delineated in Rubin et al. (1998). Because 9 of these 38 mountain lions potentially were related (siblings or offspring), we created an unrelated reference group that consisted of samples from the 29 unrelated individuals. The unrelated reference group provided allele frequency data necessary to calculate a scat match probability, which is the probability that a microsatellite DNA profile observed in 1 animal would occur in a second animal (Ernest et al. 2000). The California Department of Fish and Game (CDFG) and Fund for Animals (Ramona, California, USA) provided 21 muscle or blood samples. Fifteen DNA samples were provided by Melanie Culver (National Institutes of Health, Frederick, Maryland, USA), including those collected as part of a study conducted from 1988 to 1992 in the Santa Ana Mountains (Beier et al. 1995). We also obtained dried hide samples from 2 mountain lion carcasses discovered in the

Vallecito and Santa Rosa Mountains in 1996. All samples were stored at -20°C .

DNA Extraction and Analysis

Fecal DNA was extracted, amplified by polymerase chain reaction using primers for 12 felid microsatellite loci, analyzed, and assigned to species of origin as described by Ernest et al. (2000). To determine the probability that a genotype (the microsatellite profile of 2 alleles per locus, for all loci that amplified) observed in 1 scat sample would occur a second time (match probability), we calculated allele frequencies for the unrelated reference group. We tested this group for departures from Hardy-Weinberg and genotypic equilibria using GENEPOP software (version 3.1d; Raymond and Rousset 1995), with alpha levels set at 0.05. Beier (1993) observed that Interstate Highway 15 and urban development presented significant barriers to mountain lion migration between the Santa Ana Mountains and the rest of the Peninsular Ranges to the southeast. Therefore, we tested for population substructure between these regions by estimating F_{ST} (the amount of genetic variation that is partitioned among subpopulations) using the program GENEPOP (Weir and Cockerham 1984) and incorporated this information in match probability calculations (Ernest et al. 2000). Samples that displayed the same genotype and had match probabilities $<5 \times 10^{-4}$ were considered to be from the same mountain lion (thus limiting Type I error to $<5\%$). We determined this value by assuming that a maximum of 100 mountain lions occurred within bighorn habitat in the Peninsular Ranges (3.3 mountain lions per 100 km^2) during the study period (based on mountain lion densities reported for other desert habitats; Cunningham et al. 1995, Logan et al. 1996,).

Monte Carlo Simulation Model

Using the risk analysis software @Risk Standard Edition (version 3.5.2; Palisade Corporation, Newfield, New York, USA), we developed a Monte Carlo simulation model to quantitatively assess relative costs (losses in mountain lions) and benefits (savings in bighorn sheep and decreased probability of extinction risk) when alternative mountain lion removal strategies were implemented. To examine outcomes, we assigned probability distributions to input variables and executed 5,000 iterations for each simulated scenario. Two strategies were modeled and compared: (1) capture and removal of any mountain

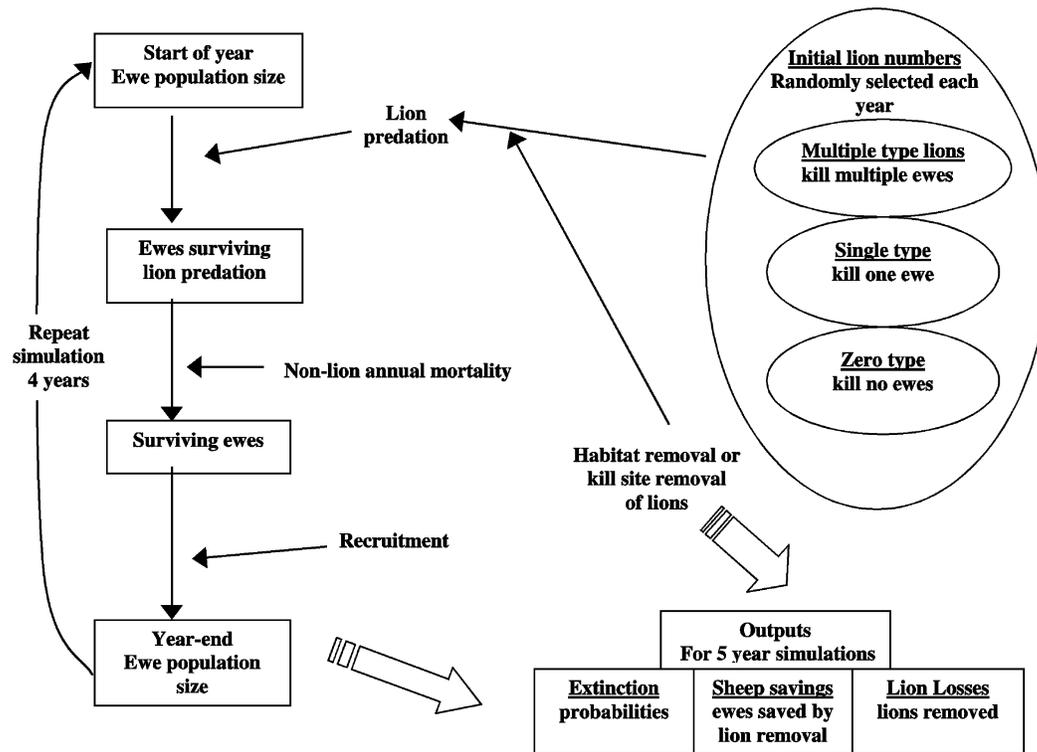


Fig. 2. Schematic diagram of Monte Carlo simulation model.

lion detected in bighorn sheep habitat (habitat removal [HR]), and (2) capture and removal of a mountain lion at the site where a bighorn sheep ewe had been killed by a mountain lion (kill site removal [KSR]). To evaluate the influence of the 2 strategies on bighorn sheep populations of different sizes, we ran simulations differing only in starting size (ranging from 5–80 ewes).

The first year of each 5-year simulation started with a set ewe population size (Fig. 2). Only ewes were considered in this model because bighorn sheep are polygynous, population dynamics are primarily driven by the number of females (Geist 1971), and simulated population viability was highly influenced by adult female survival (unpublished data). The number of ewes killed by mountain lions was subtracted from the number of ewes starting the year. We assumed that all mountain lion predation was additive and that lambs that accompanied ewes killed by mountain lions also were killed (Hayes et al. 2000). Ewes that survived mountain lion predation were randomly subjected to 1 of 17 annual non-mountain lion mortality rates ranging from 0–0.28 for each

year during each iteration based on data from Hayes et al. (2000) and U.S. Fish and Wildlife Service (2000). The survivors of non-mountain lion mortality were then allowed to reproduce using 1 of 14 randomly selected ewe recruitment rates ranging from 0.05–0.34 based on data from the U.S. Fish and Wildlife Service (2000). The surviving ewes and recruited female offspring were carried over each year and subjected to new randomly selected rates for mountain lion predation, non-mountain lion mortality, and recruitment for a total of 5 years. We assumed that movement by ewes between populations was negligible (Rubin et al. 1998, Boyce et al. 1999).

Three categories of mountain lions that may be present within bighorn sheep habitat were designated in the model (Fig. 2): (1) mountain lions that kill multiple ewes within a year (multiple type [MT]), (2) mountain lions that kill 1 ewe within a year (single type [ST]), and (3) mountain lions that did not kill any bighorn sheep ewes within a year (zero type [ZT]). The model excluded juvenile mountain lions that were dependent on their mothers for killing prey. Mountain

lion density and population dynamics in the Peninsular Ranges were unknown; therefore, the total number of mountain lions present at each yearly time step was randomly selected from a range of values (0–2 mountain lions per 100 km² area) based on our DNA data and other mountain lion studies (Cunningham et al. 1995, Logan et al. 1996). The number of ewes that each MT mountain lion killed in 1 year (ewe allotment) was determined by sampling from a truncated normal distribution based on our DNA data and other studies (Harrison 1990, Ross et al. 1997), while each ST mountain lion killed only 1 ewe in 1 year (ewe allotment = 1). By definition, 1 ewe was lost to predation for each mountain lion removed by KSR. Therefore, the number of ewes saved by this strategy was calculated by subtracting 1 ewe from the ewe allotment of each mountain lion removed.

Kill-site removal of 1–4 mountain lions per year was modeled using hypergeometric sampling (without replacement), with the probability of removing a MT or ST mountain lion at a ewe kill site set at 0.95. The probability of capturing a ZT mountain lion that did not kill the ewe, but arrived to scavenge the site after another mountain lion killed the ewe, was set at 0.05 (based on Logan et al. 1996). For HR, each mountain lion that was removed was selected from the general mountain lion population using a hypergeometric sampling distribution from all 3 mountain lion categories. No bighorn sheep were subtracted from the ewe allotment of mountain lions removed by HR because bighorn sheep mortality was not a necessary pre-condition and mountain lions were removed from the simulation year before they would have killed bighorn sheep. For either strategy, the model removed the specified number of mountain lions only if the ewe population was >0 and if there were sufficient mountain lions to remove. There were years in some iterations of a 5-year simulation during which fewer mountain lions were removed than called for by the removal strategy. At the end of the fifth year, the number of ewe populations that went extinct out of 5,000 iterations was used to calculate the probability of extinction (extinction risk). The numbers of ewes saved from predation and mountain lions lost from each of the 3 categories were tallied, with a maximum of 4 mountain lions removed per year under each strategy. The model did not assume a suppression of the mountain lion population resulting from cumulative effects of mountain lion removals over

time because a new population of lions was established at the beginning of each simulated year.

The sensitivity of the model to input values was tested by running additional simulations with best- and worst-case scenarios, and by running simulations changed by only 1 input variable or distribution at a time. In the best-case scenario, recruitment of ewe lambs was set at a deterministic value of 30% and there were no non-mountain lion mortalities (based on data for individual years of good recruitment and low mortality in the Peninsular Ranges; U.S. Fish and Wildlife Service 2000). In the worst-case scenario (based on data for individual years of poor recruitment and higher non-mountain lion mortality in the Peninsular Ranges; U.S. Fish and Wildlife Service 2000), recruitment of female lambs ranged stochastically between 0–20%, non-mountain lion sources of mortality ranged from 0–25%, ewe allotment per MT mountain lion was increased, and there was a 10% probability of a 1-year catastrophic die-off of 25–50% of adult ewes.

RESULTS

DNA Analysis

Microsatellite analysis demonstrated that each of the 38 reference mountain lions exhibited a unique genotype. None of the reference group mountain lion genotypes matched genotypes from scat or hair samples. For the unrelated reference group, there was no significant departure from Hardy-Weinberg equilibrium over all loci. Genotypic disequilibrium tests showed significant *P* values (0.016 and 0.015) at only 2 out of 45 comparisons (2.25 out of 45 would be expected by chance alone at *P* = 0.05). These findings suggest the absence of linkage disequilibrium, considering the number of comparisons that were made. F_{ST} (used for match probability calculations) across all loci was estimated to be 0.07 between the Santa Ana Mountains and the rest of the Peninsular Ranges.

We recovered 78 scats and 1 sample of hair from 39 kill sites, and collected 22 scats opportunistically. Microsatellite DNA was amplified from 54 of the kill-site scats, the single kill-site hair sample, and 9 of the opportunistically collected scats. In several cases, more than 1 scat from a kill site yielded mountain lion DNA. Seventy-seven percent of the kill sites that yielded DNA provided data with sufficient resolution to positively identify an individual mountain lion with a match probability <5 × 10⁻⁴ (Table 1). Thirty-seven percent of all kill sites

Table 1. Information on scat, hair, and hide samples yielding mountain lion DNA in the Peninsular Ranges, California, 1993–1999.

| Mountain lion genotype | Sample collection site | Mortality date or sample collection date | Match probability |
|--|------------------------------|--|----------------------|
| Samples associated with bighorn sheep mortality sites in the study area from Northern Santa Rosa Mountains in north to Interstate Highway 8 in south | | | |
| 1 | Northern San Ysidro Mtns | Jan 1996 | 1.2×10^{-4} |
| 1 | Santa Rosa Mtns/SE of Hwy 74 | Mar 1996 | 1.0×10^{-4} |
| 1 | Santa Rosa Mtns/NW of Hwy 74 | Apr 1996 | 1.0×10^{-4} |
| 1 | Santa Rosa Mtns/SE of Hwy 74 | Sep 1996 | 1.5×10^{-4} |
| 2 | Carrizo Canyon | Apr 1996 | NA |
| 3 | Carrizo Canyon | Mar 1995 | NA |
| 4 | Santa Rosa Mtns/SE of Hwy 74 | Jan 1995 | 1.5×10^{-4} |
| 5 ^a | Coyote Canyon | Mar 1995 | 2.6×10^{-4} |
| 5 ^b | Coyote Canyon | Mar 1995 | 1.3×10^{-2} |
| 5 | Northern San Ysidro Mtns | Aug 1995 | 2.6×10^{-4} |
| 5 | Santa Rosa Mtns/SE of Hwy 74 | Oct 1995 | 2.6×10^{-4} |
| 5 | Santa Rosa Mtns/SE of Hwy 74 | Mar 1996 | 2.6×10^{-4} |
| 5 | Santa Rosa Mtns/SE of Hwy 74 | May 1996 | 2.6×10^{-4} |
| 6 | Vallecito Mtns | Dec 1994 | NA |
| 7 ^c | Santa Rosa Mtns/SE of Hwy 74 | Feb 1995 | NA |
| 8 ^c | Santa Rosa Mtns/SE of Hwy 74 | Feb 1995 | NA |
| 9 | Carrizo Canyon | Feb 1996 | 4.1×10^{-5} |
| 10 | Santa Rosa Mtns/NW of Hwy 74 | Feb 1993 | NA |
| 11 | Carrizo Canyon | Jul 1996 | 6.9×10^{-5} |
| 12 | Santa Rosa Mtns/SE of Hwy 74 | Nov 1994 | NA |
| 13 | Carrizo Canyon | Jan 1995 | NA |
| 14 | Santa Rosa Mtns/NW of Hwy 74 | Apr 1993 | NA |
| 15 | Vallecito Mtns | Dec 1998 | 5.7×10^{-5} |
| 16 | Vallecito Mtns | Aug 1997 | NA |
| 17 ^b | Santa Rosa Mtns/SE of Hwy 74 | Nov 1998 | 4.5×10^{-3} |
| 17 ^b | Coyote Canyon | Feb 1999 | 5.3×10^{-3} |
| 18 | Santa Rosa Mtns/SE of Hwy 74 | Feb 1998 | NA |
| 9/15 ^b | Vallecito Mtns | Nov 1997 | 4.7×10^{-2} |
| A ^b | Vallecito Mtns | Jan 1996 | 1.2×10^{-1} |
| B ^b | Santa Rosa Mtns/SE of Hwy 74 | Nov 1996 | 7.9×10^{-1} |
| C ^b | Santa Rosa Mtns/NW of Hwy 74 | Jun 1997 | 1.2×10^{-1} |
| Samples found in canyon washes, not associated with mortality sites | | | |
| 4 | Northern San Ysidro Mtns | Jun 1996 | 1.7×10^{-4} |
| 5 | Santa Rosa Mtns/SE of Hwy 74 | Jan 1996 | 2.6×10^{-4} |
| 5 ^b | Coyote Canyon | May 1996 | 2.0×10^{-2} |
| 5/11 ^b | Carrizo Canyon | Jun 1996 | 6.9×10^{-3} |
| 9 | Carrizo Canyon | Jun 1996 | 4.1×10^{-5} |
| 19 ^d | Vallecito Mtns | Jan 1996 | NA |
| 20 ^e | Santa Rosa Mtns/SE of Hwy 74 | Jul 1996 | NA |
| 21 | Northern San Ysidro Mtns | Dec 1997 | NA |
| 22 | Santa Rosa Mtns/SE of Hwy 74 | Jan 1995 | NA |
| 23 | Coyote Canyon | Mar 1999 | NA |
| D ^b | Santa Rosa Mtns/SE of Hwy 74 | Jun 1997 | 1.0 |

^a DNA from predator hair collected from plant adjacent to mortality site.

^b Indicates that match probabilities were $>5.0 \times 10^{-4}$. Match probabilities were calculated using θ method (Ernest et al. 2000). Samples having the same genotype and match probabilities $<5.0 \times 10^{-4}$ were considered to be from the same lion (e.g., the 4 observations of genotype #1). Match probabilities $>5.0 \times 10^{-4}$ offer less evidence that the sample came from the same lion as others with the same genotype. If more than 1 genotype is listed (e.g., 9/15), the genotype was different from all other scat or hair genotypes examined, however it could not be resolved among the genotypes listed. Genotypes listed as A, B, C, and D were determined to originate from mountain lions; however, insufficient loci amplified and individual identities could not be resolved.

^c Three scats with genotype #7 and 2 scats with genotype #8 were recovered from 1 bighorn mortality site ($n = 8$ total scats collected).

^d Carcass of mountain lion found unassociated with mortality sites. Cause of death was undetermined. DNA extracted from the hide.

^e Carcass of mountain lion found within 2 m of rumen cache site of a ram mortality associated with lion scat genotype #1. Death was believed to be due to predation from another mountain lion (carcass was cached as is characteristic of lion predation) and to have occurred months prior to the bighorn mortality. The carcass was determined to be an 8- to 9-year-old male lion based on skull characteristics (Shaw 1990). DNA was extracted from the hide.

yielded a sample that could positively identify an individual mountain lion. We calculated match probabilities for those scat samples ($n = 19$ from kill sites; $n = 6$ by opportunistic sampling) and hair ($n = 1$) that yielded genotypes that were the same as 1 or more other samples. Although not every locus amplified in every scat sample, in most cases, sufficient data existed to discriminate individual mountain lions or provide match probabilities $<5 \times 10^{-4}$ for samples with the same genotype. For scats that yielded a unique mountain lion genotype, an average of 10 loci amplified, including on average 3.4 of the 4 species-specific loci (Fca 8, Fca 35, Fca 45, and Fca 77; Ernest et al. 2000). Thus, we have a high degree of confidence that the scats were from mountain lions and not from bobcats or other species.

We identified 18 individual mountain lions at 26 sites where bighorn sheep were killed (Table 1). Thirteen genotypes were each identified at only 1 kill site, while 2 genotypes were unambiguously detected at multiple kill sites. Four kill sites had samples with mountain lion genotype #1, and 5 kill sites had samples with mountain lion genotype #5. Two additional mountain lions were possibly present at more than 1 kill site; however, incomplete DNA data prevented us from confirming that these represented MT mountain lions (genotypes #9 or #15, and #17; Table 1). Three scats with genotype #7 and 2 scats with genotype #8 were recovered from the same bighorn sheep kill site (Table 1). We observed 2 different-sized sets of mountain lion tracks at that site, suggesting the presence of an adult and a cub. Furthermore, a parent-cub relationship was possible since there was at least 1 allele in common at each of the 11 loci that amplified in these samples.

We detected mountain lion DNA in scat samples collected at 3 other kill sites. However, these samples amplified too few loci to unambiguously resolve the genotype of a single mountain lion (genotypes A, B, and C; Table 1). Of the 9 mountain lion genotypes identified in the scats collected opportunistically, 3 were unique from all others; 3 were the same as genotypes #4, #5, and #9 collected at kill sites; and 3 could not be resolved due to insufficient data. We obtained 2 additional unique genotypes from the 2 mountain lion hides found within bighorn sheep habitat (genotypes #19 and #20; Table 1). Overall, 23 mountain lions (18 associated with bighorn sheep kill sites and 5 not associated with known mortalities) were identified in approximately 3,000 km² of bighorn sheep habitat over the 6-year study.

Model Results

Our DNA data indicated that at least 2 MT mountain lions (mountain lions #1 and #5), 3 ST mountain lions (mountain lions #4, #7/8, and #12), and 2 ZT (mountain lions #20 and #22) may have been present over 18 months within the habitat of 1 bighorn sheep subpopulation (Santa Rosa Mountains southeast of Highway 74; Table 1). We incorporated this information, along with data from Logan et al. (1996) and Ross et al. (1997) to construct a theoretical mountain lion population for each year of a 5-year period (Fig. 2). Thus, the model randomly selected 0–2 MT mountain lions, 0–3 ST mountain lions, and 0–3 ZT mountain lions each year to construct a population. Each MT mountain lion was allotted 2–6 bighorn sheep ewes to kill that year, and each ST mountain lion killed 1 ewe that year, based on our DNA data and those of Ross et al. (1997) and Harrison (1990). Output distributions (for number of ewes per population, number of mountain lions removed, and number of ewes saved after 5 years) were stable at 5,000 iterations, with $<1.5\%$ change with additional iterations for means, standard deviations, and percentile values. The effects of KSR and HR on bighorn sheep savings, mountain lion losses, and the probability of extinction are shown in Figs. 3 and 4. During sensitivity analyses, the worst-case scenario caused all extinction curves to uniformly shift up and to the right, and extinction probabilities were higher for each initial ewe population size. As expected, the best-case scenario caused extinction curves to shift down and to the left. In both cases, the relationships of the removal strategies (KSR, HR) relative to each other remained the same.

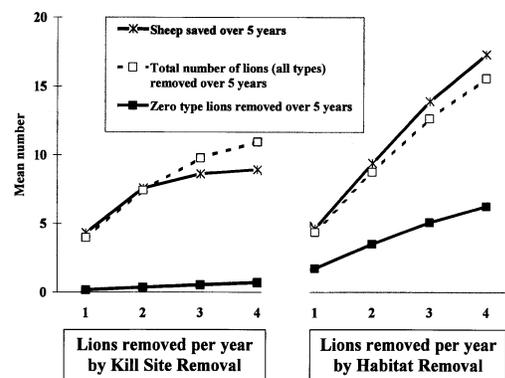


Fig. 3. Bighorn sheep savings and mountain lion losses based on Monte Carlo model simulations.

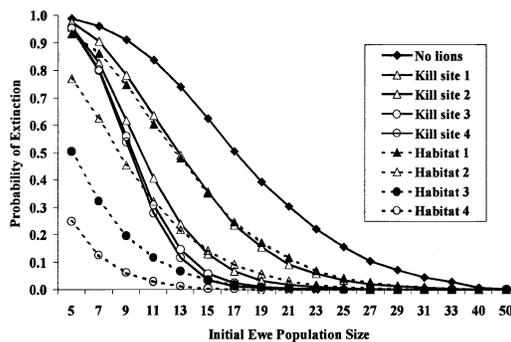


Fig. 4. Curves for probability of extinction within 5 years for bighorn sheep ewe populations in the Peninsular Ranges as modeled using Monte Carlo simulations. Curves represent the removal of no mountain lions (no lions), 1–4 mountain lions each year at bighorn sheep ewe kill sites (Kill site 1–4), and 1–4 mountain lions when they were detected in bighorn sheep habitat (Habitat 1–4).

DISCUSSION

Hayes et al. (2000) demonstrated that mountain lion predation was the major cause of mortality for radiocollared bighorn sheep in the Peninsular Ranges during 1993–1998. However, that study did not provide any information regarding the number of mountain lions preying on bighorn sheep, or the frequency with which individual mountain lions killed bighorn sheep. Based on our DNA analyses, we documented the presence of 18 different mountain lions at 26 bighorn sheep kill sites during this period. Our study lends support to the findings of Ross et al. (1997) and Linnell et al. (1999) that individual mountain lions may specialize on a single alternate prey species. We identified 2 MT mountain lions (#1 and #5) at 9 kill sites, while 2 other MT mountain lions may have been present during the study as well (Table 1). Mountain lions #1 and #5 killed bighorn sheep in several subpopulations over a relatively short time. For example, mountain lion #1 traversed at least 50 km within 1 month to kill a female bighorn sheep in the southeastern Santa Rosa Mountains (Mar 1996) and a ewe and a lamb in the northwestern Santa Rosa Mountains (Apr 1996). For an endangered population such as that in the Peninsular Ranges, the impact of MT mountain lions that specialize on bighorn sheep may be very important. For example, Ross et al. (1997) found that a single MT mountain lion that killed at least 17 bighorn sheep over 4 years likely was responsible for the population decline observed in southeastern Alberta, Canada.

In addition, Wehausen (1996) reported that the presence of 1 or more mountain lions in the Granite Mountains of California substantially reduced that bighorn sheep population.

Our data provide additional support for a growing body of evidence that not all mountain lions kill bighorn sheep repeatedly and that some mountain lions may not kill any bighorn sheep at all. For example, Ross et al. (1997) intensively monitored 5 mountain lions whose ranges overlapped with bighorn sheep range and found that 2 mountain lions did not kill bighorn sheep, while 2 others killed only a single bighorn sheep each. Hornocker (1970) identified only 1 mountain lion out of 46 that killed 2 bighorn sheep over 4 years in an area inhabited by a population of 125 bighorn sheep. Over the course of a 10-year study of 126 radiocollared mountain lions in the San Andres Mountains, New Mexico, 8 mountain lions killed 10 radiocollared bighorn sheep (Logan et al. 1996). One of those mountain lions killed 3 bighorn sheep, while the remaining 7 mountain lions killed 1 bighorn sheep each. It is important to recognize that none of these studies provided definitive information on the relative proportions of MT, ST, and ZT mountain lions that may have been present. In our study, we were unable to individually identify the mountain lions associated with 63% of bighorn sheep kill sites. Thus, it is possible that some of the animals we classified as ST or ZT mountain lions were actually MT mountain lions. Furthermore, our estimate of the proportion of ZT mountain lions was based on opportunistic sampling; thus, we may have underestimated the number of ZT mountain lions that actually were present.

It is possible that mountain lions identified by DNA analysis at kill sites may not have actually killed bighorn sheep. Scavenging mountain lions could deposit a scat at the kill site of another mountain lion (Logan et al. 1996, Pierce et al. 1999). Dependent cubs of a female mountain lion also may deposit scats at her kill (Beier et al. 1995, Pierce et al. 1998). We found evidence that an adult and cub were likely present at 1 kill site (genotypes #7 and #8; Table 1). Another possible source of misclassification is cannibalism by mountain lions (Lindzey 1988). If cannibalism occurred, there could conceivably be 2 sources of mountain lion DNA in a scat at the kill site. The cached carcass of mountain lion #20, killed by another unidentified mountain lion, was found very close to a bighorn sheep kill site (Table 1). Nevertheless, we did not find evidence of more

than 1 genotype per sample in this study or in prior work (Ernest et al. 2000).

Utilizing our DNA data and empirical data from other studies, we were able to construct a simulation model that provided insights into the costs and benefits of mountain lion removal. Not surprisingly, the model demonstrated that the extinction risk for ewe populations varied with ewe population size (Fig. 4). As population size increased above 30, the extinction risk declined to $\leq 10\%$. As population size decreased below 30, there was a corresponding increase in extinction risk such that very small populations (< 10 ewes) had a $> 90\%$ probability of extinction. For relatively large ewe populations (> 30), the removal of mountain lions by either method had a negligible effect on reducing risk of extinction for the population. As a general rule, HR was equal or superior to KSR in terms of bighorn sheep savings (Fig. 3) and reducing risk for populations < 30 (Fig. 4). The advantages of HR over KSR were most pronounced at ewe population sizes < 10 . This undoubtedly occurred because the KSR strategy required that 1 of the few remaining ewes be killed before a mountain lion was removed.

When the cost of removal (mountain lion losses) was examined relative to bighorn sheep savings and extinction risk, it was clear that mountain lion removal should be initiated before bighorn sheep populations drop too low. For example, for populations ranging from 15 to 30 ewes, removal of 1 or 2 mountain lions per year by either strategy reduced extinction risks to $< 15\%$ (Fig. 4). However, once populations fell below 15 ewes, 3–4 mountain lions had to be removed each year to accomplish sizeable reductions in risk. Furthermore, as population size decreased, the HR strategy became more effective than KSR in terms of reducing extinction risk and increasing bighorn sheep savings (Figs. 3, 4). However, this strategy also resulted in more ZT mountain lions being removed than would occur by KSR.

The model illustrated that the most effective management strategy depended on bighorn sheep population size. However, our model results depended on input variables and model assumptions; therefore, the absolute population sizes discussed above may differ from those found in nature. For example, if a portion of the mountain lion predation is compensatory (rather than additive, as we modeled), then the cost in mountain lions will increase while bighorn sheep benefits decline. We used data for mountain lion

predation on bighorn sheep fitted with radiocollars and ear tags. This may have introduced a bias if predation was influenced by a different appearance of the tagged bighorn sheep (Schaefer et al. 2000). Nonetheless, modeling of predator–prey systems can be useful even in the face of uncertainty. For example, Starfield's (1990) model provided insights regarding rainfall–predator–prey interactions for a migratory herd of antelope and African savanna lions, and Johnson and Braun's (1999) model identified population trends and predicted population dynamics for a hunted sage grouse population in Colorado. We addressed uncertainty in our input variables by integrating stochasticity, probability sampling, sensitivity analysis, and iterative computing into the model. The high number of iterations for each simulated scenario allowed outcomes with the highest likelihoods to be identified. Furthermore, sensitivity analyses showed that trends indicated by the model were robust throughout a range of input values for non-mountain lion mortality, recruitment, and number of ewes killed per MT mountain lion. It is clear, however, that additional empirical data are needed, particularly with assumptions regarding the relative proportions of MT, ST, ZT, and scavenging mountain lions.

MANAGEMENT IMPLICATIONS

Other management strategies in addition to those we analyzed might be used to reduce the impact of mountain lions on bighorn sheep. One option is to remove only mountain lions that kill more than 1 bighorn sheep (Logan et al. 1996), but that will result in a higher risk of extinction for very small populations than either the KSR or the HR strategies presented in our model. In some habitats, such as in the Sierra Nevada, mule deer (*Odocoileus hemionus*; the primary prey of mountain lions) migrate to winter ranges and are sympatric with bighorn sheep on a seasonal basis (Pierce et al. 1999). Thus, a selective strategy may be to remove mountain lions that remain in bighorn sheep habitat when deer have emigrated. Because prey availability is an important factor determining the size of a lion population (Pierce et al. 2000), an alternative strategy would be to maintain deer numbers at a lower level than carrying capacity. Ultimately, fewer mountain lions would be available to prey on bighorn sheep.

We clearly recognize that mountain lion population sizes fluctuate over time without any type of management. A decrease in mountain lion

numbers due to natural causes should have the same effect on extinction risk for bighorn sheep as the HR strategy explored in our model. Indeed, there are indications that mountain lion numbers may be declining in several areas in the western United States (Torres 2000). However, current methods for estimating trends in mountain lion numbers are very crude. The molecular methods and applications described here and in Ernest et al. (2000) offer an innovative approach to improving census methods.

The removal of mountain lions for a short time may be the best option available for bighorn sheep populations in immediate danger of extinction due to mountain lion predation. Nonetheless, mountain lions and bighorn sheep both are native species and part of our natural heritage. Beier (1993) suggested that mountain lions in the Santa Ana Mountains, at the north tip of the Peninsular Ranges, are in imminent danger of regional extinction due to habitat loss, high mortality from vehicle strikes, and loss of migration corridors. We strongly recommend that careful assessments be performed on the potential costs and benefits of predator control actions so that the rescue of 1 species or population does not jeopardize another. We have used our DNA data and findings from other studies to develop a simulation model to analyze the costs and benefits of 2 management strategies, habitat removal and kill site removal, that have been proposed or used in the western United States (U.S. Fish and Wildlife Service 2000). However, our results are best viewed as a general guide and should not be applied indiscriminately. Since mountain lion–bighorn sheep systems and management options vary, we recommend that risk assessments and models be performed that incorporate specific data and options for each particular system.

ACKNOWLEDGMENTS

Support for this study was provided by the University of California-Davis (UCD) Genetic Resources Conservation Program, UCD Department of Agriculture and Natural Resources, CDFG Wildlife Investigations Laboratory, CDFG Wildlife Forensic Laboratory, UCD Jastro-Shields Scholarship, UCD School of Veterinary Medicine fellowships, and the American Museum of Natural History Theodore Roosevelt Memorial Fund. Special thanks to M. Jorgensen of the California Department of Parks and Recreation (CDPR) for facilitating fieldwork in the Peninsular Ranges;

G. Lee, B. May, and C. Penedo (UCD) for providing advice and technical assistance on DNA analyses; and S. Torres, V. Bleich (CDFG), and J. Wehausen (UC-Riverside White Mountain Research Station) for sharing their expertise on mountain lion and bighorn sheep biology. We thank B. Collins, M. Jorgensen, P. Jorgensen, R. Mouton, J. Zuehl (CDPR), C. Hayes and C. O'Brien (UCD), C. Traisi (Fund for Animals), R. Botta, B. Gonzales, P. Swift, R. Turner (CDFG), P. Beier (Arizona State University, Flagstaff), M. Culver (Laboratory of Viral Carcinogenesis, National Cancer Institute), and volunteers for collecting field data and samples; T. Carpenter, T. Hansen, and B. Hoar (UCD) for guidance with Monte Carlo simulation modeling; and D. Kress (UCD) for technical assistance. We thank B. May and M. Syvanen (UCD), K. Logan (University of Idaho, Moscow), L. Sweanor (Hornocker Wildlife Institute, Moscow, Idaho), V. Bleich, B. Pierce, S. Torres (CDFG), J. Wehausen, and anonymous referees for comments on the manuscript.

LITERATURE CITED

- BEIER, P. 1993. Determining minimum habitat areas and habitat corridors for cougars. *Conservation Biology* 7:94–108.
- , P. D. CHOATE, AND R. H. BARRETT. 1995. Movement patterns of mountain lions during different behaviors. *Journal of Mammalogy* 76:1056–1070.
- BOYCE, W. M., R. R. RAMEY II, T. C. RODWELL, E. S. RUBIN, AND R. S. SINGER. 1999. Population subdivision among desert bighorn sheep (*Ovis canadensis*) ewes revealed by mitochondrial DNA analysis. *Molecular Ecology* 8:99–106.
- BROWN, D. E., AND C. H. LOWE. 1980. Biotic communities of the southwest. U.S. Forest Service General Technical Report RM-78.
- CALIFORNIA DEPARTMENT OF FISH AND GAME. 1992. Annual report on the status of California state listed threatened and endangered animals and plants. State of California, The Resource Agency, Sacramento, USA.
- CUNNINGHAM, S. C., L. A. HAYNES, C. GUSTAVSON, AND D. D. HAYWOOD. 1995. Evaluation of the interaction between mountain lions and cattle in the Aravaipa-Klondyke area of southeast Arizona. Arizona Game and Fish Department Technical Report Number 17, Phoenix, USA.
- DEFORGE, J. R., S. D. OSTERMANN, C. W. WILLMOTT, K. BRENNAN, AND S. TORRES. 1997. The ecology of Peninsular bighorn sheep in the San Jacinto Mountains, California. *Desert Bighorn Council Transactions* 41:8–25.
- ERNEST, H. B., M. C. T. PENEDO, B. P. MAY, M. SYVANEN, AND W. M. BOYCE. 2000. Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. *Molecular Ecology* 9:433–441.
- FEDERAL REGISTER. 1998. Endangered status for the Peninsular Ranges population segment of the desert

- bighorn sheep in Southern California. Federal Register 63:13134–13150.
- GEIST, V. 1971. Mountain sheep: a study in behavior and evolution. The University of Chicago Press, Illinois, USA.
- GOLDSTEIN, D. B., AND C. SCHLÖTTERER. 1999. Microsatellites: evolution and applications. Oxford University Press, New York, USA.
- HARRISON, S. 1990. Cougar predation on bighorn sheep in the Junction Wildlife Management Area, British Columbia. Thesis, University of British Columbia, Vancouver, Canada.
- HAYES, C., E. S. RUBIN, M. C. JORGENSEN, R. A. BOTTA, AND W. M. BOYCE. 2000. Mountain lion predation on bighorn sheep in the Peninsular Ranges, California. Journal of Wildlife Management 64:954–959.
- HORNOCKER, M. G. 1970. An analysis of mountain lion predation upon mule deer and elk in the Idaho Primitive Area. Wildlife Monographs 21:1–39.
- JOHNSON, K. H., AND C. E. BRAUN. 1999. Viability and conservation of an exploited sage grouse population. Conservation Biology 13:77–84.
- JORGENSEN, M. C., AND R. E. TURNER. 1975. Desert bighorn of the Anza–Borrego Desert State Park. Desert Bighorn Council Transactions 19:51–53.
- KOHN, M. H., AND R. K. WAYNE. 1997. Facts from feces revisited. Trends in Ecology and Evolution 12:223–227.
- LINDZEY, F. G., B. B. ACKERMAN, D. BARNHURST, AND T. P. HEMKER. 1988. Survival rates of mountain lions in southern Utah. Journal of Wildlife Management 52:664–667.
- LINNELL, J. D., J. ODDEN, M. E. SMITH, R. AANES, AND J. E. SWENSON. 1999. Large carnivores that kill livestock: do “problem individuals” really exist? Wildlife Society Bulletin 27:698–705.
- LOGAN, K. A., L. L. SWEANOR, T. K. RUTH, AND M. G. HORNOCKER. 1996. Cougars of the San Andres Mountains, New Mexico. Final Report Project W-128-R for New Mexico Department of Game and Fish, Santa Fe, USA.
- NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION. 1992–1998. Annual Climatological Summary—Borrego Desert Park. National Climatic Data Center, Asheville, North Carolina, USA.
- PIERCE, B. M., V. C. BLEICH, AND R. T. BOWYER. 2000. Social organization of mountain lions: does a land-tenure system regulate population size? Ecology 81:1533–1543.
- , ———, C. L. B. CHETKIEWICZ, AND J. D. WEHAUSEN. 1998. Timing of feeding bouts of mountain lions. Journal of Mammalogy 79:222–226.
- , ———, J. D. WEHAUSEN, AND R. T. BOWYER. 1999. Migratory patterns of mountain lions: implications for social regulation and conservation. Journal of Mammalogy 8:986–992.
- RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2)—population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.
- ROSS, P. I., M. G. JALKOTZY, AND M. FESTA-BIANCHET. 1997. Cougar predation on bighorn sheep in southwestern Alberta during winter. Canadian Journal of Zoology 75:771–775.
- RUBIN, E. R., W. M. BOYCE, M. C. JORGENSEN, S. G. TORRES, C. L. HAYES, C. S. O'BRIEN, AND D. A. JESSUP. 1998. Distribution and abundance of bighorn sheep in the Peninsular Ranges, California. Wildlife Society Bulletin 26:539–551.
- SCHAEFER, R. J., S. G. TORRES, AND V. C. BLEICH. 2000. Survivorship and cause-specific mortality in sympatric populations of mountain sheep and mule deer. California Fish and Game 86:127–135.
- SHAW, H. 1990. Mountain lion field guide. Special Report Number 9. Arizona Department of Game and Fish, Phoenix, USA.
- STARFIELD, A. 1990. Qualitative, rule-based modeling. BioScience 40:601–604.
- TORRES, S. G. 2000. Counting cougars in California. Outdoor California 61(3):7–9.
- U.S. FISH AND WILDLIFE SERVICE. 2000. Recovery plan for bighorn sheep in the Peninsular Ranges, California. U.S. Fish and Wildlife Service, Portland, Oregon, USA.
- WEHAUSEN, J. D. 1996. Effects of mountain lion predation on bighorn sheep in the Sierra Nevada and Granite Mountains of California. Wildlife Society Bulletin 24:471–479.
- WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating F statistics for the analysis of population structure. Evolution 38:1358–1370.

Received 26 December 2000.

Accepted 2 July 2001.

Associate Editor: Rattner.