

MICROSATELLITE ANALYSIS OF THREE SUBSPECIES OF ELK (*CERVUS ELAPHUS*) IN CALIFORNIA

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A total of 676 elk (*Cervus elaphus*) were genotyped at 16 tetranucleotide microsatellite loci to evaluate genetic differences among 3 subspecies of elk in California: tule (*C. e. nannodes*), Roosevelt (*C. e. roosevelti*), and Rocky Mountain (*C. e. nelsoni*) elk. Of the 13 populations analyzed, 5 represented tule elk herds, 3 were Roosevelt elk, 2 were Rocky Mountain elk, and 3 were of uncertain taxonomic status. Overall, populations averaged between 7 and 8 alleles per locus, with observed heterozygosity values ranging from 0.33 to 0.58 per population. Tule elk, which experienced a severe bottleneck in the 1870s, had consistently less genetic diversity than the other subspecies. All 3 subspecies were significantly differentiated, with the greatest genetic distance seen between the tule and Roosevelt subspecies. Assignment of individuals to subspecies using microsatellite data was nearly 100% accurate. Despite the past population bottleneck, significant differences were found among the tule elk herds. Assignment testing of elk from Modoc, Siskiyou, and Shasta counties to determine subspecific status of individuals suggested that these populations contained both Roosevelt and Rocky Mountain elk and their hybrids, indicating that these elk subspecies interbreed where subspecies coexist.

Key words: California, *Cervus elaphus*, elk, genetics, hybrid, microsatellite, population

Elk (*Cervus elaphus*) herds that roamed a large portion of North America have been reduced in both area and number due to hunting pressure and loss of habitat. Although management strategies have aimed to reintroduce elk to some of their original range, these programs are not without potential genetic consequence. Genetic bottlenecks and founder effects are of great concern, and exacerbated by harem mating structure and high variability in male reproductive success (Clutton-Brock 1989).

California contains 3 of the described subspecies of free-ranging elk: tule elk (*C. e. nannodes*; historic resident of oak woodlands and grasslands), Roosevelt elk (*C. e. roosevelti*; northwestern coastal area), and Rocky Mountain (*C. e. nelsoni*; occupying the extreme northeastern corner of California, including Modoc County) elk. The remaining extant subspecies, Manitoban elk (*C. e. manitobensis*), occurs east of the Rocky Mountains in the northern plains states and into central Canada

but does not inhabit California. Although each subspecies naturally occurs in different locations within California, there are potential geographic regions of overlap between Roosevelt and Rocky Mountain elk, allowing for the possibility of hybrid zones.

Tule elk residing in the Central Valley and oak woodlands of the foothills of California were almost eliminated after the gold rush of 1849 (McCullough et al. 1996). Historically estimated at more than 500,000 animals, tule elk were compromised by extreme hunting pressure and conversion of grass and woodland habitat into farming and agricultural operations. In 1873, when tule elk were thought to be extinct, protection was granted by the state of California (McCullough 1969; McCullough et al. 1996). Although exact numbers vary, it is believed that at least a single breeding pair of tule elk was found and protected in the southern San Joaquin Valley in Kern County, California, in 1874. Those remaining elk are believed to be the ancestors of extant tule elk populations in California (McCullough 1969; McCullough et al. 1996).

Roosevelt elk inhabit their historical range in the northwestern coastal mountain ranges of California (O'Gara 2002), mainly Humboldt and Del Norte counties. Only elk inhabiting these 2 counties are categorized as Roosevelt elk by the Boone

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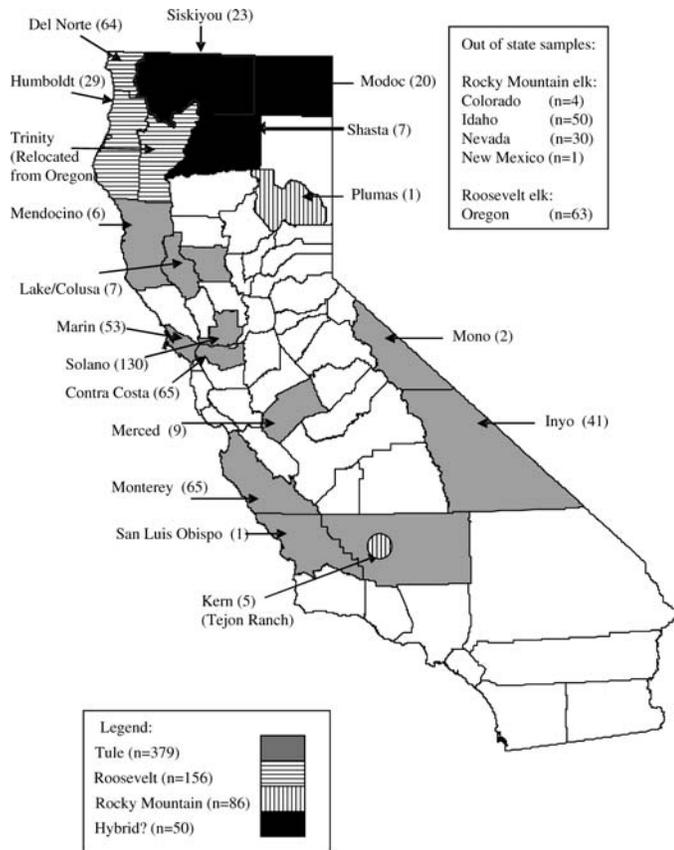


FIG. 1.—Map depicting number of individuals sampled at each herd location given by county name. Gray shaded areas represent counties that contain herds of tule elk, horizontal lines indicate counties with herds of Roosevelt elk, vertical lines indicate counties with herds of supposed Rocky Mountain elk, and diagonal lines indicate potential hybrid zones of Roosevelt and Rocky Mountain elk.

and Crockett Club (Missoula, Montana) for trophy-hunting purposes (Reneau and Reneau 1993). Discrimination of distinct herds of Roosevelt elk is difficult because of the dense forest habitat. Examination of satellite tracking data indicates restricted movement of animals and the possibility of distinct herds (R. Schaefer, in litt.).

Examination of satellite data (R. Schaefer, in litt.) provides evidence that Rocky Mountain Elk of northeastern California may migrate between Modoc County and Oregon, Idaho, and Nevada. Circa 1913, approximately 50 Rocky Mountain elk from Montana were introduced into Shasta County, California (R. Schaefer, in litt.).

Shasta, Siskiyou, and Modoc counties in northern California are considered to be potential hybrid zones for Roosevelt and Rocky Mountain elk by California Department of Fish and Game wildlife managers. For the purpose of our study, the term “hybrid” refers to an intraspecific cross. Interstate 5, a major north–south highway in Washington, Oregon, and California, has been used as an arbitrary management boundary for subspecies delineation: elk occurring west of Interstate 5 have been designated Roosevelt and those to the east of Interstate 5 as Rocky Mountain elk. Lone elk are known to wander and travel great distances (>150 miles—R. Schaefer, in litt.), and

crossing the unfenced Interstate 5 is likely, as inferred by presence of road-killed elk (R. Schaefer, in litt.). Because Roosevelt and Rocky Mountain trophy elk are recorded separately by hunting organizations, determination of the genetic lineage of animals in these areas will benefit trophy hunters and wildlife managers.

Subspecific status of North American elk has been hotly debated (see O’Gara [2002] for discussion of the taxonomy of North American elk). Overlap of morphological differences among tule, Roosevelt, and Rocky Mountain subspecies demands that other discriminating criteria, such as molecular genetic analyses, are used to address taxonomic status. Tule elk are considered the smallest subspecies of North American elk (Merriam 1905) and are typified by having lower body masses, lighter pelage, and the longest tooththrows of any North American subspecies. Roosevelt elk reportedly have the largest body mass and display different antler and jaw morphologies from the others (McCullough 1969; O’Gara 2002). Of the 3 subspecies, Rocky Mountain elk typically have the largest antlers (Reneau and Reneau 1993).

Evidence derived from mitochondrial DNA indicates that tule elk are more closely related to Rocky Mountain than Roosevelt elk, and supports the subspecific status of these 3 categories of elk (Polziehn et al. 1998, 2000; Polziehn and Strobeck 1998, 2002). Using microsatellite data, Williams et al. (2004) showed that tule elk display reduced genetic variation relative to Rocky Mountain and Manitoban elk; however, small sample size prevented robust tests of genetic differentiation among populations of tule elk.

The primary goal of our study was to measure the degree of nuclear genetic differentiation between tule, Roosevelt, and Rocky Mountain elk and evaluate whether the populations of elk in California warrant status as evolutionarily significant units. Given that Roosevelt and Rocky Mountain elk are sympatric in California, yet recorded separately for trophy records, wildlife managers will benefit from genetic information that identifies subspecies composition, particularly in potential hybrid zones. Genetic discriminators will allow identification of subspecies in trophy animals, hair samples from field sampling efforts, and forensic samples. Toward these objectives, we used 2 population assignment programs, WHICHRUN (Banks and Eichert 2000) and STRUCTURE 2.1 (Pritchard et al. 2000), to test the accuracy of assignment to subspecies from multilocus genotype data. Lastly, we assessed the risks and degree of inbreeding faced by herds of tule elk and make recommendations for monitoring and managing these herds.

MATERIALS AND METHODS

Sample collection and DNA isolation.—A total of 676 elk were analyzed in this study (Fig. 1). The majority of the samples were from a large tissue archive maintained by the California Department of Fish and Game’s Wildlife Forensic Laboratory (Rancho Cordova, California). Tissue and blood samples were collected from road-killed animals or animals legally taken at scheduled hunts and elk relocations throughout

California from 1997 through 2003. Samples were shipped frozen on ice to the Wildlife Forensic Laboratory and maintained at -20°C until DNA extraction.

Tule elk from 8 herds were sampled, including 2 of the original 3 surviving herds established in the 1930s: the Owens Valley herd (Inyo County) and the Cache Creek herd (Colusa and Lake counties). The remaining 6 herds of tule elk sampled were created by later translocations; however, all herds of tule elk are descendants from 1 original remnant population.

Samples of Rocky Mountain elk collected from Nevada and Idaho served as reference samples for comparison to Rocky Mountain elk in California. Five Rocky Mountain elk originally translocated from Wyoming to Tejon Ranch in Kern County, California, were sampled. Roosevelt elk from Jewell, Oregon, and translocated to Trinity County, California, between 1988 and 1995 were examined. The Nevada Department of Wildlife supplied muscle tissue samples of 30 Rocky Mountain elk, and the Idaho Department of Fish and Game provided 49 diluted DNA extracts ($10\text{ ng}/\mu\text{l}$) and 1 muscle tissue sample.

The DNA was isolated from all tissue and blood samples using Qiagen QIAmp tissue isolation kits and procedures (Qiagen, Chatsworth, California). After extraction, DNA was quantified using a Molecular Dynamics model 595 Fluorimager (Molecular Dynamics, Sunnyvale, California) using human DNA reference standards of known concentration. DNA from extracted tissue samples was diluted to a concentration of $10\text{ ng}/\mu\text{l}$; blood extracts were not diluted.

Microsatellite analysis.—Multiplex polymerase chain reaction was used to amplify 16 tetranucleotide microsatellite markers developed specifically for elk or mule deer (*Odocoileus hemionus*; see Table 1 for references). All loci used were developed from enriched libraries by GIS Inc. (Chatsworth, California). These primers were selected based upon their highly repeatable polymerase chain reaction products and variability within and among the 3 subspecies of elk described herein.

Forward primers were fluorescently labeled with 6FAM, VIC, or NED (Applied Biosystems, Foster City, California) and the reverse primer had a 5'-GTTTCTT-3' extension added to the 5' end to reduce split peaks and drive the reaction to the "plus A" band (Brownstein et al. 1996). Polymerase chain reaction fragments were detected using a BaseStation DNA Fragment Analyser (MJ Research, Inc., Waltham, Massachusetts).

Each amplification cocktail included up to 20 ng of template DNA, 1X PCR buffer (Applied Biosystems), 2.4 μl of multiplex specific primer concentrations (see below), 0.2 mM of each deoxynucleoside triphosphate, 2 mM MgCl_2 , and 0.2 U (Multiplex D, A, and E) or 0.25 U (Multiplex N) Amplitaq (Applied Biosystems) and double-distilled H_2O to total 20 μl per reaction. Polymerase chain reaction primer concentrations are indicated in Table 1. Reactions containing at least 5 $\text{ng}/\mu\text{l}$ DNA were run on a PTC-100 thermalcycler (MJ Research, Inc.) with the following amplification parameters: 94°C for 3 min, followed by 26 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 40 s, a final extension at 72°C for 20 min, and a final hold at 10°C . All blood samples and tissue samples containing

TABLE 1.—Summary of loci examined in this study. This table shows in which multiplex each locus was amplified, polymerase chain reaction (PCR) primer concentration (each primer), 5' fluorescent dye label used, number of alleles, heterozygosity values observed (H_O), and the reference in which the original primer sequences can be found. Note that all the reverse primers were modified with a 5'-GTTTCTT sequence to reduce split peaks and encourage the formation of "+A" bands during polymerase chain reaction. References: 1 = Jones et al. (2002); 2 = Meredith et al. (2005); 3 = Jones et al. (2000).

Locus	Multiplex	PCR concentration (μM)	5' dye label	No. alleles	Size range (base pairs)	H_O	Reference
T108	D	0.100	6Fam	8	136–181	0.540	1
T26	D	0.483	6Fam	12	328–398	0.565	1
T172	D	0.017	Vic	7	174–198	0.450	1
T501	D	0.600	Ned	9	252–290	0.576	1
T268	N	0.092	6Fam	6	228–256	0.437	1
T156	N	0.062	Vic	15	143–249	0.545	1
T507	N	0.062	Ned	11	148–202	0.390	1
C273	N	0.985	6Fam	8	132–166	0.553	2 and 3
T193	A	0.706	6Fam	10	184–220	0.599	1
C217	A	0.212	Vic	2	185–193	0.415	1
T123	A	0.282	Ned	4	155–186	0.399	1
C180	E	0.048	6Fam	4	156–168	0.507	2
T107	E	0.144	Vic	4	242–265	0.326	2
C229	E	0.144	6Fam	5	299–319	0.363	2
C143	E	0.240	Ned	4	166–178	0.492	2
C01	E	0.624	Ned	5	342–358	0.433	2

less than 5 $\text{ng}/\mu\text{l}$ DNA were amplified for 30 cycles. One microliter of polymerase chain reaction product was then added to 4 μl of loading buffer (double-distilled H_2O , formamide, blue dextran, Genescan 400HD ROX [Applied Biosystems], and Genescan 500 ROX [Applied Biosystems] mixed in a ratio of 220 μl :155.2 μl :51.7 μl :12 μl :12 μl). Polymerase chain reaction products were separated using a denaturing 5.5% acrylamide gel (Long Ranger Gel Solution, Cambrex Bio Science Rockland Inc., Rockland, Maine). Gel data analysis and allele sizing were performed using Cartographer (MJ Research, Inc.).

Statistical methods.—Genotypic data were collected on all 676 samples. However, only those counties or states (Idaho, Nevada, and Oregon) with at least 20 animals ($n = 632$) were used in frequency-based analyses, specifically the calculation of F -statistics and log-likelihood statistics of population differentiation. Because the alleles were not sequenced to determine the actual number of tetranucleotide repeat units, statistical models conforming to the infinite alleles model were used.

Allele frequencies, unique alleles, and observed and expected heterozygosities within counties or states ("populations") with a minimum of 20 individuals and within each of the 3 subspecies were calculated using GENEPOP on the Web (<http://www.biomed.curtin.edu.au/genepop>—Raymond and Rousset 1995). For frequency-based analyses, the populations of Roosevelt elk used were from Humboldt and Del Norte counties (California) and Jewell, Oregon; the populations of Rocky Mountain elk used were from Nevada and Idaho. Deviations from linkage equilibrium between all pairs of loci

across all populations and conformation to Hardy–Weinberg equilibrium on a locus-by-locus basis within populations also were tested using GENEPOP. The P -value for a significant deviation from Hardy–Weinberg equilibrium using the exact test (Guo and Thompson 1992) was adjusted from 0.05 to 0.00027 using a Bonferroni adjustment for 186 tests of the same hypothesis (16 loci by 12 populations with 6 loci being monomorphic in a population). A Bonferroni-adjusted P -value of 0.0014 was used to assess significance for multiple tests of deviation from Hardy–Weinberg equilibrium at the subspecies level (3 subspecies and 16 loci).

Quantitative measures of population differentiation (F_{ST}) and inbreeding (F_{IS}) were made among subspecies and among populations within subspecies using the software package FSTAT (FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3, J. Goudet, 2001; <http://www.unil.ch/izea/software/fstat.html>) as described in Weir and Cockerham (1984) after Bonferroni-adjusted pairwise significance levels. Samples from Modoc, Shasta, and Siskiyou counties were not used in the comparisons of subspecies populations because the taxonomy of elk from these 3 counties was uncertain.

Analysis of molecular variance (AMOVA; ARLEQUIN—Schneider et al. 2000) was used to evaluate the degree of population differentiation based on the relative number of repeats. Genotypic data were analyzed using subspecies, populations within subspecies, and individuals within populations as sources of variation.

The measure of genetic distance among 12 of the county or state sampling groups was Nei's standard distance (D_s —Nei 1972), calculated in PHYLIP, version 3.5c (Felsenstein 1993) using GENDIST. The neighbor-joining method was used in NEIGHBOR (PHYLIP, version 3.5c—Felsenstein 1993).

Animals were assigned to subspecies using genotypic data and 2 population assignment software packages, WHICHRUN (Banks and Eichert 2000) and STRUCTURE 2.1 (Pritchard et al. 2000), to test accuracy of assigning to presumptive subspecies. Elk from the hybrid zones were excluded because of the confounding effects of uncertain lineage. A baseline genotype data file was constructed using known reference animals, including 367 tule elk, 156 Roosevelt elk, and 80 Rocky Mountain elk. The tule elk baseline reference samples consisted of animals from Contra Costa County ($n = 65$), Inyo County ($n = 41$), Lake County ($n = 5$), Marin County ($n = 53$), Monterey County ($n = 65$), and Solano County ($n = 130$). Roosevelt elk baseline samples included Del Norte County ($n = 64$), Humboldt County ($n = 29$), and Oregon ($n = 63$). Rocky Mountain elk baseline samples included elk from the states of Idaho ($n = 50$) and Nevada ($n = 30$).

In WHICHRUN, the probability of a given sample belonging to a “critical population” was generated by a likelihood ratio log of odds score of the probabilities of the 1st and 2nd most probable population assignment given that sample's genotype. The baseline data file of the 603 samples was jackknifed, a log of odds score was generated for the most probable population assignment, and each sample was assigned to that subspecies with log of odds score of ≥ 1.0 .

WHICHRUN was then used to assign individual elk from Modoc, Siskiyou, and Shasta counties to Rocky Mountain or Roosevelt subspecies with log of odds score of ≥ 1.0 . Five elk from the Tejon Ranch (Kern County) and 6 elk from Mendocino County also were analyzed for subspecies verification. The 6 elk from Mendocino County were collected in 2 different locations. An individual was assumed to be a possible hybrid if the log of odds score for both Roosevelt and Rocky Mountain was ≤ 1.0 . The same analysis parameters were used for assignment testing of baseline data and for animals of unknown ancestry.

The baseline genetic data also were tested for assignment accuracy using the program STRUCTURE using 100,000 rounds of iteration after a 10,000-round burn-in. The STRUCTURE genetic analysis program also was used to test assignment of reference elk and samples from Modoc, Siskiyou, and Shasta counties. STRUCTURE was used to estimate the number of lineages that comprise the counties or states without using a priori population information. The number of populations (K) was evaluated for 1–20 populations. Most likely number of populations was determined by $\Delta(K)$ as described in Evanno et al. (2005).

Elk were classified as potential hybrids if the most probable subspecies was <10 times more likely than the 2nd most probable subspecies, indicative of past introgression. This is mathematically equivalent to the log of odds score threshold of 1.0 used in WHICHRUN for subspecies assignment.

RESULTS

Measures of genetic diversity.—Within the 676 samples, loci possessed from 2 alleles (locus C217) to 15 (locus T156; average = 7.3) with observed heterozygosity values ranging from 0.33 (locus T107) to 0.60 (locus T193). F_{IS} estimated for the 5 herds of tule elk analyzed ranged from -0.038 (Contra Costa County) to 0.079 (Inyo County). Tule elk displayed the lowest allelic diversity and showed no more than 5 alleles at each locus (average number of alleles = 3.2), with several loci being monomorphic in some of the tule elk herds. Rocky Mountain elk averaged 6.8 alleles per locus and Roosevelt elk were intermediate with an average of 5.2.

The 16 loci did not show departures from Hardy–Weinberg equilibrium within analyzed counties or states after a Bonferroni correction. However, when data were pooled by subspecies, several loci departed from Hardy–Weinberg equilibrium. No loci deviated significantly from Hardy–Weinberg equilibrium in the 80 samples of Rocky Mountain elk, 6 loci deviated from Hardy–Weinberg equilibrium within the samples of tule elk, and 1 locus deviated significantly from Hardy–Weinberg equilibrium within the samples of Roosevelt elk.

Relationships among subspecies and populations (Table 2).—There were significant differences in allele frequencies among populations of tule elk. Exact tests of population differentiation yielded a P -value of <0.0002 and significance at all pairwise comparisons of the tule elk herds (1% level after Bonferroni corrections). The overall value of F_{ST} for the 5 populations of tule elk was 0.11.

TABLE 2.—Genetic distances among the 3 subspecies of elk (*Cervus elaphus*) in California and their populations. Data are presented for both the population and subspecific levels of comparison. Nei's standard genetic distance values are above the diagonal and F_{ST} values are below. Significance levels for pairwise tests are: *** $P = 0.001$, ** $P = 0.01$, and * $P = 0.05$ after a Bonferroni correction. The Oregon samples were collected from animals released into California from Oregon. Sample sizes for each population or herd are given in Fig. 1.

	Tule elk herds					Roosevelt elk populations			Rocky Mountain elk populations		Subspecies		
	Contra Costa	Inyo	Marin	Monterey	Solano	Del Norte	Humboldt	Oregon	Idaho	Nevada	Tule	Roosevelt	Rocky Mountain
Tule													
Contra Costa	—	0.03	0.12	0.03	0.07	0.49	0.64	0.42	0.46	0.62			
Inyo	0.06**	—	0.11	0.02	0.08	0.54	0.74	0.50	0.47	0.63			
Marin	0.19**	0.14**	—	0.10	0.08	0.42	0.61	0.34	0.37	0.45			
Monterey	0.07**	0.03**	0.13**	—	0.06	0.55	0.71	0.45	0.45	0.56			
Solano	0.12**	0.12**	0.10**	0.10**	—	0.41	0.59	0.39	0.39	0.53			
Roosevelt													
Del Norte	0.37**	0.33**	0.25**	0.34**	0.29**	—	0.18	0.09	0.31	0.53			
Humboldt	0.47**	0.42**	0.34**	0.42**	0.37**	0.12*	—	0.25	0.47	0.61			
Oregon	0.40**	0.37**	0.27**	0.37**	0.31**	0.06*	0.16*	—	0.17	0.31			
Rocky Mountain													
Idaho	0.33**	0.28**	0.21**	0.28**	0.27**	0.14**	0.19**	0.13**	—	0.09			
Nevada	0.38**	0.33**	0.25**	0.33**	0.31**	0.20**	0.24**	0.18**	0.03*	—			
Subspecies													
Tule											—	0.55	0.48
Roosevelt											0.30*	—	0.31
Rocky Mountain											0.28*	0.14*	—

Exact tests of population differentiation, as measured by allele frequencies, were highly significant ($P < 0.0002$) among populations of Roosevelt elk (Oregon and Humboldt and Del Norte counties) and among populations of Rocky Mountain elk (Nevada and Idaho). F_{ST} values among populations of Roosevelt elk ($F_{ST} = 0.096$) and between populations of Rocky Mountain elk ($F_{ST} = 0.03$) were less than those observed among herds of tule elk. Individual populations of Roosevelt and Rocky Mountain elk showed significant differentiation at the 5% nominal level after Bonferroni corrections.

Data from the 3 subspecies were analyzed as a whole and tested for population differentiation using subspecies as the source of variation (Table 2). A highly significant Exact test ($P < 0.0002$) suggested that there were greater differences in allele frequencies among the 3 subspecies than among populations or herds within any of the 3 subspecies. Pairwise tests of differentiation between the 3 subspecies were all significant at the 5% nominal level of significance after a Bonferroni correction. The AMOVA results (Table 3) indicated that the subspecies are well differentiated.

STRUCTURE yielded results, both in terms of K populations and $\Delta(K)$, that suggested the sampled elk are from 2 "populations": tule and Roosevelt–Rocky Mountain elk lineages. Although the likelihood values for $K = 1$ –20 populations approached a maximum at $K = 3$ populations, the $\Delta(K)$ values spiked at $K = 2$ populations.

Subspecies clustered distinctly, with 100% bootstrap support between tule elk and the other 2 subspecies (Fig. 2). The node separating the 2 Rocky Mountain elk populations (Idaho and Nevada) from the other subspecies populations had a 94% level of bootstrap support.

Assignment testing.—All of the 367 samples presumptively categorized by wildlife managers as tule elk assigned correctly using both WHICHRUN and STRUCTURE (Table 4). STRUCTURE was slightly more accurate in assigning reference elk to their presumptive subspecies, although both programs yielded a very high success rate of correct assignment. Population assignment of Roosevelt and Rocky Mountain elk had a small error rate (<5%), which varied by analysis program. One presumptive Roosevelt elk collected from eastern Oregon (Bend, Oregon) was assigned to the Rocky Mountain subspecies with >3.0 log of odds score.

Assignment testing of individual elk using both STRUCTURE and WHICHRUN (Table 5) revealed that Modoc, Shasta, and Siskiyou counties were inhabited by Rocky Mountain, Roosevelt, and hybrid elk. The same individuals were identified as hybrids by both programs. The 5 individuals from the Tejon Ranch in Kern County were correctly assigned as Rocky Mountain elk. The 6 elk from Mendocino County consisted of 2 Roosevelt elk and 4 tule elk.

TABLE 3.—Analysis of molecular variance of 3 subspecies of elk (*Cervus elaphus*) in California using subspecies, populations within subspecies, and individuals as sources of variation. Samples were collected from 1997 through 2003.

Source of variation	<i>df.</i>	Sum of squares	Variance components	Percentage of variation (%)
Among subspecies	2	905.12	1.253 Va	24.18
Among populations within subspecies	7	319.94	0.3631 Vb	7.00
Within populations	1,170	4,174.93	3.568 Vc	68.81
Total	1,179	5,399.99	5.185	

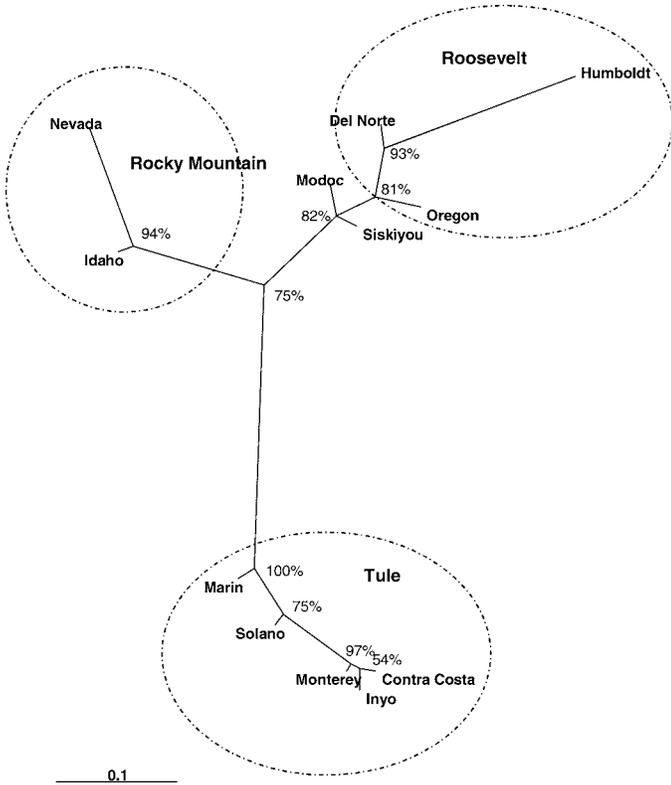


FIG. 2.—Unrooted tree of Nei's standard genetic distance after bootstrapping the data 1,000 times. The bootstrap level of support (out of 1,000) is indicated at each node. Included are all populations of elk with at least 20 samples.

DISCUSSION

Tule elk have much reduced microsatellite variation compared to the Roosevelt and Rocky Mountain elk subspecies, as expected given the severe population bottleneck in the late 1800s. The low level of genetic variability in the tule elk was likely due to the low numbers of founders rather than insufficient sampling, because sampling collections were well distributed among herds. Thus, the molecular genetic uniqueness of the tule elk resulted from lack of genetic variation, not from novel genetic variability.

Tule elk may have been reduced to 1 breeding pair in 1874 (McCullough et al. 1996). Barring a mutation event or experimental error, the presence of 5 alleles at 1 locus requires that the tule elk subspecies was reduced to no fewer

TABLE 4.—Assignment test results for 3 subspecies of elk (*Cervus elaphus*) in California using programs WHICHRUN and STRUCTURE 2.1. The numbers of correct assignments are on the diagonal and incorrect assignment counts are off the diagonal for each program.

Software	Subspecies	n	Tule	Roosevelt	Rocky Mtn.
WHICHRUN	Tule	367	367	—	—
	Roosevelt	156	—	151	5
	Rocky Mountain	80	—	1	79
STRUCTURE 2.1	Tule	367	367	—	—
	Roosevelt	156	—	154	1
	Rocky Mountain	80	—	—	80

TABLE 5.—Assignment tests of elk from Modoc, Siskiyou, Shasta, and Kern counties, California, using programs WHICHRUN and STRUCTURE. Animals are noted as potential hybrids using WHICHRUN when the log of odds score of assignment was less than 1.0, and when the probability of assignment was less than 10 times the 2nd most probable subspecies using STRUCTURE.

Program	County			
	Modoc (n = 20)	Siskiyou (n = 23)	Shasta (n = 7)	Kern (n = 5)
WHICHRUN				
Roosevelt	9	15	1	0
Rocky Mountain	10	2	5	5
Hybrid	1	5	1	0
STRUCTURE 2.1				
Roosevelt	9	15	1	0
Rocky Mountain	10	2	5	5
Hybrid	1	5	1	0

than 1 female and 2 males, or vice versa. Allele frequencies varied significantly among the herds of tule elk. The results also suggest that the herds in Contra Costa, Inyo, and Monterey counties were more closely related than the other 2 herds of tule elk; the Marin herd was the most distantly related. This also was reflected in the phylogenetic results (Fig. 2) and follows logically from historical information on relocations (McCullough et al. 1996). Because all tule elk originated from the same herd, founder effects and genetic drift likely caused the herds to diverge genetically in spite of relocation efforts.

Although tule elk do not currently display the effects of reduced fitness, such as low reproductive output and morphological deformities, the individual herds are definitely at risk if they remain genetically isolated. However, reduced genetic variation at neutral loci does not necessarily indicate a lack of adaptability (Hedrick 1999, 2001) and would not warrant intentional crossbreeding with Roosevelt or Rocky Mountain elk.

We propose the following management recommendations for tule elk given the genetic data and their life-history characteristics. Management of tule herds should continue to involve the movement of animals, preferably mature females, between the tule herds. Adult female elk would be much more likely to contribute genetically because of the harem mating structure, because an introduced male elk would likely have to establish dominance before breeding. Translocating elk among Inyo, Contra Costa, and Monterey counties should not negatively impact genetic diversity of these 3 herds, because they are closely related.

Periodic monitoring of the physical health and genetics of the tule herds is required in order to detect a rise in frequency of deleterious inherited phenotypes, reduced fitness, and other effects of inbreeding. Although the 6 elk samples from Mendocino County were either pure tule or pure Roosevelt and did not indicate crossbreeding, the elk in the Mendocino and Lake county areas should be monitored for hybridization. The tule and Roosevelt elk sampled were from 2 differ-

ent locations and did not occur sympatrically. Tule elk in Mendocino County have recently been detected in close proximity to Roosevelt elk (R. Schaefer, in litt.). Introgression of Roosevelt elk into these tule herds should prohibit their use for future transplants.

The reproductive strategy of elk makes this species vulnerable to the loss of genetic diversity. Williams et al. (2002, 2004) applied theory and computer simulation to conclude that elk in small isolated herds tend to lose genetic variation and heterozygosity. The effect of small population size is magnified by the highly polygynous nature of elk, and even brief bottlenecks can have a large effect on the number of alleles and heterozygosity of species with this mating system.

The effects of a small population size on a mammal are well illustrated by research on Florida panthers (*Puma concolor coryi*). Hedrick (2001) suggested that populations that remain small over a long time period would incur a large genetic load from fixation of many deleterious alleles of small effect, as seen in the Florida panther. Even with an effective population size of 30–50, this subspecies of panther so rapidly accumulated deleterious alleles through drift and inbreeding that it was in serious danger of extinction (Hedrick 1995).

Population assignment for individual reference elk with known source populations using multilocus genotype data was concordant with source population records because of highly significant differences in allele frequencies observed between the subspecies. Two population assignment software programs, WHICHRUN and STRUCTURE, yielded nearly identical assignment accuracies. This high degree of accuracy is important from a forensic standpoint because tule elk are a heavily managed subspecies within California; recaptured escapees from game refuges and evidence from suspected cases of tule elk poaching now can be reliably identified to subspecies.

Elk present in the northern California counties of Modoc, Siskiyou, and Shasta are genetically Roosevelt elk, Rocky Mountain elk, or hybrids of these 2 subspecies. Thus, trophy elk taken by sportsmen from these counties cannot be reliably assigned to subspecies in the absence of molecular genetic information. The unique genetic character of Roosevelt elk from California merits careful monitoring of translocations of elk if new animals are moved into the existing herds in Humboldt and Del Norte counties from areas containing elk of mixed ancestry.

Our analyses lend strong support to previously published work suggesting that tule, Roosevelt, and Rocky Mountain elk should be designated as discrete subspecies (Polziehn et al. 1998, 2000; Polziehn and Strobeck 1998, 2002) and as evolutionarily significant units. Values of F_{ST} and log-likelihood values for tests of population differentiation were highly significant. AMOVA results indicated that the subspecies are well differentiated and gene flow has likely occurred among populations within the subspecies.

The criteria used for determining which populations comprise an evolutionarily significant unit have been the topic of considerable debate (i.e., Crandall et al. 2000; Fraser and Bernatchez 2001; Moritz 1994, 2002). We incorporated

criteria from these studies and propose evolutionarily significant units for elk in California. Tule elk displayed highly significant differences in nuclear allele frequencies relative to other elk populations, consistent with the criteria of Waples (1991) and Moritz (1994, 2002). Given its unique ecological niche, evolutionarily significant unit status is warranted under the “ecological exchangeability” concept of Crandall et al. (2000).

We propose evolutionarily significant unit status for Roosevelt elk of the north coast of California (Humboldt and Del Norte counties). Again, significant genetic divergence was observed between this group and the other sampled populations. Because Roosevelt elk from the Olympic Peninsula in Washington State may have some Rocky Mountain introgression (Polziehn and Strobeck 2002), care (and perhaps genetic testing) is essential before translocating elk from the Olympic Peninsula to augment Roosevelt elk in other regions, including California.

Rocky Mountain elk are the least populous elk in California, although they exist in great numbers in the mountains of the western United States. They are genetically distinct from both the Roosevelt and tule elk and inhabit environments where the tule elk are absent. The only pure population of Rocky Mountain elk within California identified from this study occurs at Tejon Ranch (Kern County). These animals originally were imported from Yellowstone National Park, Wyoming. California Department of Fish and Game managers had expressed concern that these animals had bred with tule elk at 1 point in time; this concern appears unfounded. Rocky Mountain elk and tule elk are held at 2 physically separated ranches in Kern County. Although Rocky Mountain elk are sympatric with Roosevelt elk in northern California, their range extends beyond that of Roosevelt elk east into the Rocky Mountains. Elk taken from the counties containing hybrids should be genetically tested on an individual basis to determine the subspecies of their source. Polziehn et al. (2000) documented that population subdivision and restricted gene flow occurs in herds of Rocky Mountain elk, many of which were relocated or reintroduced. Considering that this subspecies covers a large geographic area, future studies covering larger geographic areas are likely to identify additional Rocky Mountain elk evolutionarily significant units.

To date, our study is the most comprehensive population genetic analysis of the 3 subspecies of elk inhabiting California and should provide valuable information for elk managers and wildlife law enforcement. Future conservation efforts should focus on ensuring connectivity between herds or populations within each evolutionarily significant unit to ensure that adaptive genetic variation is maintained in a large population and not removed by genetic drift or fixed by inbreeding in small isolated populations. Current population management efforts focus primarily on the protected tule elk, maintained as several distinct, isolated herds across the state. We recommend the continued translocation of tule elk between the herds in order to maintain the genetic diversity of the tule subspecies and avoid the potential inbreeding that can occur in small polygynous herds.

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