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CONSERVATION GENETICS OF THE SONOMA TREE VOLE (*ARBORIMUS POMO*) BASED ON MITOCHONDRIAL AND AMPLIFIED FRAGMENT LENGTH POLYMORPHISM MARKERS

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We used a comparative, multimarker approach to investigate the conservation genetics of an arboreal vole (the Sonoma tree vole [*Arborimus pomo*]) in the Pacific Northwest of North America. We compared geographic patterns and overall levels of genetic diversity based on 55 amplified fragment length polymorphism (AFLP) loci with those based on a single, commonly used mitochondrial locus, the control region. Although examination of the control region data revealed the presence of 2 distinct mitochondrial DNA (mtDNA) clades within *A. pomo* (1 in the north and 1 in the south of the species' range), the nuclear perspective provided by AFLP did not reveal a similar geographic division within the species, supporting instead that *A. pomo* consists of a single panmictic population. Genetic diversity estimates based on the mtDNA data (gene diversity = 0.79 and 0.80 for the 2 clades) were much greater than those based on AFLP (gene diversity = 0.31 and 0.19 for the 2 clades). These contrasting results reflect inherent differences between mitochondrial and nuclear loci in mutation rate, effective population size, expected time to monophyly, and mode of inheritance, and highlight the utility of using the combination of AFLP and mtDNA when assessing the genetic status of wild populations and species of mammals, especially those of conservation concern. In the case of *A. pomo*, our combined AFLP and mtDNA data support the recognition of the southern Sonoma tree voles as a distinct management unit within the species.

Key words: amplified fragment length polymorphism (AFLP), *Arborimus pomo*, genetic diversity, mitochondrial DNA (mtDNA), Sonoma tree vole

Biological diversity is threatened worldwide (Koh et al. 2004; Thomas et al. 2004), yet for many taxonomic groups we still lack the fundamental ecological and evolutionary information necessary to develop effective conservation strategies (Hammond 1994; May 1990; Tear et al. 1995). Most conservation efforts to date have been ecological, ranging from those designed to maintain or restore viable populations of imperiled species to more community-level approaches designed to identify and preserve geographic areas that maximize species or habitat diversity (Primack 2002). These types of conservation approaches provide essential information on contemporary levels and patterns of biodiversity on earth, as well as detailed information on the natural history and demographic characteristics of select species (Lande 1988). However, the preservation of genetic diversity also may be crucial to the long-term survival of species (Spielman et al. 2004). The rarest and most critically endangered species are, by definition, comprised of small and often fragmented populations, making them particularly susceptible to the loss of genetic variation resulting from random genetic drift and bottleneck events (Frankham et al. 2002; Gaines et al. 1997). In turn, reduced levels of genetic variation are often directly related to a reduction in fitness and also may limit the ability of a population to respond to changes in its environment over time (Frankham 1996; Frankham et al. 1999; Mills and Smouse 1994; Nei et al. 1975; O'Brien 1994; O'Brien et al. 1985). Given that most threatened species must contend with an increasing number of novel situations in their environments, such as loss of habitat, invasive species, and climatic changes, a detailed understanding of their underlying genetic diversity and population genetic structure is critical for the long-term conservation of the species (Bowen 1999; Nielsen 1999; Olsen et al. 2003; Spielman et al. 2004).

In mammals (and animals in general), direct sequencing of regions within the mitochondrial DNA (mtDNA) genome has emerged as a widely used and powerful tool for examining genetic variation, population structure, and phylogenetic

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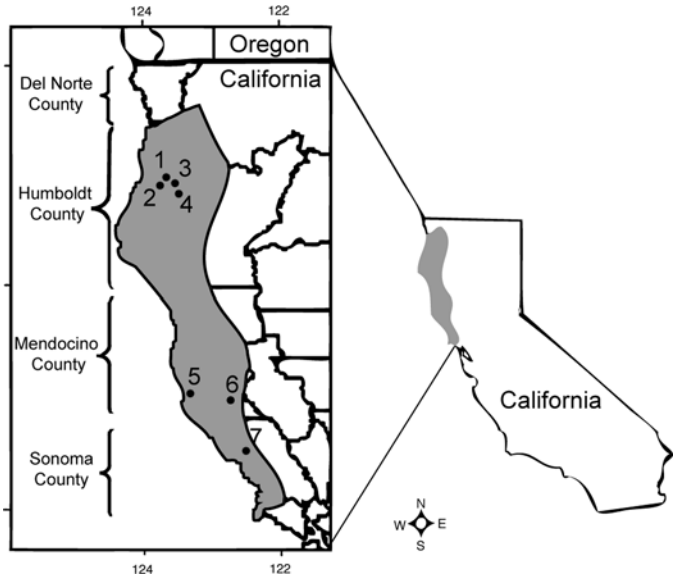

Fig. 1.—Geographic range of *Arborimus pomo* in northern California (shaded region). The numbered dots indicate general location of sampling areas with respect to counties in northern California and correspond to those given in Tables 1 and 2. Sites: 1 = Highway 299; 2 = Korbel; 3 = Maple Creek; 4 = Boulder Creek; 5 = Manchester; 6 = Galbreath Ranch; 7 = Sonoma County. The range map was adapted from Adam and Hayes (1998).

relationships across a range of spatial, temporal, and taxonomic scales (Avise et al. 1987). However, there are some potentially important limitations to this molecule, especially for conservation purposes (Ballard and Whitlock 2004). First, mtDNA represents a very small part of the mammalian genome; the mitochondrial molecule is about 16,000 bases long in mammals, which represents just 0.00055% of the total human genome, for example (Ballard and Whitlock 2004). Thus, issues of sampling alone could be problematic for inferring the evolutionary history of a species based solely on mtDNA, even if the entire molecule was sequenced. Second, it is unknown how well variation within the mitochondrial genome of mammals reflects variation across the nuclear genome because of their different modes of transmission. Potentially, the maternally inherited mtDNA of a given species of mammal could have a very different genealogical and demographic history than that of its biparentally inherited nuclear DNA (Chappell et al. 2004; Miller et al. 2005; Patton and Smith 1994). In particular, because the effective population size of mtDNA is much smaller than that of nuclear genes, mtDNA may be more sensitive to bottleneck events even when the genes have the same genealogical history (Edwards and Beerli 2000). This may lead to patterns of geographic structuring and levels of genetic diversity that differ greatly between the 2 genomes (Creer et al. 2004; Shaw 2002).

To gain a more comprehensive understanding of population genetic structure and diversity within a species, researchers have begun to explore newly developed methods for rapidly assessing genetic diversity at large numbers of nuclear loci; one such method is amplified fragment length polymorphism (AFLP—Vos et al. 1995). AFLPs are widely used in plants (reviewed by Blears et al. [1998]), but in only a few cases have they been examined in wild populations of mammals (Byrnes 1999; Dragoo et al. 2003; Kingston and Rosel 2004; Polyakov et al. 2004; Sipe and Browne 2004). AFLP has the potential to be a powerful technique for assessing genetic variation within and between new taxa because it can be used at different taxonomic levels, is relatively inexpensive, and requires no initial time investment associated with primer design (Bensch and Akesson 2005). Furthermore, because the same set of primers can be used on different taxa, the potential of this approach for comparative studies, such as comparative phylogeography (Arbogast and Kenagy 2001), is particularly appealing. As with all techniques, there are some drawbacks to AFLPs (namely, they are unable to detect codominant loci) but overall, AFLPs represent a promising, but as yet largely untapped complement to traditional single-locus approaches (such as mtDNA sequencing) in phylogeographic and conservation studies of mammals (see Bensch and Akesson [2005] for an in-depth review of AFLPs).

In this paper, we combine AFLPs and mtDNA sequence data to infer population genetic structure and genetic variability across the geographic range of a unique rodent, the Sonoma tree vole (*Arborimus pomo*). *A. pomo* (formerly *Arborimus* [*Phenacomys*] *longicaudus*) is a highly arboreal member of the Arvicolinae endemic to the temperate coniferous forests of northwestern California (Fig. 1; Bellinger et al. 2005; Johnson and George 1991). *A. pomo* nests, forages, eats, and travels almost exclusively in Douglas-fir (*Pseudotsuga menziesii*) trees (Carey 1991; Carey et al. 1991; Hamilton 1939), and although not strictly dependent on old-growth forest, it prefers habitat with larger trees (Meiselman and Doyle 1996; Thompson and Diller 2002). The Sonoma tree vole is currently listed as a Species of Special Concern by the California Department of Fish and Game and has several characteristics typical of many endangered species; it has a highly specialized ecology, a very limited distribution, and has suffered extensive habitat loss throughout its range (Norse 1990). The dependence of Sonoma tree voles on Douglas-fir makes the widespread loss and fragmentation of this habitat type alarming, especially given that no data are available on how the extensive fragmentation of northern California forests may be affecting the demographic and genetic structure of *A. pomo*. Only 2 studies have examined the molecular structure and evolutionary history of the genus (Bellinger et al. 2005; Murray 1995) and no molecular studies have focused on *A. pomo*. Because of the highly specialized, arboreal habits of this species, in situ sampling of individuals is difficult (Swingle et al. 2004) and molecular methods may be particularly valuable in assessing population structure and historical gene flow. *A. pomo* is thus a good model species for assessing genetic variation within rare or endangered species, where sample sizes are also likely to be small and traditional sampling restricted.

Our major goal for this study is to conduct a multimarker molecular analysis of *A. pomo* (using both the mtDNA control region and AFLPs) to assess population structure and genetic diversity within this species for conservation and management purposes.

Table 1.—Locality information for *Arborimus* and *Phenacomys* samples from the Pacific Northwest used in this study. The mitochondrial DNA (mtDNA [group]), mtDNA haplotype name, amplified fragment length polymorphism (AFLP) genotype name, sampling area, and GenBank accession number are given for each sample. The sampling area (Fig. 1) is given as the county (state) of collection for all specimens except *A. pomo*, for which more specific locations are given. Museum voucher numbers associated with specific samples can be found on GenBank. Symbols: * indicates the sample was used only in the mtDNA analysis; † indicates the sample was used only in the AFLP analysis. State abbreviations: WA = Washington, OR = Oregon, CA = California. GenBank numbers starting with "AY" correspond to samples in Miller et al. (2006).

Species	mtDNA group	mtDNA haplotype name	AFLP genotype name	Sampling area	GenBank accession number
Phenacomys intermedius	Phenacomys	Phenacomys1	*	Whatcom (WA)	DQ198847
		Phenacomys2	*	Okanogan (WA)	DQ198846
Arborimus albipes	Albipes	Albipes1	*	Lane (OR)	DQ198848
		Albipes1	*	Lincoln (OR)	DQ198849
		Albipes2	*	Lane (OR)	DQ198850
A. longicaudus	Longicaudus North	Longicaudus1	*	Benton (OR)	DQ324546
		Longicaudus2	*	Lincoln (OR)	DQ198853
		Longicaudus3	*	Lincoln (OR)	AY836263
		Longicaudus4	*	Tillamook (OR)	AY836255
	Longicaudus South	Longicaudus5	*	Douglas, Curry, Josephine (OR)	AY836314
		Longicaudus6	*	Douglas, Coos (OR)	AY836289
		Longicaudus7	*	Del Norte (CA)	DQ198851
		Longicaudus7	*	Del Norte (CA)	DQ198852
		Longicaudus7	*	Curry, Josephine (OR)	AY836321
A. pomo	Humboldt	†	AFLP 5	Highway 299, Humboldt (CA)	
		†	AFLP 5	Highway 299, Humboldt (CA)	
		Pomo3	AFLP 6	Highway 299, Humboldt (CA)	DQ198821
		Pomo4	AFLP 13	Korbel, Humboldt (CA)	DQ198834
		Pomo5	*	Highway 299, Humboldt (CA)	DQ198836
		Pomo6	AFLP 2	Highway 299, Humboldt (CA)	DQ198820
		Pomo6	AFLP 10	Highway 299, Humboldt (CA)	DQ198823
		Pomo6	AFLP 3	Highway 299, Humboldt (CA)	DQ198830
		Pomo6	AFLP 3	Highway 299, Humboldt (CA)	DQ198831
		Pomo7	AFLP 15	Korbel, Humboldt (CA)	DQ198819
		Pomo7	AFLP 12	Maple Creek, Humboldt (CA)	DQ198824
		Pomo7	AFLP 12	Boulder Creek, Humboldt (CA)	DQ198825
		Pomo7	AFLP 12	Maple Creek, Humboldt (CA)	DQ198826
		Pomo7	AFLP 16	Maple Creek, Humboldt (CA)	DQ198828
		Pomo7	AFLP 17	Maple Creek, Humboldt (CA)	DQ198832
		Pomo7	AFLP 18	Boulder Creek, Humboldt (CA)	DQ198839
		Pomo7	AFLP 14	Korbel, Humboldt (CA)	DQ198841
		Pomo7	AFLP 7	Highway 299, Humboldt (CA)	DQ198844
		Pomo8	AFLP 4	Highway 299, Humboldt (CA)	DQ198822
		Pomo9	AFLP 1	Highway 299, Humboldt (CA)	DQ198827
		Pomo10	AFLP 12	Korbel, Humboldt (CA)	DQ198829
		Pomo10	AFLP 11	Korbel, Humboldt (CA)	DQ198833
		Pomo10	AFLP 9	Highway 299, Humboldt (CA)	DQ198845
		†	AFLP 8	Highway 299, Humboldt (CA)	
		†	AFLP 12	Highway 299, Humboldt (CA)	
		†	AFLP 12	Highway 299, Humboldt (CA)	
	Mendocino	Pomo14	AFLP 22	Manchester, Mendocino (CA)	DQ198835
		†	AFLP 21	Manchester, Mendocino (CA)	
		Pomo16	AFLP 20	Manchester, Mendocino (CA)	DQ198838
		Pomo16	AFLP 23	Manchester, Mendocino (CA)	DQ198843
		Pomo16	*	Galbreath, Mendocino (CA)	DQ198842
		Pomo17	AFLP 19	Manchester, Mendocino (CA)	DQ198837
		Pomo18	AFLP 14	Sonoma (CA)	DQ198840

Materials and Methods

Sample collection and extraction.—We obtained tissue samples of *A. pomo* from direct field sampling of extant populations in Humboldt County, California, and from private and museum collections (Table 1). The samples came from 4 main sampling areas within Humboldt County, 2 sampling areas within Mendocino County, and 1 sampling area from Sonoma County (Fig. 1). Direct sampling was accomplished by accessing arboreal nests via single-rope tree-climbing techniques (Perry 1978) and probing the nest with a blunt-tipped, metal probe until the vole exited. In some cases, the nest tree was small enough that the tree was shaken until the vole left the nest. Voles were captured by hand after exiting their nests. All animals were handled in accordance

with guidelines of Humboldt State University and the American Society of Mammalogists (Animal Care and Use Committee 1998). Samples consisted of either tail snips preserved in ethanol or body tissues stored at −80°C. We extracted genomic DNA from the samples using either a QIAGEN DNeasy tissue extraction kit (Qiagen Inc., Valencia, California) or a standard phenol–chloroform extraction (Ausubel and Brent 1992).

Mitochondrial DNA.—A 362–base pair segment of the mtDNA control region was amplified using the primers CTRL-L (Bidlack and Cook 2001) and TDKD (Kocher et al. 1993) with the following polymerase chain reaction profile: an initial 45-s DNA denaturation step at 94°C, then 35 cycles of a 10-s denaturation step at 94°C, a 15-s annealing step at 48°C, and a 45-s extension step at 72°C, followed by a final extension of 3 min at 72°C. Most purified polymerase chain reaction products were sequenced at the Microchemical Core Facility of San Diego State University (San Diego, California). However, some individuals were sequenced at Humboldt State University by cloning the amplified fragment following the protocol outlined in the Promega pGem-T Easy Vector System (Promega Corp., Madison, Wisconsin) and sequencing using a LI-COR DNA 4200 automated DNA sequencer (LI-COR Biosciences, Lincoln, Nebraska). In most cases (except the cloned fragments), both strands were sequenced to minimize sequencing error. We visually examined all sequences for errors and aligned them using Sequencher software (Gene Codes Corp., Ann Arbor, Michigan).

To evaluate the samples of *A. pomo* within a phylogenetic framework, we also obtained mtDNA data from specimens of *A. longicaudus*, *A. albipes*, and *Phenacomys intermedius*, 3 closely related species (these samples were not used in the AFLP analysis because it was not the main focus of this study to examine intraspecific patterns of genetic variation within any species other than *A. pomo* [Table 1]). We examined the phylogenetic relationships among mtDNA haplotypes of *A. pomo*, *A. longicaudus*, and *A. albipes* using a combination of likelihood and distance methods. *P. intermedius* was used as the outgroup for *Arborimus* following Bellinger et al. (2005). We used MODELTEST (version 3.5—Posada and Crandall 1998) to determine the model of nucleotide substitution that provided the best fit to the control region data based on the Akaike information criterion. We then used PAUP* (version 4.0b10—Swofford 2003) to infer a maximum-likelihood gene tree of all unique control region haplotypes using the best-fit model conditions with starting trees determined by 10 random addition sequence replicates. Nodal support was estimated by performing 100 maximum-likelihood bootstrap replicates. We also estimated Bayesian posterior probabilities for each node using MRBAYES (version 3.1—Huelsenbeck and Ronquist 2001) with the best-fit model conditions specified under MODELTEST. We ran the analysis for 1×10^6 generations, with trees sampled every 100 generations (a total of 10,000 trees), and then discarded the first 2,500 trees for burn-in (inspection of likelihood scores indicated that the scores had stabilized by this point).

We estimated genetic diversity parameters for *A. pomo* from the mtDNA data using Arlequin (version 2.00—Schneider et al. 2000). Specifically, we estimated gene diversity following the method of Nei (1987) and nucleotide diversity following the methods of Tajima (1983) and Nei (1987). We also performed a hierarchical analysis of molecular variance (AMOVA—Excoffier et al. 1992) to examine the partitioning of genetic variance among the phylogenetic lineages detected using PAUP and MRBAYES. We tested for isolation by distance by performing a Mantel test using the Vegan package (Oksanen et al. 2005) in the statistical program R (R Development Core Team 2005). Genetic distances were calculated in Arlequin as the squared Euclidean pairwise difference between individuals. Geographic distances were measured as the approximate straight-line distance between individuals using a topographic map.

Amplified fragment length polymorphisms.—We used the AFLP method (Vos et al. 1995) to assess variation across the nuclear genome in *A. pomo*. Vos et al. (1995) found that AFLP was reliable over a range of initial DNA quantities but was sensitive to DNA quality. Thus, we 1st gel purified the samples using a QIAGEN QIAquick Gel Extraction Kit (Qiagen Inc.) to ensure high-quality template DNA. We followed the AFLP protocol outlined in the LI-COR IRDye Fluorescent AFLP Kit for Large Plant Genome Analysis (LI-COR Biosciences) with some modifications. Most importantly, the initial digestion was longer (3 h instead of 2 h) than initially outlined in the protocol because the banding pattern was not reproducible with a shorter digestion period. Additionally, we quantified and standardized the amount of DNA in each sample immediately before final amplification to ensure a uniform DNA quantity of 250–300 ng of DNA per 1-μl sample. We initially screened 12 primer combinations and used 4 primer combinations in the final amplification step: MseI-CAA and EcoRI-AGC, MseI-CAT and EcoRI-AGC, MseI-CAT and EcoRI-ACC, and MseI-CAT and EcoRI-ACG. These primer combinations produced clean, scorable banding patterns and revealed some level of variability between individuals at at least some of the AFLP loci. Fragments were separated on an 8% polyacrylamide gel and detected using a LI-COR DNA 4200 automated DNA sequencing machine. Fragments were scored for presence or absence using RFLPscan Plus (version 3.0; Scanalytics, Fairfax, Virginia). Each sample was verified by eye at each locus.

We used PAUP* (version 4.0b10—Swofford 2003) to infer an unrooted neighbor-joining tree for *A. pomo* based on total pairwise character differences between unique AFLP genotypes, with ties randomly broken. The inclusion of all individuals rather than just unique AFLP genotypes did not change our conclusions. We estimated genetic diversity among all individuals using the software program AFLP-SURV (Vekemans 2002). AFLP-SURV computes several estimates of population genetic diversity based on the method of Lynch and Mulligan (1994) for random amplified polymorphic DNA data: the number and proportion of polymorphic loci, and expected heterozygosity (H_E or Nei's gene diversity). Additionally, AFLP-SURV uses the Lynch and Mulligan (1994) method to assess the total population genetic structure by estimating total gene diversity (H_T), average gene diversity within phylogenetic lineages (H_W), average gene diversity between phylogenetic lineages (H_B), and Wright's fixation index (F_{ST}). We tested for isolation by distance by performing a Mantel test based on squared Euclidean distances as described above.

Results

Mitochondrial DNA.—We obtained clean mtDNA sequences from 27 individual *A. pomo* distributed across 7 sampling areas (Tables 1 and 2), in addition to 14 sequences from the other taxa. The TIM+I model of nucleotide substitution best described the control region data. This model is based on a symmetric nucleotide substitution rate matrix with the following substitution rates: A–C = 1.000, A–G = 8.828, A–T = 4.796, C–G = 4.796, C–T = 28.961, and G–T = 1.000. Additionally, the estimated proportion of invariable sites was 0.799 and the observed nucleotide frequencies were A = 0.312, C = 0.263, G = 0.088, and T = 0.337. The resulting gene tree (Fig. 2) generally supported the phylogeny of Bellinger et al. (2005), with *A. pomo* monophyletic and sister to *A. albipes*. Our samples from Del Norte County, California, grouped with

TABLE 2.—The number of samples and unique mitochondrial DNA (mtDNA) haplotypes and amplified fragment length polymorphism (AFLP) genotypes for each *Arborimus pomo* sampling area in northern California. The names correspond to those given in Table 1. When more than 1 individual has the same haplotype or genotype in a given sampling area, the number of individuals with that haplotype or genotype is noted in parentheses.

		mtDNA			AFLP		
mtDNA group	Sampling area	Samples	Haplotypes	mtDNA haplotype names	Samples	Genotypes	AFLP genotype names
Humboldt	Highway 299	10	7	Pomo3	14	11	AFLP 1
				Pomo5			AFLP 2
				Pomo6 (4)			AFLP 3 (2)
				Pomo7			AFLP 4
				Pomo8			AFLP 5 (2)
				Pomo9			AFLP 6
				Pomo10			AFLP 7
							AFLP 8
							AFLP 9
							AFLP 10
							AFLP 12 (2)
	Korbel	5	3	Pomo4	5	5	AFLP 11
				Pomo7 (2)			AFLP 12
				Pomo10 (2)			AFLP 13
							AFLP 14
							AFLP 15
	Maple Creek	4	1	Pomo7 (4)	4	3	AFLP 12 (2)
							AFLP 16
							AFLP 17
	Boulder Creek	2	1	Pomo7 (2)	2	2	AFLP 12
							AFLP 18
Mendocino	Manchester	4	3	Pomo14	5	5	AFLP 19
				Pomo16			AFLP 20
				Pomo17 (2)			AFLP 21
							AFLP 22
							AFLP 23
	Galbreath	1	1	Pomo16			
	Sonoma	1	1	Pomo18	1	1	AFLP 14

those forming a southern clade of *A. longicaudus* as reported by Miller et al. (2006; see "Discussion"). However, the monophyly of *A. longicaudus* was only weakly supported in our Bayesian analysis (posterior probability = 0.63) and not supported in the maximum-likelihood analysis (Fig. 2). *A. albipes* formed a strongly supported lineage based on both Bayesian and maximum-likelihood analyses (posterior probability = 1.00, bootstrap support = 99%) and was sister to *A. pomo* with strong Bayesian support but only weak maximum-likelihood support (posterior probability = 0.99, bootstrap support = 63%; Fig. 2). The monophyly of all samples of *A. pomo* was strongly supported by the Bayesian analysis (posterior probability = 0.92), but not maximum-likelihood analysis. Rather, maximum-likelihood analysis placed *A. albipes*, a southern *A. pomo* lineage, and several northern *A. pomo* haplotypes as an unresolved polytomy.

Two phylogenetic lineages within *A. pomo* were identified by the Bayesian analysis, with 3.14% mean corrected sequence divergence between them (Fig. 2). One lineage, comprised of individuals from Mendocino and Sonoma counties in the southern part of the species' range (hereafter referred to as the Mendocino group), was strongly supported (Bayesian posterior probability of 1.00 and 100% maximum-likelihood bootstrap support). The remaining samples, all from the northern part of the species' range, formed a 2nd lineage (hereafter referred to as the Humboldt group) with moderate nodal support (Bayesian posterior probability = 0.78). AMOVA further supported the distinctiveness of the 2 *A. pomo* lineages ($F_{ST} = 0.811$, $P < 0.00001$). The Humboldt and Mendocino groups had estimated gene and nucleotide diversity values of 0.786 ± 0.075 and 0.007 ± 0.004 (Humboldt), and 0.800 ± 0.172 and 0.011 ± 0.007 (Mendocino), respectively (Table 3). The relative proportion of distinct haplotypes was greater within the Mendocino group, which consisted of 4 haplotypes shared by 6 individuals, although sample size for this group was small (Table 2). The Humboldt group consisted of 8 distinct haplotypes shared among 21 individuals (Table 2). The Mantel test showed a significant relationship between geographic and genetic distances ($r = 0.944$, $P < 0.001$) when all individuals were considered a single group. However, within the Humboldt and Mendocino groups, individuals showed no pattern of isolation by distance (Humboldt: $r = 0.038$, $P = 0.275$; Mendocino: $r = -0.169$, $P = 0.612$).

Amplified fragment length polymorphisms.—We obtained purified DNA from 31 individuals of *A. pomo* distributed across 6 sampling areas (Tables 1 and 2). The 4 primer combinations generated a total of 55 clear, scorable loci, 37 (Humboldt) and 35 (Mendocino) of which were polymorphic.

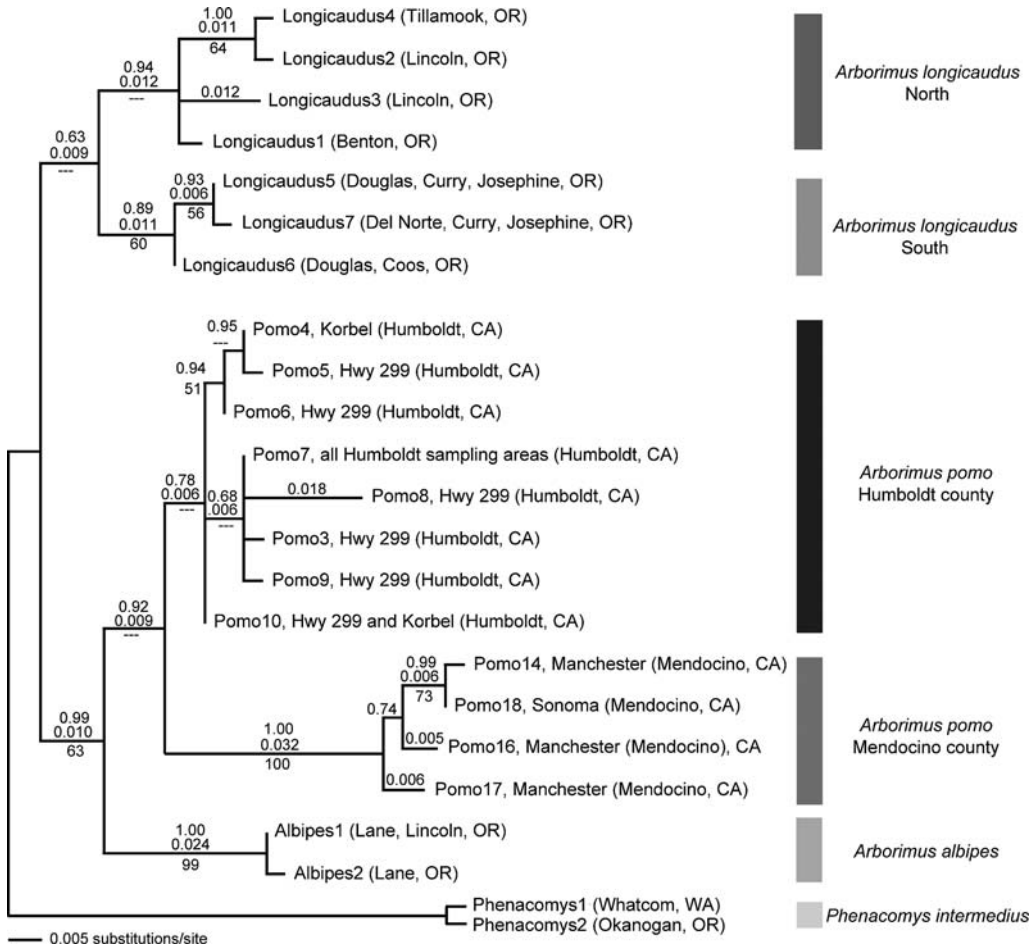

FIG. 2.—Phylogenetic relationships among *Arborimus* taxa in the Pacific Northwest of North America based on Bayesian analysis of sequence data from the mitochondrial DNA (mtDNA) control region. The mtDNA haplotype identification and sampling area (county, state) are given for each unique haplotype. The mtDNA group (Table 1) is given rather than county for all haplotypes of *A. pomo*. All haplotype and group names correspond to those in Table 1. Note that the Mendocino group contains samples from Sonoma County. For each node, the Bayesian posterior probability (top number) and maximum-likelihood bootstrap support (bottom number) are given, along with the Bayesian estimate of the length of the branch leading to the node (middle number). Dashes indicate that the node was not recovered in the maximum-likelihood analysis. Only maximum-likelihood bootstrap values greater than 50% and branch lengths greater than 0.005 substitutions per site are shown. (OR = Oregon, CA = California.)

We did not exclude the monomorphic loci from our analyses so as not to bias the diversity estimates. Based on results from the mtDNA analysis, samples were initially divided into the 2 mtDNA groups (Humboldt and Mendocino) for comparative purposes. However, examination of a neighbor-joining tree based on the total pairwise character difference matrix showed no clear geographic structuring within the species (Fig. 3). Overall, the Humboldt group had slightly higher diversity levels than the Mendocino group; in the former 67.3% of the loci were polymorphic and expected heterozygosity (H_E) or Nei's gene diversity was 0.319 ± 0.027, compared to 63.6% and 0.192 ± 0.025, respectively, for Mendocino (Table 3). Within the Humboldt group, 18 of 25 individuals had a unique AFLP genotype, whereas all 6 individuals within the Mendocino group had AFLP genotypes that were distinct from one another. In one case, individuals belonging to the 2 different mtDNA groups shared the same AFLP genotype (AFLP 14; Tables 1 and 2; Fig. 3).

Total gene diversity (H_T) for the combined Humboldt and Mendocino groups of *A. pomo* was 0.295, with most of the gene diversity occurring within (0.256 or 86.7%) rather than between groups (0.039 or 13.3%). Wright's fixation index (F_{st} = 0.127), based on 500 random permutations of individuals among groups, was not significant. There was not a

TABLE 3.—Diversity estimates based on sequence data from the mitochondrial DNA (mtDNA) control region and from the amplified fragment length polymorphism (AFLP) method for the Humboldt and Mendocino groups within *Arborimus pomo* in northern California.

	mtDNA		
Group	Nucleotide diversity (± variance)	Gene diversity (± variance)	AFLP gene diversity (± *SE*)
Humboldt	0.007 ± 0.004	0.786 ± .075	0.319 ± 0.027
Mendocino	0.011 ± 0.007	0.800 ± .172	0.192 ± 0.025

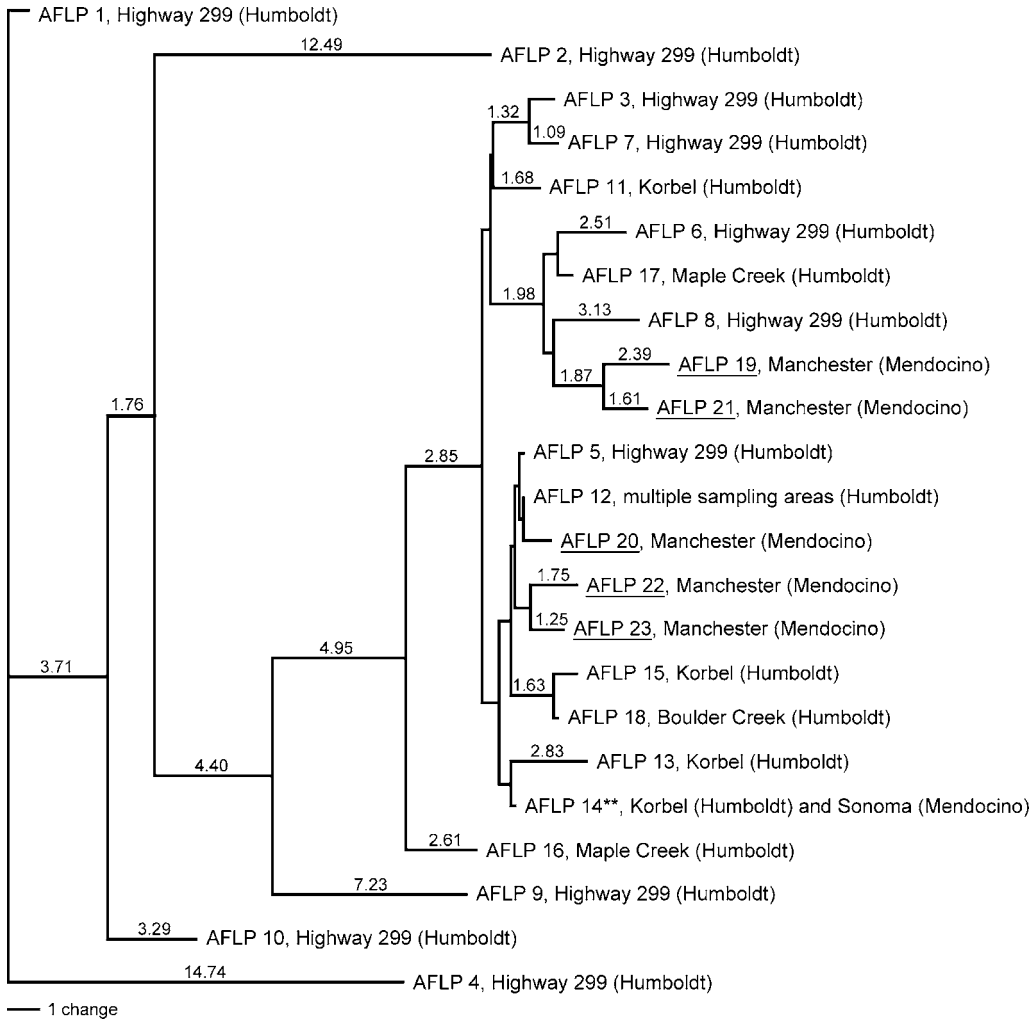

Fig. 3.—Amplified fragment length polymorphism (AFLP) neighbor-joining tree for *Arborimus pomo* in northern California, based on the total pairwise character differences among AFLP genotypes. AFLP genotype identification, sampling area, and mitochondrial DNA (mtDNA) group from Table 1 are indicated. AFLP genotypes from the Mendocino mtDNA group are underlined and the AFLP genotype shared by individuals in the Humboldt and Mendocino mtDNA groups is indicated by 2 asterisks (**). All other taxa belong to the Humboldt mtDNA group. Numbers above branches correspond to total character length of that branch. Only branches with >1.0 change in length are shown.

significant pattern of isolation by distance in the AFLP data ($r = -0.103$, $P = 0.746$).

Discussion

Phylogenetic relationships among Arborimus voles.—The mtDNA control region gene tree (Fig. 2) is consistent with the cytochrome-*b* gene tree of Bellinger et al. (2005) in supporting a monophyletic *A. pomo* and a sister relationship between *A. pomo* and *A. albipes*. Recently, Miller et al. (2006) identified 2 major clades within Oregon members of *A. longicaudus* (southern and northern clades). However, they did not include tree voles from Del Norte County, California (Fig. 1) in their study and the relationship of these voles to *A. longicaudus* has been uncertain, in part because they are geographically located at the boundary between *A. longicaudus* and *A. pomo*. Johnson and George (1991) placed them within *A. pomo* because of karyotype similarities, but Murray (1995) placed them within *A. longicaudus* based on mtDNA restriction enzyme analysis. The samples of *A. longicaudus* (including the samples from Del Norte County, California) in this study fell within the 2 *A. longicaudus* clades identified by Miller et al. (2006), with samples from Tillamook and Lincoln counties, Oregon, falling within the northern clade as expected and samples from Del Norte County, California, falling within the southern clade (they were identical to haplotype 67 from Miller et al. [2006]). Thus, our analysis supports inclusion of Del Norte County, California, tree voles within *A. longicaudus*. However, the monophyly of *A. longicaudus* and its sister relationship to the *A. pomo–A. albipes* group was only weakly supported (posterior probability = 0.63; Fig. 2) in our analyses.

Genetic population structure of A. pomo.—Analysis of the mtDNA control region and AFLP data revealed contrasting geographic patterns of genetic variation within *A. pomo*. The Humboldt and Mendocino groups formed reciprocally monophyletic mtDNA clades (Fig. 2) and there was a significant

amount of geographic structuring, with a large portion of the total observed mtDNA variation between the Humboldt and Mendocino groups (81.1%) rather than within each lineage (18.9%). Examination of these data suggests that gene flow between the northern and southern parts of the species' distribution may have been limited in the past. In contrast to the mtDNA data, very little of the overall genetic variation detected using AFLP was due to variation between the Humboldt and Mendocino groups. For example, only 13.3% of the total AFLP variation observed within *A. pomo* can be attributed to variation between these groups, whereas 86.7% can be attributed to variation within groups. Furthermore, unlike the reciprocal monophyly observed for the Humboldt and Mendocino groups based on the mtDNA control region data (Fig. 2), there was little geographic structure apparent in the AFLP neighbor-joining tree (Fig. 3), highlighted by the fact that in one case the same AFLP genotype was shared between individuals representing the 2 different mtDNA groups (AFLP 14; Tables 1 and 2).

The discordant patterns of genetic population structure based on the mtDNA and ALFP data could be the result of several nonexclusive factors. Foremost among these is the expected time to monophyly for mitochondrial versus nuclear loci. Because the diploid nuclear genome has a 4-fold larger effective population size than the haploid mitochondrial genome, the coalescent process is expected to proceed much more slowly for the former (on average, taking approximately four times longer than mtDNA to reach reciprocal monophyly—Avise 2000). Although the Humboldt and Mendocino mtDNA clades are reciprocally monophyletic (Fig. 2), this divergence is relatively shallow (mean pairwise sequence divergence = 3.14%), especially considering the rapid rate of evolution typical of the control region of mammals (Larizza et al. 2002). This, along with only moderate support for the monophyly of the Humboldt group (posterior probability = 0.78), suggests that the mtDNA divergence in *A. pomo* is relatively recent; based on estimates for the rate of divergence in the control region of other arvicoline rodents (i.e., ~13% per million years—Galbreath and Cook 2004), this divergence is likely to be only a few hundred thousand years old (mid–late Pleistocene). As such, even if gene flow completely ceased between the Humboldt and Mendocino groups at this time, it is likely that the gene trees of many nuclear loci would not yet have had enough time to achieve reciprocal monophyly. This would be reflected in many shared ancestral polymorphisms between the Humboldt and Mendocino groups at nuclear loci and a lack of strong differentiation between them (Avise 2000).

Although only a handful of studies (e.g., Irwin et al. 2005; Sipe and Browne 2004) have compared phylogeographic patterns based on mtDNA with those based on AFLPs, a range of genealogical concordance has been observed between the 2 types of markers. For example, Sipe and Browne (2004) used these 2 markers to compare the phylogeography of 2 species of shrews (*Sorex cinereus* and *S. fumeus*) in the Appalachians. Although for *S. fumeus* they found clear phylogeographic structuring in both mtDNA and AFLPs, there was no significant phylogeographic structure in either marker for *S. cinereus*. In these cases, the mtDNA and AFLP markers exhibited general genealogical concordance within each species, although the observed degree of phylogeographic structuring varied greatly between species. In contrast, Irwin et al. (2005) found genealogical discordance between mtDNA and AFLP markers in greenish warblers (*Phylloscopus trochiloides*); although this taxonomic group exhibits multiple deeply divergent mtDNA clades, the AFLP data are consistent with an isolation-by-distance model. Irwin (2002) found that this type of genealogical discordance is expected to arise in a continuously distributed species as long as individual dispersal distances are small relative to the overall geographic range of the species.

In the case of *A. pomo*, a lack of detailed data makes it difficult to infer how dispersal dynamics might influence the shapes of gene genealogies. However, examination of data from the ecologically and taxonomically closely related *A. longicaudus* indicates that the mean daily minimum distance moved by both sexes is less than 7 m and does not differ significantly between the sexes, although males move more often than females (J. K. Swingle, pers. comm.). If the same is true for *A. pomo*, low individual dispersal distances may be contributing to the observed discordant patterns of genetic population structure based on the mtDNA and ALFP data in a similar fashion to that observed in greenish warblers (Irwin et al. 2005).

Sex-biased dispersal (e.g., strong female philopatry coupled with high male dispersal) is another mechanism commonly invoked to explain genealogical discordance between mitochondrial and nuclear loci (Avise 2004). In their study of wolverines (*Gulo gulo*), Chappell et al. (2004) concluded that although males and females have the same dispersal capabilities, strong mtDNA structure coupled with the lack of nuclear genetic structure supports the idea of female site fidelity and male dispersal. In their study of the Oregon slender salamander (*Batrachoseps wrighti*), Miller et al. (2005) also invoked sex-biased dispersal as one of the main mechanisms for producing the discordant pattern of genetic structure detected by mtDNA and random amplified polymorphic DNA techniques. However, unless *A. pomo* differs greatly from *A. longicaudus* in this respect, sex-biased dispersal seems unlikely to have played an important role in shaping the genealogical discordance observed in the former species.

Because samples from localities intermediate to the Humboldt and Mendocino groups were not available in the present study, it remains unclear if the observed divergence in the mtDNA control region between these groups reflects a distinct phylogeographic discontinuity or is simply a product of isolation by distance. There was a highly significant pattern of isolation by distance based on the mtDNA data, but not on the AFLP data. However, the mtDNA relationship appears to be driven by the large geographic distance between the Humboldt and Mendocino groups, because there was no pattern of isolation by distance within each group; additional sampling in the center of the range is warranted. Additionally, although we were able to use the AFLP method to sample 55 presumably independent loci across the genome, it is possible that even this was inadequate to detect subtle geographic patterns of genetic structure in *A. pomo*. Other recent studies in different taxa have generated substantially more polymorphic loci using a similar number of primer pairs (e.g.,

240 polymorphic loci using 3 primer pairs [plants, *Phyteuma globulariifolium*—Schönswetter et al. 2002], 525 polymorphic loci using 5 primer pairs [vipers, *Trimeresurus stejnegeri*—Creer et al. 2004], 650–664 polymorphic loci using 5 primer pairs [shrews, *S. cinereus* and *S. fumeus*—Sipe and Browne 2004], and 62 polymorphic loci using 3 primer combinations [greenish warblers, *P. trochiloides*—Irwin et al. 2005]).

Levels of genetic variability within A. pomo.—Quantification of genetic variation within species is of central importance in conservation genetics (Frankham et al. 2002). Genetic variation is an important factor in determining the ability of a species to adapt to new environmental conditions and therefore may be an important measure of the evolutionary potential and long-term viability of a species. Despite the high geographic resolution typically provided by mtDNA control region data, the mtDNA control region provides little information on genome-wide levels of diversity within species because it represents such a small portion of the total genome. To assess genetic variation representative of the entire genome of an organism, it is necessary to survey a large number of independent loci, which is one of the strengths of AFLP. In this study, AFLPs provided data from 55 presumably unlinked nuclear loci, whereas direct sequencing of the mtDNA control region provided information from only 1 locus. The data resulting from the 2 techniques provided very different pictures of levels of genetic diversity within *A. pomo*. Overall, levels of diversity based on the mtDNA data were substantially higher than the AFLP data (Table 3). This highlights the fact that levels of genetic variability based only on the rapidly evolving mtDNA control region are likely to give large overestimates of genome-wide genetic variability.

Conservation implications.—Conservation of species is an important concern today because of the rapid rate of extinction the world is currently experiencing (Koh et al. 2004; Thomas et al. 2004). Although traditional conservation strategies such as habitat preservation are vitally important for ensuring both the short- and long-term conservation of species, conservation of genetic diversity also plays an important role in helping ensure species existence through evolutionary time. Reliable and informative molecular methods are necessary to accurately assess the evolutionary history and genetic variation of species in order to make sound conservation decisions. Additionally, molecular techniques may be especially important when studying species that are rare or difficult to sample using traditional, field-based methods. In this study, the unique diet (dependent almost entirely on Douglas-fir needles—Benson and Borell 1931) and highly arboreal lifestyle of the Sonoma tree vole rendered traditional methods of livetrapping ineffective. Instead, each vole had to be hand-captured by climbing into the tree canopy, a very labor-intensive method (Swingle et al. 2004). For example, our exhaustive survey of 1 local population within Humboldt County yielded a rough estimate of a large local population (23 active nests $\approx$ 23 individuals), yet extensive climbing and capture efforts yielded only 3 captures (Blois 2005). Thus, it was impractical to accurately estimate population size or detect movement and activity patterns within 1 small population of voles, let alone across the entire species. In contrast, molecular methods provided practical information on the genetic population structure of *A. pomo*. With the advent of new and increasingly efficient molecular techniques, integrating genetic information from a large number of loci distributed throughout the genome into conservation decisions should be a top priority. This study demonstrates that the AFLP technique can be a valuable tool for quantifying genetic variation in groups that are not well studied or when information is needed quickly (Bensch and Akesson 2005).

The use of both mitochondrial and nuclear markers is gaining widespread support in phylogeography and conservation biology. Moritz (1994) provided a theoretical framework for the inclusion of genetic information in species conservation and management with his delineation of evolutionarily significant units (Ryder 1986; Waples 1991) and management units. Moritz recognized that information from both nuclear and mitochondrial genomes provides different and complementary information in conservation biology. Evolutionarily significant units represent deeply diverged lineages and Moritz (1994) proposed that evolutionarily significant unit designation requires reciprocal monophyly at mitochondrial loci and significant divergence at nuclear loci. Management units, on the other hand, are defined as having significant divergence at either mitochondrial or nuclear loci. This framework provides a practical method for incorporating genetic information into species conservation and management of *A. pomo*, a California Species of Special Concern. This study demonstrates that the Mendocino group is clearly a distinct phylogenetic unit based on the mitochondrial control region locus but not the nuclear loci and is best described as a separate management unit within the species. This indicates that the differences in allele frequencies between the Mendocino group and the rest of the species are not necessarily fixed, but there is probably such a low level of gene flow that the Mendocino group is in the process of becoming a distinct evolutionarily significant unit. However, more sampling needs to be done in the central part of the species' geographic range to clarify the management status of the non-Mendocino samples and determine whether the division between the Mendocino and Humboldt groups suggested by the mtDNA tree (Fig. 2) is simply the result of historically recent demographic processes or reflects a deeper evolutionary divergence (Avise 2004). Overall, consideration of the Mendocino group as a separate management unit is recommended until further sampling is completed to ensure conservation of the evolutionary trajectory of this lineage.

Four main conclusions emerge from this research. First, the Del Norte County, California, tree voles fall within the southern *A. longicaudus* clade, although the sister relationship of *A. longicaudus* to the *A. pomo–A. albipes* group was only weakly supported. Second, the Mendocino lineage within *A. pomo* should be considered a separate management unit. This group exhibits significant mtDNA divergence but no substantial divergence across the nuclear loci we examined. The conservation status of the Humboldt lineage is uncertain and requires additional genetic analysis of geographically intermediate populations. Third, this study emphasizes how the use of mitochondrial markers alone is likely to provide a biased picture of the evolutionary history and genetic diversity of a species. In this case, use of only the mtDNA data could lead to overestimates

of both the differentiation between the Humboldt and Mendocino groups of *A. pomo* as well as levels of genetic diversity within each group. Finally, AFLP shows promise for several applications—in the rapid assessment of total genetic diversity in mammals and as a nuclear-marker complement to mtDNA for comparative phylogeographic studies focused on taxonomically diverse, codistributed species.

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