PHYLOGEOGRAPHY OF THE WESTERN RATTLESNAKE (*CROTALUS VIRIDIS*) COMPLEX, WITH EMPHASIS ON THE COLORADO PLATEAU

MICHAEL E. DOUGLAS[1], MARLIS R. DOUGLAS[1], GORDON W. SCHUETT[2,3], LOUIS W. PORRAS[4], AND ANDREW T. HOLYCROSS[5]

ABSTRACT: The Western Rattlesnake (*Crotalus viridis*), the most widespread and phenotypically variable rattlesnake in North America, has been partitioned into nine subspecies based on size, color, pattern, scutellation, and geographic distribution. Several subspecies, especially those occurring in the western portion of the range, have proved difficult to identify due to similarities in scutellation, color and pattern ontogeny, and because specimens fade in preservative. Over a half-century has elapsed since Laurence Klauber's extensive work on the *C. viridis* group. Since that time, researchers have used a range of phenotypic characters to further elucidate relationships among members of this group, but a comprehensive phylogenetic analysis incorporating a wide range of morphological characters has not been accomplished. Analyses of snake lineages using mitochondrial (mt) DNA genes as molecular markers appear promising in reconstructing relationships, substantiating previously identified clades, and revealing new ones. Since 1987, three studies using mtDNA gene sequences have partitioned the *C. viridis* complex into eastern and western groups, but researchers have been unable to resolve relationships in the latter. The goal of this study was to evaluate earlier results and to resolve relationships in the western clade by utilizing two rapidly evolving mtDNA genes (ATPase 8 and 6). We sampled from a wide range of populations, emphasizing those from the Colorado Plateau because it is an area pivotal to understanding the evolution of *C. viridis*. In this region, six taxa (*C. v. abyssus*, *C. v. cerberus*, *C. v. concolor*, *C. v. lutosus*, *C. v. nuntius*, and *C. v. viridis*) potentially contact one another in the area of the Grand Canyon. The remaining three taxa occur west of the Colorado Plateau. *Crotalus v. helleri* and *C. v. oreganus* are found along the Pacific Coast and inland, and *C. v. caliginis* is restricted to Isla Sur of the Islas de los Coronados off the coast of Baja California Norte.

Our study includes all nine subspecies of the *C. viridis* complex plus two outgroups (153 individuals, 111 from the Colorado Plateau), and results were derived from 669 base pairs of sequence data. Net percent sequence divergence ranged from 0.4 ± 0.2 (*viridis* and *nuntius*) and 1.0 ± 0.3 (*abyssus* and *lutosus*), to 7.3 ± 0.9 (*lutosus* and *nuntius*). Weighted and unweighted maximum parsimony (MP), maximum likelihood (ML), and distance analyses rooted with *Agkistrodon contortrix* and *Crotalus scutulatus* all supported the same clades. Unweighted MP produced 99 equally most-parsimonious trees. A strict consensus, resampled 1,000 times, supported (94%) the monophyly of the *C. viridis* species group. The eastern clade (*C. v. viridis* and *C. v. nuntius*) was 100% diagnosable, and in this clade *C. v. nuntius* was resolved at only 52%. Eastern and western clades differed at 6.1 ± 0.9 percent sequence divergence, and thus are on separate evolutionary trajectories. The eastern clade is most appropriately viewed as a distinct species, with *C. v. nuntius* placed in synonomy with *C. viridis*. The western clade is also well-defined (87%) and contains five Colorado Plateau lineages: (1) *C. v. cerberus* (72%), (2) *C. v. concolor* (92%), (3) a *C. v. lutosus* and *C. v. abyssus* clade (92%) that contains *abyssus* (88%) and a paraphyletic *lutosus*, (4) a paraphyletic *C. v. oreganus*, and (5) a *C. v. helleri* clade (89%) within which *C. v. caliginis* is nested. *Crotalus v. cerberus* is the basal-most taxon in our western clade and distinct from the other western clades. Relationships among the other western clades are less robust, suggesting a more-recent evolutionary history. Nonetheless, because these lineages are also well-defined and on separate evolutionary trajectories, we propose the elevation of *C. v. abyssus*, *C. v. cerberus*, *C. v. concolor*, *C. v. helleri*, *C. v. lutosus*, and *C. v. oreganus* to specific status. Furthermore, because *C. v. caliginis* is nested in *C. v. helleri*, we propose that this taxon should be placed in synonomy with *C. helleri*. The two undescribed clades within our western group (*lutosus*-like, L3; *oreganus*-like, O1) will require additional sampling and molecular, morphological, and natural historical analyses to clarify their taxonomy (i.e., potential new species).

INTRODUCTION

The genetic architecture of a species is shaped by both contemporary and historic components. The former represents phenomena that are primarily ecological and population genetic, and thus by definition microevolutionary. Historical components, on the other hand, epitomize biogeographic factors acting over evolutionary time, and include both vicariant and dispersal events (Avise et al., 1987). The relationship between these factors is often best manifested within broadly distributed species (Walker and Avise, 1998). Species with broad distribution experience a welter of habitats and can reflect numerous phenotypes, and thus many are regarded as polytypic (i.e., they occur in various forms in different parts of their ranges). For over a century, biologists have been aware that phenotypes of broadly distributed taxa are most often

[1]Department of Fishery and Wildlife Biology, Colorado State University, Ft. Collins, Colorado 80523-1474, USA
E-mail (MED): michael.douglas@colostate.edu
[2]Department of Herpetology, Zoo Atlanta, 800 Cherokee Avenue, S.E., Atlanta, Georgia 30315-1440, USA
[3]Department of Biology, Center for Behavioral Neuroscience, Georgia State University, 33 Gilmer Street, S.E., Unit 8, Atlanta, Georgia 30303-3088, USA
[4]7705 N. Wyatt Earp Ave., Eagle Mountain, Utah 84043, USA
[5]Department of Biology and Museum, Arizona State University, Tempe, Arizona 8528-1501, USA

habitat-specific. Adams (1901: quoted in Walker and Avise, 1998) stated that over time "…the fauna comes to fit the habitat as a flexible material does a mold." Hence, it would appear that the best opportunity for scientists to examine contemporary and historical processes and their effects upon differentiation of populations is to evaluate taxa with broad distributions.

Biologists have parceled variation found in polytypic species in a variety of ways (see Endler, 1977:4). The most contentious, however, has been the formal recognition of subspecies. Gould and Johnson (1972:488) established the groundwork for future arguments by stating, "In non-quantitative studies that used the comparative method of analyzing geographic variation, there was virtually no alternative to the formal establishment of subspecies and the enumeration of differences among them. This had a host of unfortunate consequences. It buried some of the most fascinating cases of dynamic adaptation under a thicket of names. It allocated the study of a central phenomenon in evolutionary theory to [those individuals] more adept at cataloguing than analyzing. It partitioned continuity into more or less arbitrary packages of convenience. It imposed an inherently static nomenclature upon the most dynamic aspect of evolution." These arguments have been amplified and extended during the last decade, primarily by utilization of molecular data within a phylogenetic framework. They now encompass the proper method of delineating this variability (Shaffer et al., 1991; Miththapala et al., 1996; Miyamoto, 1996; Baker et al., 1998), the model best reflecting speciation (Cracraft, 1997; Avise and Wollenberg, 1997), and the very nature of intraspecific taxa themselves (Frost and Hillis, 1990). As a result of these changes, investigations of broadly distributed taxa have shifted from the realm of empiricists to that of theoreticians, and recent research (Rodríguez-Robles et al., 1999; Rodrígues-Robles and De Jesús-Escobar, 2000) may shift this focus back to field-related evaluations.

The Western Rattlesnake (*Crotalus viridis*) is a widely distributed polytypic species in North America (Klauber, 1972; Stebbins 1985). Originally described as *Crotalinus viridis* (Rafinesque, 1818), this taxon has undergone a convoluted history of nomenclatorial changes (see McDiarmid et al., 1999; Ashton and de Queiroz, 1999). Klauber (1956, 1972) viewed *C. viridis* as a monospecific taxon with nine subspecies based largely on size, color and pattern, scutellation, and geographic distribution.

Klauber (1956) was first to construct a phylogenetic tree for all taxa of *Crotalus* and *Sistrurus* based on a wide range of characters, which included cranial and vertebral osteology, body size, head and tail proportions, form and unit growth of the rattle, hemipenis, lungs, venom, squamation, color and pattern, ecological preferences, and geographic range. In his tree, Klauber (1956) relied on the unpublished osteological work of B. H. Brattstrom. Later, Brattstrom (1964) suggested a phylogeny of *Crotalus* and *Sistrurus* (including extinct taxa) based exclusively on osteological characters, but did not provide a phylogeny of the intraspecific relationships of *C. viridis*. Nonetheless, based on the examination of 67 specimens of the *C. viridis* group [*C. v. concolor* (N = 1), *C. v. helleri* (N = 31), *C. v. lutosus* (N = 4), *C. v. oreganus* (N = 12), and *C. v. viridis* (N = 170], he speculated on the relationships of five of the nine subspecies by claiming (p. 245) "On the basis of osteology, intraspecific relationships in *viridis* are difficult to determine. *Crotalus v. concolor* and *C. v. lutosus* seem closely related, as do *C. v. helleri* and *C. v. oreganus*. In many characters, however, *C. v. oreganus* is more like *C. v. viridis* than like either *C. v. lutosus* or *C. v. helleri*."

Klauber (1972) modified his earlier phylogeny of rattlesnakes as a result of the work of Brattstrom (1964) and the description of new taxa. A subsequent study by Foote and MacMahon (1977) incorporated biochemical information on venom proteins and used numerical taxonomic tools (e.g., methods based on overall similarity) to suggest changes in the Klauber-Brattstrom rattlesnake phylogenies. With respect to the *C. viridis* complex, the Foote and MacMahon "similarity tree" shows a sister relationship between *C. v. viridis* and *C. v. cerberus*, and *C. v. lutosus* as sister to all other members of *C. viridis*. In contrast, Klauber (1972) indicated *C. v. viridis* as sister to *C. v. concolor* + *C. v. lutosus*, and *C. v. oreganus* is sister to *C. v. cerberus* + *C. v. helleri*. The phylogenies of Brattstrom (1964), Klauber (1972), and Foote and MacMahon (1977), however, all differ with respect to the relationship of *C. viridis* to other species of *Crotalus*. In addition to their proposed similarity tree, Foote and MacMahon (1977: Fig. 6) provided a suggested phylogeny of *Crotalus* and *Sistrurus* based on Brattstrom (1964), indicating *C. v. cerberus* as sister to *C. v. helleri*. Brattstrom (1964) did not report on specimens of *C. v. cerberus* (see above), and thus the sister relationship between those taxa attributed to Brattstrom is incorrect.

Aird (1984) analyzed certain aspects of population-level problems (alpha taxonomic issues) of several *C. viridis* subspecies using morphological (scutellation), biochemical (venom, erythrocyte proteins), and environmental (climate, habitat) characters. Although his analyses were hampered by limitations of geographic scope and lack of a formal phylogenetic hypothesis, his results, nonetheless, are noteworthy. Importantly, Aird (1984) showed that differentiation in the populations of *C. viridis* he inspected was greater than formerly stated by Klauber (1972), and he arrived at the following conclusions, "Based upon morphological, venom elution profile, and genetic distance data, *concolor*, *viridis* and *lutosus* appear to be legitimate species. I suspect that eventually all of the *viridis* subspecies, except *abyssus* and possibly *caliginis*, will be recognized as species."

Quinn (1987) was the first of three studies to incorporate mtDNA gene sequence data to reconstruct relationships in the *C. viridis* complex. Quinn evaluated the subspecies of *C. viridis* by using morphological (i.e, scutellation, coloration) and molecular (i.e., isozymes and mtDNA) markers. Despite certain shortcomings of his analyses (e.g., a strict phenetic approach, small sample sizes) we consider Quinn's contribution as valuable. He was able to discern two distinct lineages in *C. viridis* (i.e., eastern and western clades), and suggested synonomization of the eastern entity *C. v. nuntius* to *C. v. viridis*. Although he was unable to clarify relationships among members of the western group, several aspects of his results are congruent with subsequent analyses, including our own. Unfortunately, because the studies by Aird (1984) and Quinn (1987) are unpublished dissertations, they have been largely overlooked.

Pook et al. (2000) evaluated the relationships of 68 individuals of all nine subspecies of *C. viridis* by using sequence data derived from the mtDNA genes cytochrome-*b* (cyt-*b*) and ND4L. Their study supported Quinn's proposal of eastern and western clades, but these researchers further subdivided the western clade into three groups: southwestern United States (*C. v. cerberus*), Great Basin (*C. v. abyssus* and *C. v. lutosus*), and Pacific (*C. v. caliginis, C. v. concolor, C. v. helleri,* and *C. v. oreganus*). Although there was some ambiguity in their results, Pook et al. suggested that the basal-most member of the western clade was *C. v. cerberus*. These workers did not propose taxonomic changes.

Ashton and de Queiroz (2001) evaluated 25 individuals of the *C. viridis* complex (plus several outgroups) using ND2 and D-loop regions of mtDNA. As in the above studies, these researchers recognized the division of *C. viridis* into eastern and western groups, and made the following taxonomic recommendations: an eastern group (*C. viridis*, composed of the subspecies *C. v. nuntius* and *C. v. viridis*), and a western group (*C. oreganus*, composed of the subspecies *C. o. abyssus*, *C. o. caliginis*, *C. o. cerberus*, *C. o. concolor*, *C. o. helleri*, *C. o. lutosus*, and *C. o. oreganus*).

In our analyses, evaluated the results of earlier studies and strived to better-define and interpret relationships in the largely unresolved western clade by using two rapidly evolving mtDNA genes (ATPase 8 and 6) as molecular markers. Although we sampled populations throughout the range of the species (see Appendix I), we emphasized the region of the Colorado Plateau because it is an area we consider pivotal to understanding the evolution of this group. In this region, six subspecies of *C. viridis* potentially contact one another at or near the Grand Canyon, and three potentially enter it (Figs. 1–2; Plate 12c): the Great Basin Rattlesnake (*C. v. lutosus*) from the north, the Hopi Rattlesnake (*C. v. nuntius*) from the east, and the Arizona Black Rattlesnake (*C. v. cerberus*) from the south; the Grand Canyon Rattlesnake (*C. v. abyssus*) is essentially found within and near (e.g., north and south rims) the Grand Canyon; the Midget Faded Rattlesnake (*C. v. concolor*) is associated with major canyons and rivers of the Colrado Plateau; and the Prairie Rattlesnake (*C. v. viridis*) enters the Colorado Plateau from the east. The remaining three subspecies are distributed west of the Colorado Plateau: the Southern Pacific (*C. v. helleri*) and Northern Pacific (*C. v. oreganus*) rattlesnakes are found along the Pacific Coast and inland; and the Coronado Island Rattlesnake (*C. v. caliginis*) is restricted to Isla Sur of the Islas de los Coronados, off the coast of Baja California Norte, Mexico.

MATERIALS AND METHODS

Sampling and Laboratory Protocols

Data on sampling localities are provided in Appendix I and their cartographic locations are detailed in Figures 1–2. Sampling was accomplished from 1979 to the present, often with the assistance of agency personnel. Samples were primarily aliquots of blood taken by syringe from the caudal vein of each specimen. All blood samples were preserved in 100% EtOH or Queen's lysis buffer. Occasionally, liver and muscle were obtained from recent road kills, from

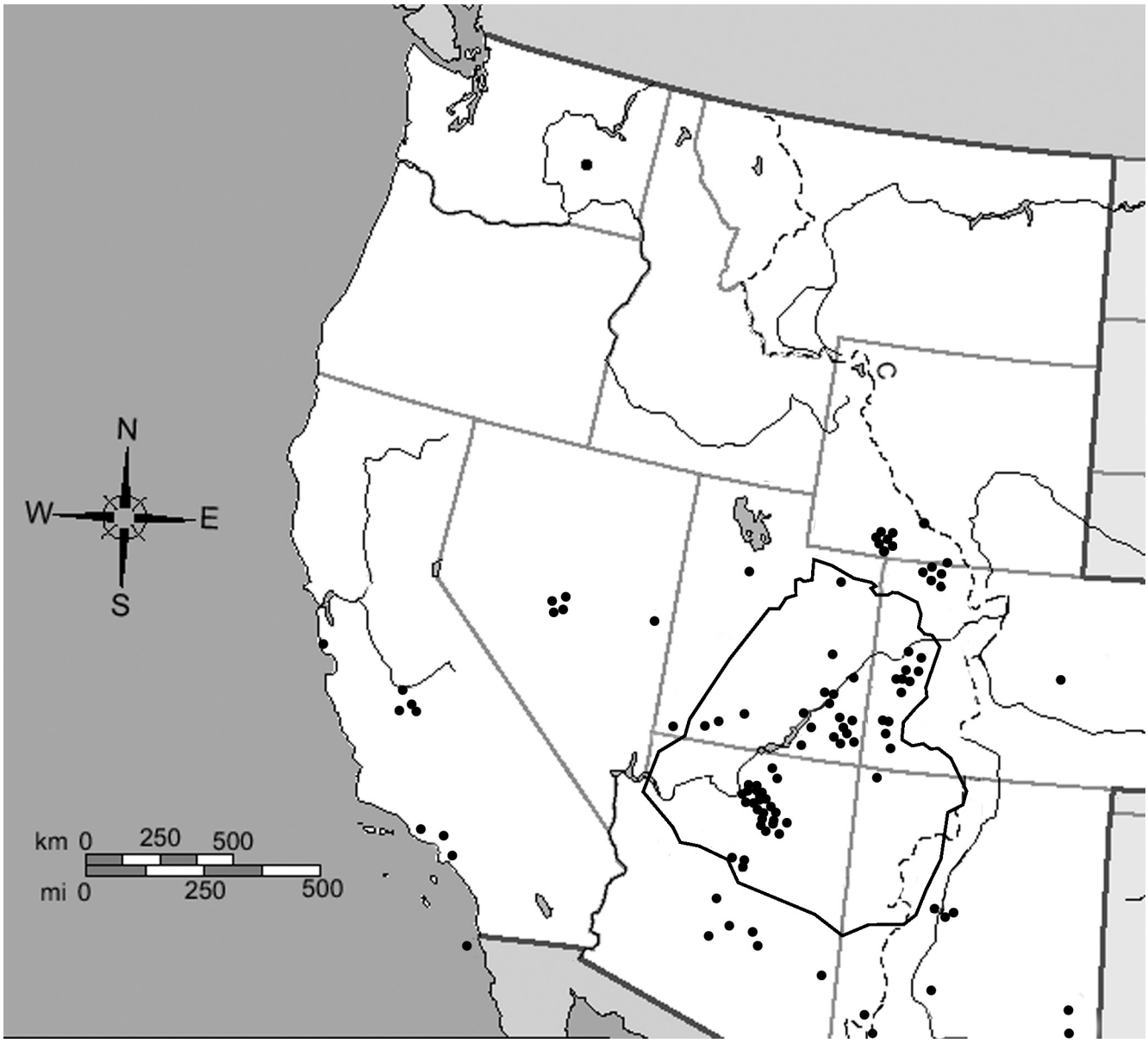

Fig. 1. Map of western North America depicting localities for individual rattlesnakes used in this study (see Appendix I). The Colorado Plateau is outlined.

voucher specimens, or from earlier sampling efforts, and these were either frozen or preserved in 100% EtOH. In total, the study involved 153 specimens (149 ingroup and four outgroup specimens). Outgroup taxa were *Crotalus scutulatus* and *Agkistrodon contortrix*, respectively.

Total genomic DNA was isolated using the PureGene DNA Isolation Kit (D-70KB; Gentra Systems, Inc., Minneapolis, Minnesota) and stored in DNA hydrating solution (same kit). The ATPase 8 and ATPase 6 genes were amplified using primers specified in Bermingham and Martin (1998).

Single-stranded sequencing reactions were conducted with fluorescent-labeled dideoxy terminators according to manufacturer's recommendations [Applied Biosystems Inc. (ABI), Forest City, California]. Labeled extension products were gel-separated and analyzed with an automated DNA sequencer (ABI model 377) located in the sequencing facility at Arizona State University. All samples were sequenced in the forward direction, and problematic sequences were re-sequenced in a forward direction until resolved.

Analytical Protocols

To judge levels of saturation we plotted uncorrected individual pairwise sequence divergences (*p*-distances) for transitions and transversions at each codon position against Tamura-Nei (TN) estimates

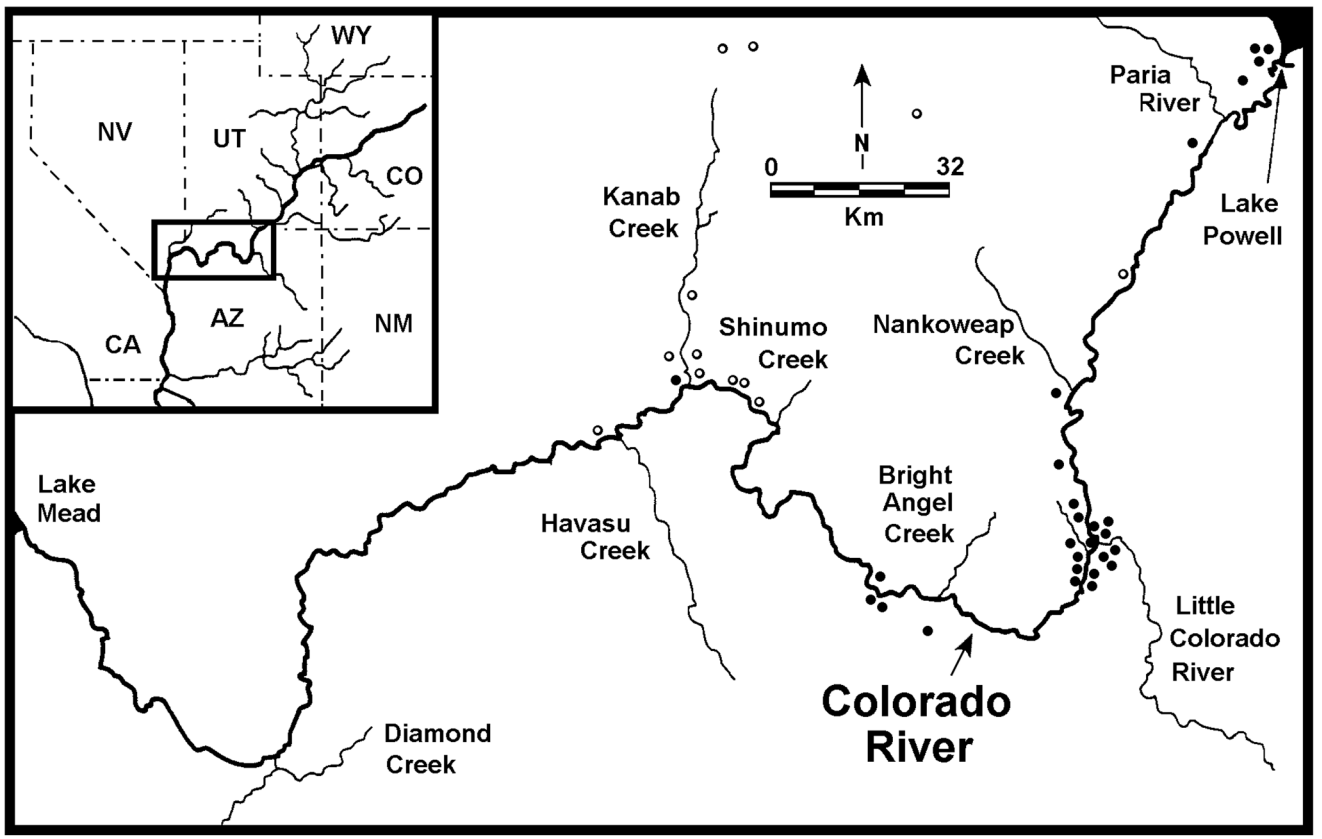

Fig. 2. Map of the Grand Canyon National Park (rectangle within inset) showing locations of *Crotalus abyssus* (closed circles) and *C. lutosus* (open circles) used in this study.

of relative divergence, as per Zamudio et al. (1997), Parkinson (1999) and Parkinson et al. (2000). This was done for each site using the program MEGA2 (S. Kumar, K. Tamura, I. Jakobsen, and M. Nei: http://www.megasoftware.net/). The *p*-distance is the proportion (*p*) of nucleotide sites at which the two sequences compared are different, and it is obtained by dividing the number of nucleotide differences by the total number of nucleotides compared. The TN model, on the other hand, is more parameter-rich and corrects for multiple hits (see below) by taking into account substitutional rate differences among nucleotides and inequality of nucleotide frequencies. Also, it can distinguish between transitional changes of purines (adenine and guanine) vs pyrimidines (thymine and cytosine) and assumes equality of substitution rates among sites. Saturation effects appear as an increase in the more parameter-rich TN distances at the expense of the simpler *p*-distances, when both are plotted against one another. This yields a nonisometric plot (i.e., one that is deflected towards the TN axis). Where this occurs, then classes of substitutions for saturated codons must be weighted appropriately (Zamudio et al., 1997).

We compiled haplotypes from raw sequence data using MacClade 4.03 (Maddison and Maddison, 2001). These served as unweighted input to the maximum parsimony (MP) algorithm of PAUP* (version 4.0b8: Swofford, 2002). Shortest trees were sought by using heuristic searches that employed accelerated character transformation (ACCTRAN) optimization, tree bisection-reconnection (TBR) branch swapping, retention of minimal trees (MULPARS), and collapse of zero-length branches to yield polytomies. Sequence deletions were considered as an alternative character state under the assumption that these (and insertions) represented evolutionary changes rather than merely missing data. Support for individual nodes was evaluated by nonparametric bootstrapping using 1,000 pseudoreplicates per analysis with 100 random addition sequences per pseudoreplicate. A node with a bootstrap value > 70% was considered strongly supported (Hillis and Bull, 1993; Wiens and Hollingsworth, 2000).

We also performed a similar series of analyses as above, but using a dataset of haplotypes with transitions downweighted 4x and 8x that of transversions. To accomplish this, *A. contortrix* was removed as an outgroup and *C. scutulatus* was retained. This proce-

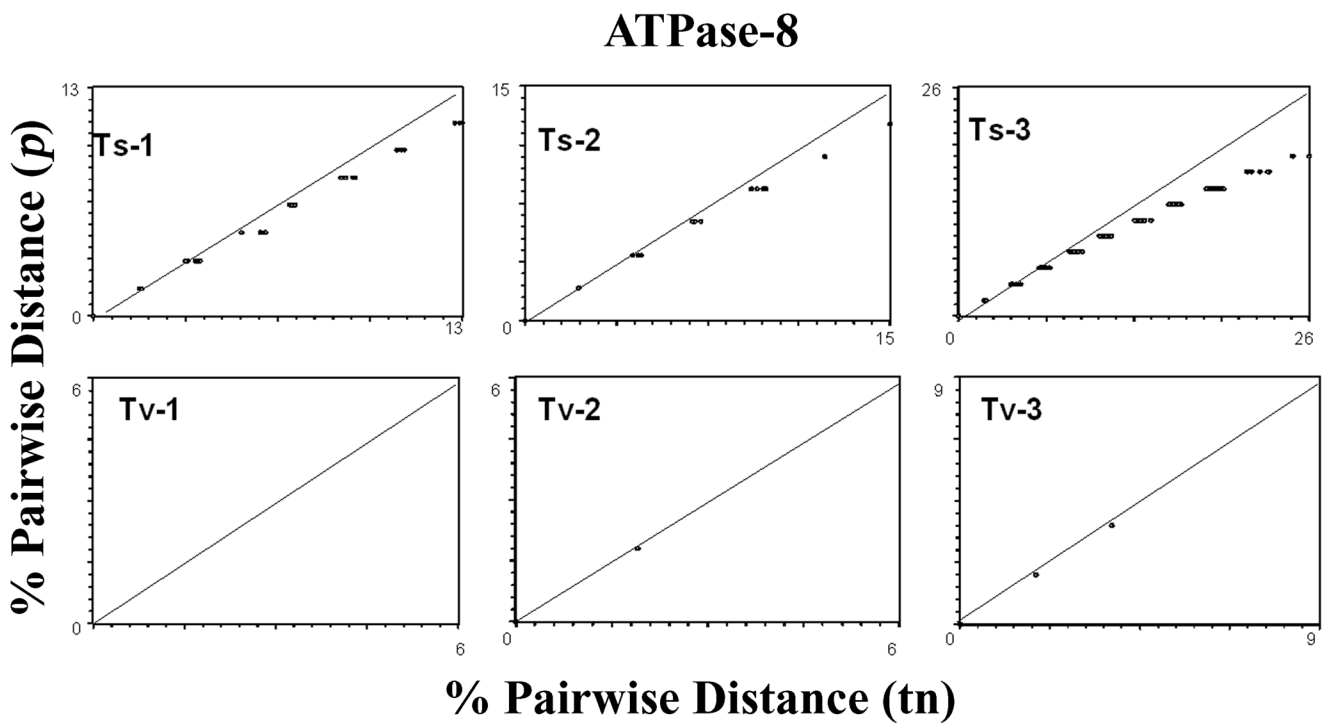

Fig. 3. Plot of uncorrected individual pair-wise sequence divergences (*p*-distances) for transitions and transversions at each codon position for ATPase 8 against Tamura-Nei (TN) estimates of relative divergence. Isometry is indicated by straight line.

dure was perfomed because inclusion of *A. contortrix* forced an insertion and a deletion into the dataset, which made determination of transitions and transversions problematic according to their codon location.

Similarly, a weighted MP analysis was done by first using MEGA2 to classify nucleotide sites as 4-fold or 0-fold degenerate. A site is 4-fold degenerate if all possible changes are synonymous (i.e., they cause no alteration in the specified amino acid). A site is 0-fold degenerate if all possible changes are nonsynonymous (i.e., they alter the amino acid produced) (Graur and Li, 2000). We downweighted the 4-fold degenerate sites by a factor of eight.

Finally, we used maximum likelihood (ML) to compare the relative likelihoods of trees derived from the MP analyses. In this approach (Swofford et al., 1996:445), one of the equally parsimonious MP trees was randomly selected as a starting tree. Probabilities of the six possible nucleotide transformations, the proportion of invariable sites, and the shape parameter of the gamma distribution of rate heterogeneity across nucleotide positions were fixed according to empirical values calculated from the starting tree. The most parameter-rich of the general time-reversible models of nucleotide substitution (GTR+I+Γ: reviewed in Yang, 1996) was used to search for an ML tree with a higher log-likelihood value. When such a tree was found, parameters were re-optimized and fixed for a subsequent ML search. This procedure was repeated until the same tree was found in successive iterations (Rodríguez-Robles et al., 1999; Rodríguez-Robles and De Jesús-Escobar, 2000).

We determined the model of sequence evolution that best fit our data by using the program MODELTEST (Posada and Crandall, 1998), which evaluates 56 different models using two separate statistics [the likelihood ratio test ($-2 \log \Lambda$), and the Akaike information criterion ($-2\ln L + 2n$, where L = maximum value of the likelihood function for a specific model, and n = number of independently adjusted parameters within the model)]. The former is a widely accepted statistic for assessing goodness-of-fit across a variety of models, and the latter rewards models for good-fit but penalizes them for unnecessary parameterization. Interestingly, the log-likelihood test and the AIC criterion can each favor different models of sequence evolution. When this occurs, the user has the opportunity to interpret these results in light of the data.

We also developed a distance matrix prior to tree construction for several reasons. First, it focuses attention on the model of sequence evolution to be used (as above). Second, it is often more efficient to estimate

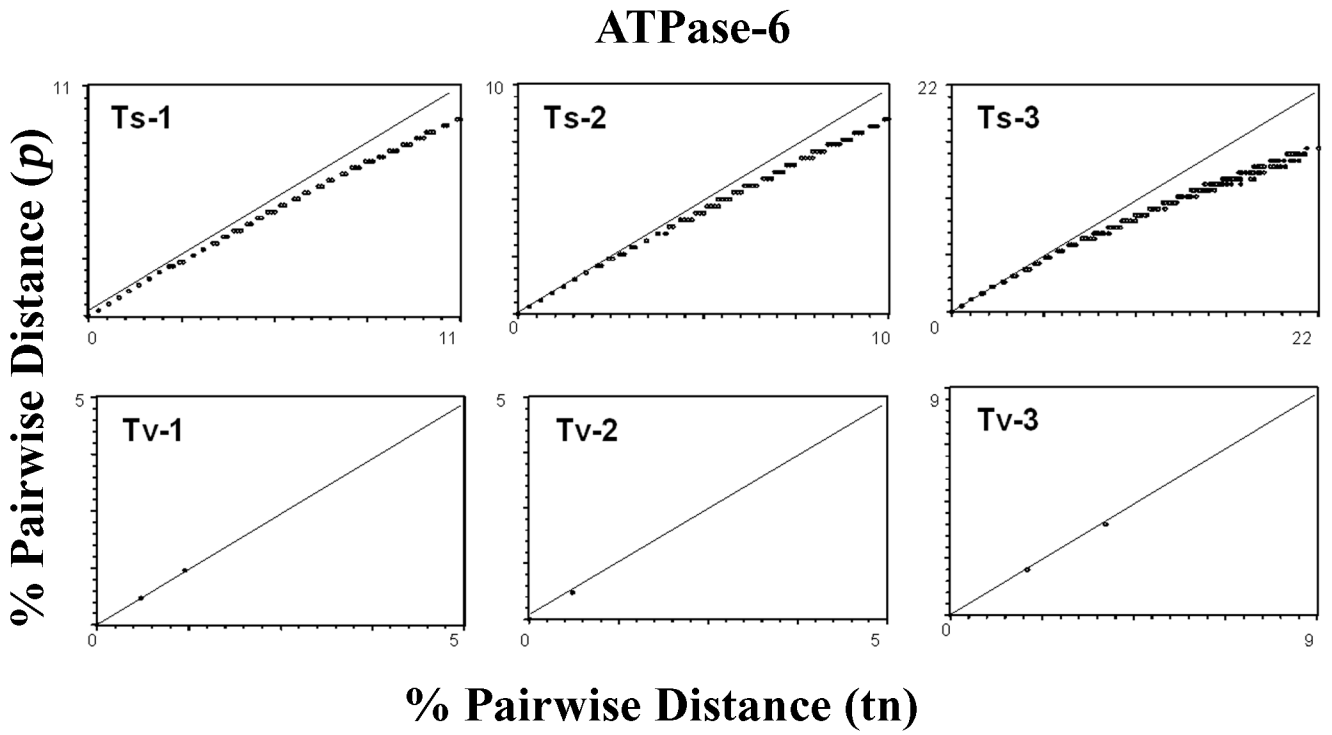

Fig. 4. Plot of uncorrected individual pair-wise sequence divergences (*p*-distances) for transitions and transversions at each codon position for ATPase 6 against Tamura-Nei (TN) estimates of relative divergence. Isometry is indicated by straight line.

topologies from a distance matrix rather than by searching for minimum evolution trees among a universe of possible topologies. Last, it is often easier to calculate standard errors for each divergence than to evaluate the support for nodes using a bootstrap approach, which is the case for those sequences approaching saturation. To derive distances among haplotypes, we employed the Tamura-Nei model (Tamura and Nei, 1993) with the shape parameter of the gamma distribution (i.e., TrN+Γ) serving as additional input (as indicated by MODELTEST). Neighbor-joining trees were constructed from 1,000 bootstrapped sequences using MEGA2, and trees were rooted at outgroups (as above).

Sequence divergence (*p*) values were generated for each subspecies from 1,000 bootstrapped sequences using MEGA2. Values were corrected for within-group variation then converted to provisional estimates of genealogical separation times (Avise et al., 1998) using three different mtDNA clocks: a standard clock [i.e., 2% sequence divergence per million years (Ma); Brown et al., 1979; Klicka and Zink, 1997], a fish clock (i.e., 1.3% sequence divergence/Ma calibrated for the study markers using fishes separated by the rise of the Isthmus of Panama; Bermingham et al., 1997), and a 4-fold-slower clock recommended for ectothermic vertebrates (i.e., 0.5% sequence divergence/Ma; Avise et al., 1992; Mindell and Thacker, 1996). Sequence divergence (*p*) values were also produced as above to delineate within-group divergence.

GenBank accession numbers for the *Crotalus viridis* clades listed in Figures 5–8 are: A1-A2 (*Crotalus v. abyssus*) AF462362, AF462363; C1 (*C. v. cerberus*) AF462374; H1 (*C. v. helleri*) AF462375; K1-K2 (*C. v. concolor*) AF462360, AF462361; L1-L2 (*C. v. lutosus*) AF462364, AF462365; L3 (undescribed, *C. v. lutosus*-like) AF462366; N1 (*C. v. nuntius*) AF462371; O2 (*C. v. oreganus*) AF462373; O1 (undescribed, *C. v. oreganus*-like) AF462372; V1-V4 (*C. v. viridis*) AF462367, AF462368, AF462369, AF462370.

RESULTS

Summary Statistics

Polymerase chain reaction (PCR) amplifications and subsequent automated sequencing of all 153 specimens resulted in 169 base pairs (bp) of unambiguously readable sequences for ATPase 8, and 509 bp for ATPase 6. Each gene was aligned using Clustal X, a modification of Clustal W (Thompson et. al., 1994), and examined by eye. The outgroup *A. contortrix* demonstrated one insertion and one deletion within the ATPase 8 gene relative to *C. scutulatus* and ingroup taxa. All sequences were evaluated by MED and re-evaluated independently by MRD. Within the mitochondrial

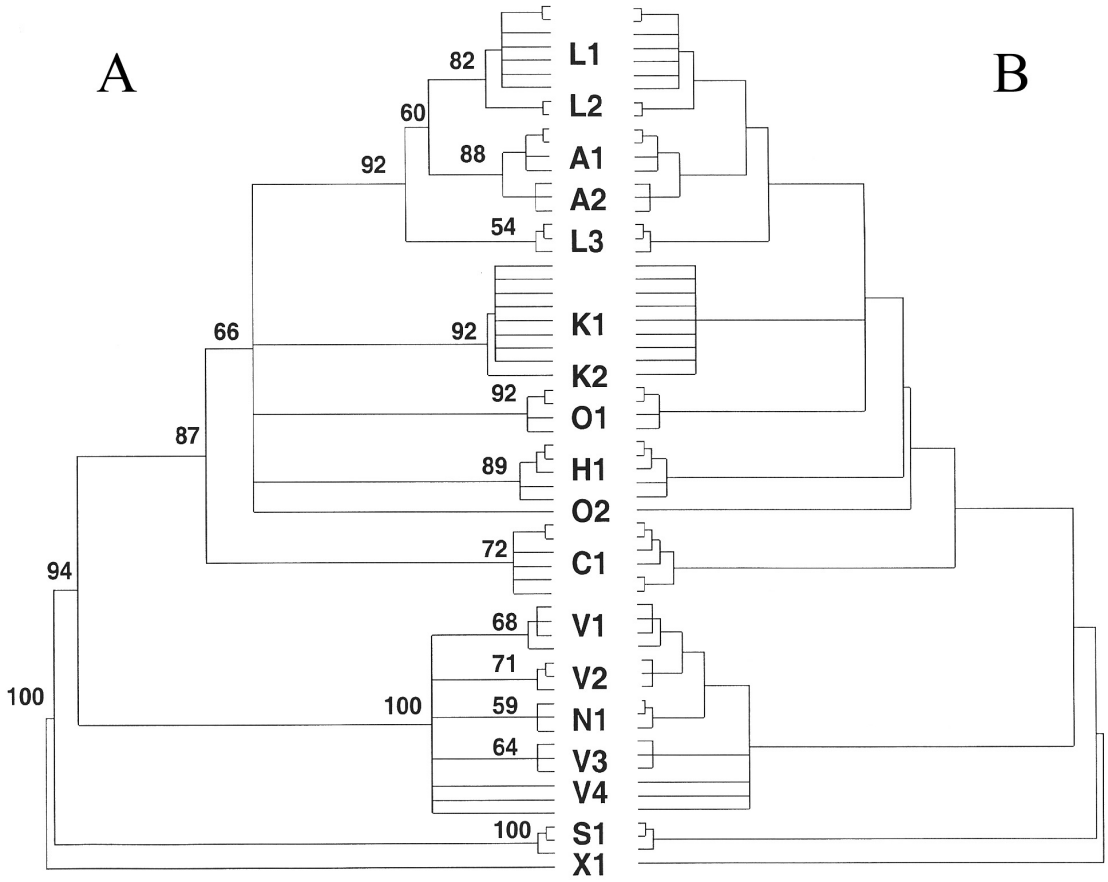

Fig. 5. (A) Bootstrap results (1,000 pseudoreplicates per analysis with 100 random addition sequences per pseudoreplicate) of an unweighted maximum parsimony analysis. Clades are: A1, A2 (*Crotalus viridis abyssus*); C1 (*C. v. cerberus*); H1 (*C. v. helleri*); K1, K2 (*C. v. concolor*); L1, L2, L3 (*C. v. lutosus*); N1 (*C. v. nuntius*); O1, O2 (*C. v. oreganus*); V1, V2, V3, V4 (*C. v. viridis*); S1 (*C. scutulatus*); X1 (*Agkistrodon contortrix*); (B) Strict consensus of 99 most-parsimonious trees resulting from a maximum parsimony analysis. Clades same as in (A).

genome, genes ATPase 8 and ATPase 6 overlap each other by 9 bp. Prior to analyses, both genes were separated and the overlapping sequence was retained at the 3' end of the lead ATPase 8 and added to the 5' end of the trailing ATPase 6. Both genes were then recombined in a non-overlapping format, and all subsequent analyses were performed on this composite.

Comparisons among the 149 ingroup individuals revealed 541 monomorphic sites and 124 polymorphic ones. Parsimony-informative polymorphic sites totaled 99. There were 60 unique haplotypes. Haplotype (gene) diversity = 0.950 (SD = 0.009) and nucleotide diversity = 0.04281 (SD = 0.0009). Base frequencies were tabulated as: A = 0.34; G = 0.31; C = 0.10; and T = 0.25. The transition/transversion (Ti/Tv) ratio = 8.05:1. Plots of transitions vs transversions were done for each codon position (Figs. 3–4).

The MP analyses, using equally weighted characters, provided 99 most-parsimonious trees each 362 steps in length (L) with a consistency index (CI) of 0.696 and a retention index (RI) of 0.907. As per Archie (1996:184–185), we computed SC (the average number of steps per character on the tree), where SC = 1/CI = 1.436, and HC (the average homoplasy per character), where HC = SC – 1 = 0.436.

The weighted MP analyses, with transitions down weighted 4x and 8x that of transversions, each produced 702 most-parsimonious trees. For the 4x analysis, CI = 0.622 and RI = 0.917 (SC = 1.607 and HC = 0.607), and for the 8x analysis CI = 0.629 and RI = 0.919 (SC = 1.589 and HC = 0.589). The weighted MP analysis of the 0-fold/4-fold sites produced 48 most-parsimonious trees each 571 steps with CI = 0.68 and RI = 0.929 (SC = 1.471 HC = 0.471).

The bootstrap tree for the unweighted MP analysis is shown in Figure 5a, and Figure 5b shows the strict consensus of the 99 MP trees. Similarly, the bootstrap tree of the 8x weighted MP analysis is in Figure 6a, and Figure 6b denotes the strict consensus of the 702 8x weighted MP trees. (Comparable figures for the 4x

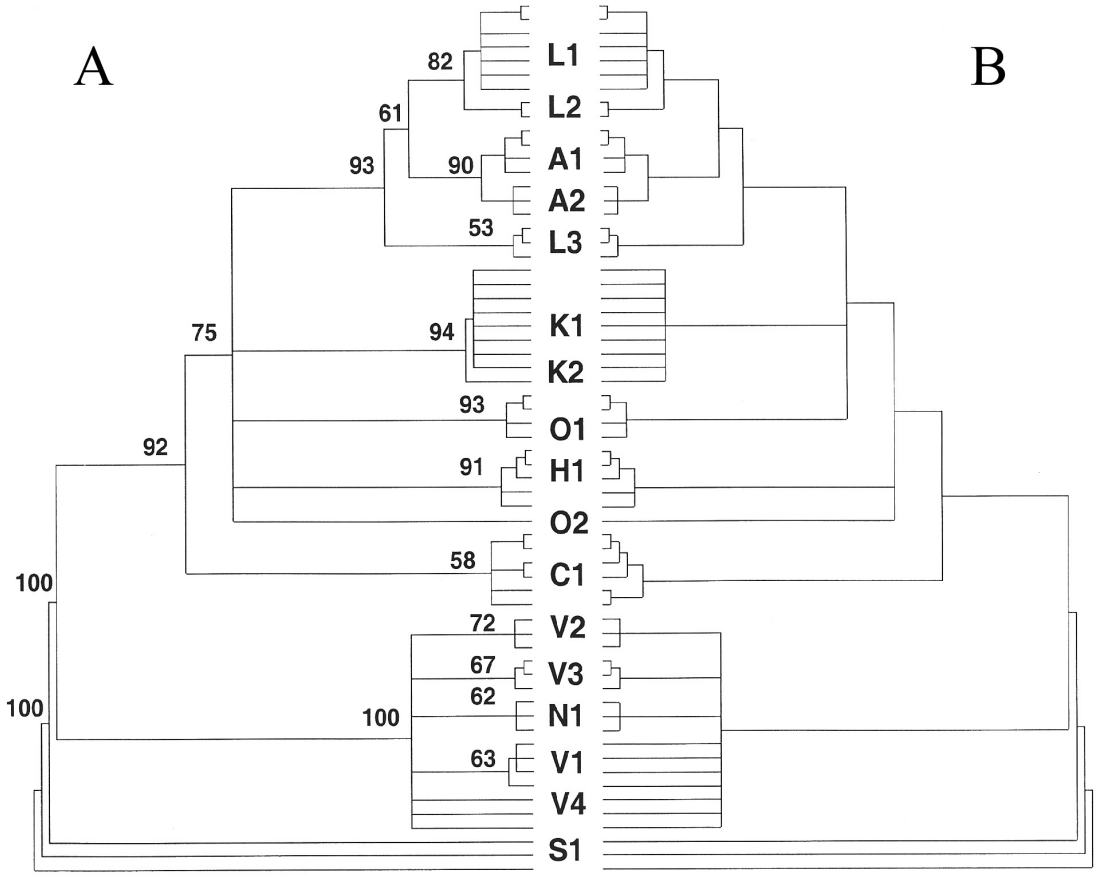

Fig. 6. (A) Bootstrap results (1,000 pseudoreplicates per analysis with 100 random addition sequences per pseudoreplicate) of a maximum parsimony analysis with transitions weighted 8x that of transversions. Clades are: A1, A2 (*Crotalus viridis abyssus*); C1 (*C. v. cerberus*); H1 (*C. v. helleri*); K1, K2 (*C. v. concolor*); L1, L2, L3 (*C. v. lutosus*); N1 (*C. v. nuntius*); O1, O2 (*C. v. oreganus*); V1, V2, V3, V4 (*C. v. viridis*); S1 (*C. scutulatus*). (B) Strict consensus of the 702 equally most-parsimonious trees resulting from a maximum parsimony analysis with transitions weighted 8x that of transversions. Clades same as in (A).

weighting scheme are identical but not presented.) The strict consensus of the 48 most-parsimonious trees from the 8x analysis of the 0-fold/4-fold sites is presented in Figure 7a, and the bootstrap for this analysis is shown in Figure 7b.

The log-likelihood score for the best ML tree (Fig. 8a) is LnL = 2293.3029. The neighbor-joining tree of Tamura-Nei distances with gamma correction is shown in Figure 8b. All three methods (distance, unweighted, and weighted MP, ML) recovered the same major nodes that are supported in each analysis by high bootstrap values. All further discussion of relationships within the *C. viridis* complex will focus primarily on the unweighted MP tree (Fig. 5a).

Unweighted Maximum Parsimony Tree

The most basal split within the *C. viridis* complex is supported at 94% and produces eastern and western clades (Fig. 5a). The eastern clade is diagnosable at 100% and contains *C. v. viridis* and *C. v. nuntius*. It has five main divisions. The V1 branch represents four haplotypes from southeastern Utah, northwestern New Mexico, and southwestern Colorado. The V2 branch is composed of three haplotypes from northwestern Colorado, southcentral Wyoming, southwestern and west-central New Mexico, and southeastern Arizona. The V3 branch is composed of three haplotypes from central and southwestern New Mexico. The V4 branch is comprised of three unresolved haplotypes. Finally, the N1 branch contains three haplotypes of *C. v. nuntius*.

In the western clade, the split between *C. v. cerberus* and the remainders of the clade is supported at 87%, with *cerberus* composed of six haplotypes and defined at 72%. The remaining members of the western clade form a polytomy at 66%. In the latter, *C. v. oreganus* is paraphyletic with a single haplotype (O2) from Grant County, Washington, separated from the O1 haplotypes. The remaining four *oreganus* haplotypes (O1) are supported at 92% and contain individuals from central and western California. A third branch of

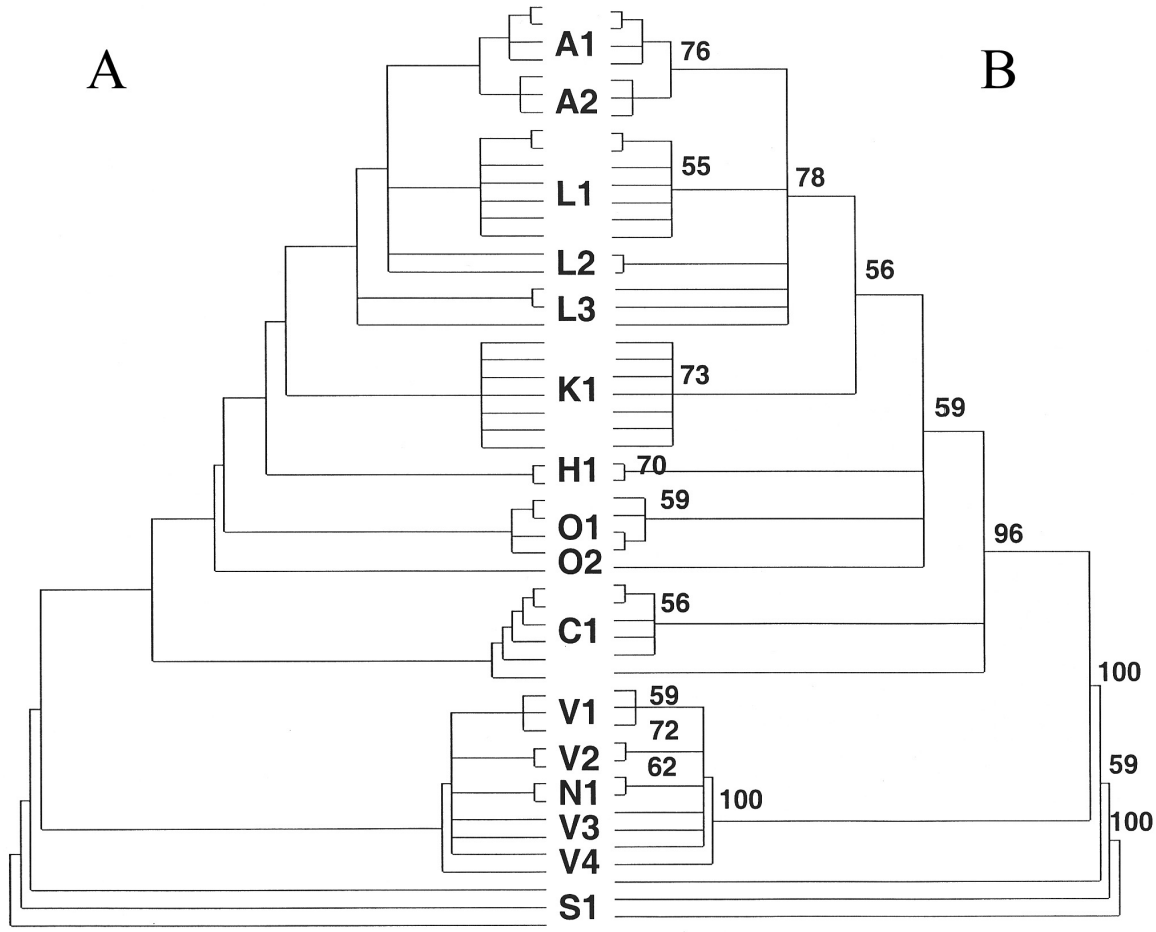

Fig. 7. (A) The strict consensus of the 48 most-parsimonious trees resulting from an analysis of 0-fold/4-fold degenerative sites where the 0-fold sites were weighted 8x that of the 4-fold sites. Clades are: A1, A2 (*Crotalus viridis abyssus*); C1 (*C. v. cerberus*); H1 (*C. v. helleri*); K1, K2 (*C. v. concolor*); L1, L2, L3 (*C. v. lutosus*); N1 (*C. v. nuntius*); O1, O2 (*C. v. oreganus*); V1, V2, V3, V4 (*C. v. viridis*); S1 (*C. scutulatus*). (B) Bootstrap results (1,000 pseudoreplicates per analysis with 100 random addition sequences per pseudoreplicate) of 0-fold/4-fold degenerative sites where the 0-fold sites were weighted 8x that of the 4-fold sites. Clades same as in (A).

the polytomy (H1, at 89%) is composed of four haplotypes of *C. v. helleri* and a single haplotype of *C. v. caliginis*. A fourth branch (K1 and K2, at 92%) is composed of nine *C. v. concolor* haplotypes. A fifth branch is supported at 92% consists of *C. v. abyssus* and a paraphyletic *C. v. lutosus*. The L3 branch (at 54%) consists of three *lutosus* haplotypes from southern Nevada, southwestern Utah and western Grand Canyon. The interior clade of *C. v. lutosus* (L1 and L2, at 82%) is comprised of nine haplotypes from northern Arizona, southern Utah, and northern and central Grand Canyon. The other interior group (i.e., A1 and A2, at 88%) is composed of seven *C. v. abyssus* haplotypes from extreme southcentral Utah through the northern and central areas of the Grand Canyon.

The among-group *p*-distances (with standard errors) for the *C. viridis* complex are presented in Table 1. Among clades the *p*-distances (net between clade averages) range from 1.0 ± 0.3 for *C. v. abyssus*-*C. v. lutosus*, to 7.3 ± 0.9 for *C. v. nuntius*-*C. v. lutosus*. Using the standard clock, divergence times for *C. v. abyssus*-*C. v. lutosus* range from 0.65–0.35 (average 0.5) mya. When a fish clock is utilized, divergence is at 1.0–0.54 (average 0.77) mya. Finally, when a slow mtDNA clock is employed, divergence is at 2.6–1.4 (average 2.0) mya. The same approach was used to bracket the divergence of eastern from western clades of *C. viridis*. Under the standard clock, this divergence is at 6.6–3.9 (average 5.25) mya. When a fish clock was used, divergence times increased to 10.2–6.0 (average 8.1) mya, while the slow clock showed divergence times of 26.4–15.6 (average 21) mya.

In Table 2 we present within-group *p*-distances for eight of nine subspecies, with standard errors and sample sizes. These range from 0 ± 0 (*C. v. nuntius*) to 1.5 ± 0.2 (*C. v. cerberus*) and 1.3 ± 0.2 (*C. v. lutosus*).

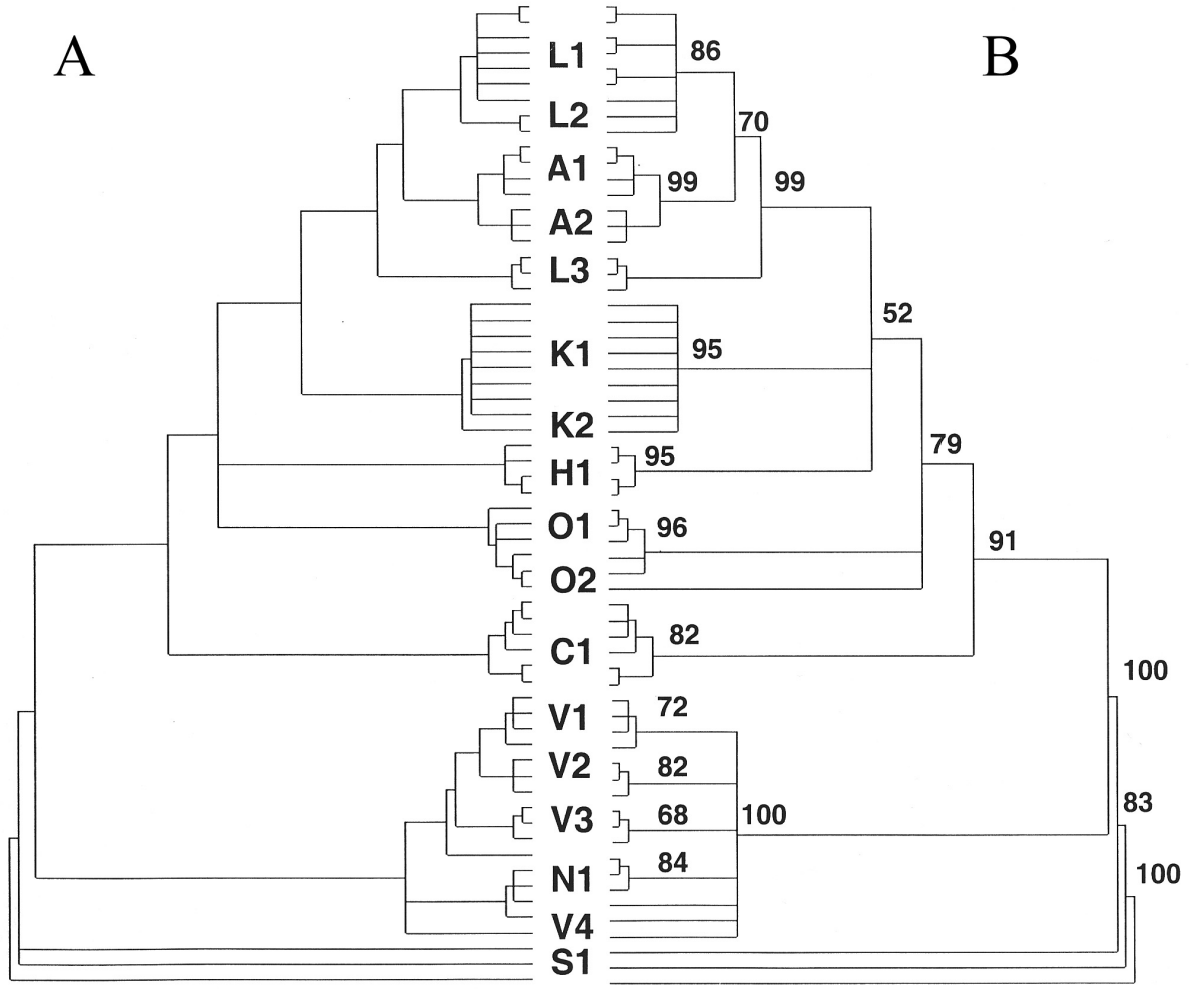

Fig. 8. (A) Maximum likelihood tree of the 60 unique haplotypes in this study. Clades are: A1, A2 (*Crotalus viridis abyssus*); C1 (*C. v. cerberus*); H1 (*C. v. helleri*); K1, K2 (*C. v. concolor*); L1, L2, L3 (*C. v. lutosus*); N1 (*C. v. nuntius*); O1, O2 (*C. v. oreganus*); V1, V2, V3, V4 (*C. v. viridis*); S1 (*C. scutulatus*). (B) Neighbor-joining tree of Tajima-Nei distances with gamma correction. Clades same as in (A).

DISCUSSION

The phenotypic variability displayed by polytypic species has long intrigued naturalists. Darwin (1859:47) noted, "Those forms which possess in some considerable degree the character of species, but which are so closely similar to some other forms, or are so closely linked to them by intermediate gradations, that naturalists do not like to rank them as distinct species, are in several respects the most important to us." This interest has increased in the last decade with the advent of molecular methods that can parsimoniously describe population genetic structure within polytypic species, and recover the historical components of their matrilinear hierarchy.

We will discuss our results at a variety of levels: (1) gene trees vs species trees; (2) the choice of a molecular marker; (3) the evolutionary rate of particular genes; (4) the problem of sequence saturation; (5) statistical and analytical protocols; (6) our current understanding of rattlesnake phylogeny; (7) species and infraspecific variability; and (8) the application of our results to conservation and management. Before these aspects are discussed, however, we will first describe the ecological setting within which the majority of our study was conducted.

The Colorado Plateau

The Colorado Plateau is a unique physiographic province located in western North America between the Rocky Mountains (east and north) and the Basin and Range province (west and south) (Hunt, 1967; Fig. 1). It spans four states (Arizona, Colorado, New Mexico, Utah), has an area of ca. 337,000 km^2, and an average elevation of 1,525 m (minimum = 360 m; maximum = 3,850 m). The Colorado Plateau was formed by extensive geological formations of nearly

Table 1. Lower triangle (shaded area) represents nucleotide *p*-distances (net between-group average) between the subspecies, and upper triangle represents standard errors (estimated by bootstrap method with random number seed and 1,000 replications). Subspecies abbreviations are: CVH = *helleri*; CVO = *oreganus*; CVK = *concolor*; CVL = *lutosus*; CVA = *abyssus*; CVN = *nuntius*; CVV = *viridis*; CVC = *cerberus*.

	CVH	CVO	CVK	CVL	CVA	CVN	CVV	CVC
CVH	–	0.5	0.5	0.6	0.6	0.9	0.9	0.6
CVO	1.7	–	0.4	0.5	0.5	0.8	0.8	0.5
CVK	2.1	1.8	–	0.5	0.6	0.9	0.9	0.5
CVL	2.8	2.1	2.5	–	0.3	0.9	0.9	0.6
CVA	3.1	2.1	2.7	1.0	–	0.9	0.9	0.6
CVN	6.6	5.4	6.3	7.3	6.9	–	0.2	0.9
CVV	6.5	5.3	6.1	7.1	6.8	0.4	–	0.8
CVC	2.7	2.3	2.7	3.2	3.5	6.0	5.7	–

horizontal sedimentary rock. These areas are locally interrupted by volcanic uplifts and extensive areas of bare rock that are drained by deeply incised canyons.

Elevation influences the distribution of vegetation across the Colorado Plateau and its effects are reasonably well understood (Betancourt, 1990). Several "Life Zones" (as per Merriam, 1890) are recognized: alpine (> 3,480 m); sub-alpine (i.e., spruce-fir, 2,900–3,480 m); mixed conifer (2,600–2,900 m); Ponderosa Pine (2,100–2,600 m); pinyon-juniper (1,600–2,100 m); desert-scrub (1,500–1,600 m); mixed shrub (1,400–1,500 m); Mojave Desert (1,100–1,400 m); and Sonoran Desert (< 1100 m). The spatial overlay of vegetation is variable in its density and composition, and this diversity offers numerous opportunities for local adaptation of rattlesnakes and their prey (see Plate 16e).

The seasonal climate of the Colorado Plateau is heterogeneous (Mock, 1996). Its geographic position and elevation influence the North American monsoons in much the same manner as the Tibetan Plateau influences the monsoon of Southeast Asia (Adams and Comrie, 1997). In both situations, a high elevation land mass provides an efficient summer heat source for the normally cooler but higher altitude air mass, which induces an atmospheric trough that drives monsoonal flow. Its onset and duration varies among years. A large springtime snow pack in the southern Rocky Mountains, for example, requires colder continental temperatures that, much like the Tibetan Plateau system, inhibit monsoon development. A similar effect must have occurred during glacial periods when large snow packs were produced on the Colorado Plateau (Anderson et al., 2000). In general, precipitation in the Colorado Plateau decreases from higher to lower elevations, and in summer from the southern areas northward (Higgins et al., 1997). The amount of summer precipitation falling in the north is related to the strength of the summer monsoon. These complex climatological relationships impact modern biotic distributions on the Colorado Plateau, and likewise must have strongly influenced the past distribution of biota.

The Colorado River originates in the Rocky Mountains and dissects the Colorado Plateau from northeast to southwest, essentially forming the major axis. Its larger tributaries (i.e., Green, San Juan, and Little Colorado rivers) drain it from the north, east, and southeast, respectively, while the smaller Virgin River drains it from the southwest. Riparian corridors along these tributaries likely have provided dispersal routes for biotic components (Benson and Darrow, 1981). In addition, the Green River is believed to have been a significant dispersal route southward into the Colorado Plateau, and likewise from the Colorado Plateau northward into the vast interior of North America (Anderson et al., 2000).

Large portions of the Colorado Plateau are now protected (or semi-protected) federal lands and include national parks such as Arches, Bryce, Canyonlands, Capitol Reef, Grand Canyon, and Zion. There are also the newly established Grand Staircase-Escalante (0.7 million ha) and Grand Canyon-Parashant (0.8 million ha) national monuments, as well as the proposed Vermillion Cliffs National Monument (1.2 million ha) on the Paria Plateau.

The Colorado Plateau forms a complex ecological landscape within which *C. viridis* has diversified. Six clades are recognized to occur within its boundaries, but their distributions are inconsistent among various authors (e.g., Wright and Wright 1957; Klauber, 1972; Glenn and Straight, 1982; Stebbins, 1985; Cox and Tanner, 1995; Bartlett and Tennant, 2000). Thus, one goal of our study is to delineate the geographic boundaries for these clades, as well as define the genetic diversity inherent in each. Given the apparent broad distributions of some clades (as per Klauber, 1972), we argue that it would not be unusual to find paraphyletic sister groupings within each nominal clade. Due to the preliminary nature of this study, our sample sizes for several clades are reduced. Although our data will suffice to detect large-scale patterns among the most frequent haplotypes, a more finely

structured history and the underlying processes that yield such a pattern will require substantially increased sample sizes and re-evaluation. In this report we focus on the genetic validity of those clades identified by Klauber (1972).

Gene Trees vs Species Trees

A phylogeny of a species is a multitude of nested component trees, each reflecting the history of populations as determined by single characters. When a component tree is derived from DNA information (e.g., haplotype sequences) it is referred to as a "gene-tree" (Avise, 1994, 2000; Templeton, 2001). On the other hand, a "species tree" can be viewed as (Avise, 1994:126) "…a single pedigree that extends [historically] as an unbroken chain of parent-offspring genetic transmission…." Hence, gene trees are histories of traits, and species trees are histories of organisms or pedigrees (Avise, 1994, 2000).

In phylogenetic reconstruction it is important to understand that a gene tree does not necessarily reflect a species tree. Not only can gene trees differ in their topology from each other, but also from species trees as a result of a variety of biological factors (e.g., stochastic lineage sorting, introgressive hybridization, horizontal transfer; Avise, 1994, 2000). The process of stochastic lineage sorting (SLS), for example, involves random extinction of haplotypes through time. Originally, SLS was believed to be problematic for only those lineages that had diverged relatively recently, but lineage splitting in deep history can also reflect ambiguities (e.g., genealogical discordance) (Wu, 1991). Despite difficulties associated with interpreting gene trees, a common practice in reconstructing phylogenies is to use them to estimate species tree. Some consider this association to be tenuous in that there are ample reasons for gene trees and species trees to be discordant. Nonetheless, topologies derived from gene trees are frequently congruent with species trees (Avise, 2000:326). How is this possible? How can gene trees show congruence among themselves and with species tree? A possible (and reasonable) answer would involve past fluctuations in effective population sizes (i.e., N_e) for component populations of a species (Frankham, 1995). Most likely, this is due to occurrence of past environmental oscillations that impacted census sizes, ranges, recruitment, and so on. Given these vagaries, within- and among-population genetic variance may be substantially reduced, particularly when compared with distances in the tree (i.e., internodal distances) that separate speciation events. In other words, genetic diversity is low whereas topographic distances separating species are high; hence, congruence among gene trees (in particular those derived from loci that exhibit the appropriate evolutionary rate), as well as these gene trees and species trees, is expected (Avise, 2000).

Table 2. Mitochondrial DNA (ATPase 8 and 6) analyses for the *Crotalus viridis* complex. Nucleotide *p*-distance values = d (within-group average for a given subspecies); SE = standard error estimated by bootstrap method with random number seed and 1,000 replications; N = sample size. Subspecies abbreviations are: CVH = *helleri*; CVO = *oreganus*; CVK = *concolor*; CVL = *lutosus*; CVA = *abyssus*; CVN = *nuntius*; CVV = *viridis*; CVC = *cerberus*.

Taxon	d	SE	N
CVH	0.7	0.3	4
CVO	0.9	0.2	6
CVK	0.7	0.1	23
CVL	1.3	0.2	22
CVA	0.4	0.1	29
CVN	0.0	0.0	23
CVV	1.0	0.3	32
CVC	1.5	0.3	10

We should not, however, base taxonomic decisions a priori on the above arguments simply because they offer a convenient rationale that allows a gene tree to be equated with a species tree. Instead, it is more important to clarify that a gene tree shows agreement with a previous taxonomy derived from independent data, where possible, regardless of the methodological processes that generated them. In this regard, the species listed in our proposed taxonomy for *C. viridis* (Table 3) were not described by us as new, since they were described previously as species or subspecies based on morphological and biogeographical data (summarized in Klauber, 1972). But based on our current molecular analyses (e.g., high degree of sequence divergence) and contemporary shifts in perspectives relating to the very nature of species themselves (Avise, 1994, 2000), we contend that the taxa we list in Table 3 are indeed distinct and best viewed as evolutionary or phylogenetic species. Along similar lines we suggest that the paraphyletic taxa recovered in our analyses should be left for future studies to clarify.

Choice of Molecular Markers

Mitochondrial (mt) DNA has properties that are eminently desirable for an evolutionary marker, and

these qualities often lead to efficient and reliable recovery of gene trees, especially when compared to nuclear markers (Avise, 2000; DeFilippis and Moore, 2000). The high substitution rate of mtDNA, for example, is useful in studies of recently evolved clades (as in this study), and also has a relatively conserved gene order (but see below). This promotes the use of "universal" PCR primers that often cross-amplify across divergent groups. Further, mtDNA is believed to be non-recombinant, which allows lineages to be traced historically and reduces the sample size needed to quantify within- and among-lineage variability. The mtDNA genome consists of 13 protein-coding genes, 22 transfer RNA (tRNAs) genes, a single control region, and several intergenic spacers. Synonymous substitutions in the protein coding genes are 10x that for nuclear genes. Non-synonymous substitutions vary greatly in mtDNA protein coding genes, but their rate is always higher than the average rate for nuclear DNA (Graur and Li, 2000:157). Thus, a mitochondrial haplotype tree also has a higher probability of congruence with a species tree than does a nuclear gene tree (Moore, 1995). Because mtDNA is maternally inherited, an important caveat is that the breeding biology and/or population structure of a species does not curtail male effective population size. Last, the mitochondrial genome is inherited as a single linkage group that allows evaluation of additional mtDNA genes without the difficulties that separate evolutionary histories, and may stem from recombination or lineage sorting.

Despite the above, mtDNA has concomitant shortcomings that can limit its potential in recovering phylogenetic signal. One is saturation by multiple substitutions at low levels of divergence (discussed below), and mtDNA also has a strong base compositional bias. Both factors can diminish phylogenetic signal, particularly at levels of deep history. Last, because mtDNA is inherited as a single linkage group, there is only a single independent estimate of the species tree regardless of the number of mtDNA genes sequenced.

Choice of Specific mtDNA Genes

The selection of a particular mtDNA gene (or genes) as a marker should be based on the time scale of the divergences being studied. Fu (2000), for example, was unable to unravel the evolutionary history of the lizard lineage Lacertidae in spite of using four slow-evolving mtDNA genes and 4,708 bp of sequence. Apparently, recent and explosive speciation in this group has obscured relationships within subfamilies. Similarly, Kraus et al. (1996:768) hypothesized that mtDNA evolution is much faster in crotalines than in other snakes. These researchers employed a gene (ND4L) that had resolved relationships among snakes much older than the diversification of crotalines (Forstner et al., 1995). Yet, Kraus et al. (1996) found that this locus resulted in levels of saturation and homoplasy that were greater than expected, which led them to caution researchers that mtDNA genes with high rates of evolution must be employed to evaluate relationships among populations and subspecies of pitvipers.

The displacement loop (i.e., D-loop) of the mtDNA genome is a non-coding region that is the origin of replication for the molecule. In most animals, D-loop is much more variable than protein coding regions of the mtDNA genome, and is, therefore, a useful marker for studies of recently diverged populations or species (Parker et al., 1998). Kumazawa et al. (1998), however, discovered a functional duplication of the control region in the Japanese colubrid snake, *Dinodon semicarinatus*. Each is 1,018 bp in length with termination-associated sequences (TASs) located in a region where hairpin-like secondary structure and repetitive sequences occur. This is a typical situation for vertebrate D-loop. But upstream of these at the 5'-end is a cytocine-rich sequence common to all snakes examined (Kumazawa et al., 1998). Sequencing reactions for both strands stall in this region, suggesting a structural barrier for mtDNA polymerization. In addition, snake mtDNAs also possess just 5'-upstream of the control region, rather stable hairpin-like structures derived from the tRNA-pro gene (or pseudogene), or an intergenic spacer. This hairpin seemingly serves as a structural barrier to DNA polymerization, and if the amplification were successful, duplicate sequences may be produced that would not necessarily juxtapose, thus providing signals that conflict. It would appear that the mtDNA control region in snakes is a poor choice for amplification in that numerous structural barriers are present that impede polymerization (see Burbrink et al., 2000; Ashton and de Queiroz, 2001).

Which of the 13 mtDNA protein-coding genes exhibit rapid evolution? Kumar (1996:Fig. 5b) used first and second codons of different mtDNA genes to evaluate the relationship between ä (the shape parameter of the gamma distribution) and S (total number

of substitutions that have occurred per site in the evolutionary history of the gene). He found that highly conserved mtDNA genes show greater among-site variation than do faster evolving genes. Only a small proportion of sites in the highly conserved genes were free to change, whereas a greater proportion were free to change in more rapidly evolving genes. Of the 13 protein-coding mtDNA genes examined, ATPase 8 demonstrated the most rapid rate of evolution, while the commonly used cyt-*b* gene was one of the slowest. ATPase 6 was intermediate. Other difficulties exist with cyt-*b* as well. Meyer (1994) noted that it exhibits unequal rates of evolution among distantly related lineages, and that this tendency was problematic at higher taxonomic levels. Wiens and Hollingsworth (2000) recorded similar difficulties, and suggested the problem may even apply to analyses of genera within a single lineage. The above underscores the utility of ATPase 8 and 6 as molecular markers exhibiting evolutionary rates comparable to those needed for interpreting intraspecific divergence.

The Problem of Sequence Saturation

As genetic mutations occur over time, a pair of homologous sequences will gradually differentiate. At first this occurs almost linearly and observed divergence between the two correctly reflects actual divergence, but, over time, some sites will begin to absorb multiple substitutions (i.e., multiple "hits"). The rates by which identical nucleotides are produced by new changes or by multiple substitutions will gradually equilibrate over time. At this point, the sequences have become "saturated" in that they cannot achieve greater sequence divergence in spite of the fact that additional substitutions continue to occur. This saturation phenomenon is problematic because it masks true divergence among sequences, as well as their true evolutionary rate. Superimposed substitutions also increase levels of homoplasy, thereby deteriorating the historic signal. Kocher and Carleton (1997) showed the effects of saturation in the ND2 region of mtDNA among cichlid fishes after two million years of divergence. This underscores not only the pervasiveness of saturation effects, but also the importance of correcting for multiple substitutions, particularly when constructing phylogenies of distantly related taxa.

Parsimony analysis does not include an explicit evolutionary model (Steel and Penny, 2000). Information on dynamics of sequence evolution, nonetheless, can be incorporated within a parsimony approach by employing character and character-state transition weighting, thus potentially improving phylogenetic estimation. Yet, there is little theoretical (or empirical) indication as to which of several approaches to weighting might be most appropriate. When data are weakly structured (i.e., "noisy"), then different weighting schemes will yield different hypotheses of relationship, none of which will be strongly supported. Similarly, when data are strongly structured and each weighting scheme produces similar strongly supported hypotheses, then weighting has little effect on overall inference. The problem arises when alternate weighting schemes produce strongly supported yet different hypotheses of relationship. In these cases, character weighting becomes a significant factor and the choice of correct weights is imperative. Thus, the question of which weighting scheme is most appropriate for analysis of a particular data set bcomes important only if alternative weighting schemes significantly affect the results obtained (Barker and Lanyon, 2000). Ambiguity in defining optimal parsimony weighting schemes is in sharp contrast to choosing optimal models within the maximum likelihood framework.

Many phylogenetic studies employing mtDNA have suggested downweighting transition substitutions relative to transversions, especially in cases of deep divergences where multiple superimposed substitutions are likely (Reeder, 1995; Nei, 1996; Griffiths, 1997), yet few researchers have indicated at what level downweighting should occur. First, second, and third positions in a codon are often weighted inversely with regard to their variability, because third position substitutions are, in many cases, synonymous changes, while second position substitutions always result in an amino acid replacement. There are reasons to suspect that in most coding genes purifying selection is stronger on second rather than third positions, due to the fact that second position changes are rare and perceived as reliable, whereas third position changes are more common and judged less reliable. Bjorklund (1999), however, rejected the hypothesis that second positions provide better phylogenetic signal than third positions. Moore and DeFilippis (1997) suggested that third positions may not be neutral in their mutation patterns, but instead are biased and under considerable selection. It would thus appear that some third positions are seemingly as inert from a mutational stance as first and second positions (Xia, 1998), and consequently of considerable value in phylogenetic studies. Bjorklund (1999) concluded

that indiscriminate downweighting of third positions as a single character class is not appropriate.

Our approach to this problem was twofold: we downweighted transitions relative to transversions in the commonly accepted manner by using the Ti/Tv ratio as a qualitative benchmark. We also approached the problem by first determining 0-fold and 4-fold degenerate sites in our data then regardless of position or type of substitution downweighted 4-fold relative to 0-fold sites. Since 0-fold sites always result in an amino acid change and 4-fold sites do not, we correctly address the problem of base substitutions but escape the recognized difficulties identified by Bjorklund (1999) that pertain to codon position and type of substitution. Using this technique, both MP consensus and bootstrapped MP consensus trees (Fig. 5a, b, respectively) recovered those major clades identified using other approaches.

Barker and Lanyon (2000) argued that the precise ratio of weights for transition vs transversion substitutions is of little overall consequence, and saw no significant impact on the results of their phylogenetic analyses when different types of weights were employed. Weights between 0 and 5, for example, yielded similar phylogenetic hypotheses (Barker and Lanyon, 2000). We followed standard convention in our analyses by downweighting transitions 4x and 8x that of transversions, because transitions were clearly saturated at all positions whereas transversions were not (Figs. 3–4). Although our choice of weights was arbitrary, they reflected at the high end the approximate Ti/Tv ratio inherent in our data. Interestingly, our weighting schemes had little effect on the overall arrangement of our parsimony topologies (see Barker and Lanyon, 2000). This suggests (as above) that our data are indeed strongly structured, for each weighting scheme produced strongly supported hypotheses that are quite similar to those produced by unweighted MP, ML, and NJ analyses.

Analytical Methods

In our examination of parsimony trees we provided two indices per tree as evaluative standards: the consistency index (CI) and the retention index (RI). These statistics are commonly cited in most phylogenetic studies. CI (also called the homplasy index) represents the fit of an entire data set to a tree, and trends from one (if there is no convergence) towards zero as the amount of convergence on the tree increases. The minimum possible value of CI on minimum length trees is correlated with the numbers of taxa and characters, and CI is often inappropriately scaled to permit meaningful comparisons among studies that employed different sets of characters or different taxa (Archie, 1996:171). RI, on the other hand, measures the proportion of apparent homoplasy in the data that is retained in the phylogenetic tree.

Few investigators actually discuss implications of either high or low CI and RI values relative to the expected accuracy of their tree. Furthermore, it is not apparent from the literature just how these values should be used. Similarly, the statistical significance of g1 values for tree-length distributions are also often reported in spite of the fact that use of g1 as a measure of phylogenetic signal is deemed inappropriate (Kállersjõ et al., 1992).

Archie (1996:184–185) recommended two new statistics for interpretation of evolutionary change on a tree and indicated that both are more useful than CI as a direct measure of homoplasy. These are SC (average number of steps per character), and HC (average homoplasy per character). SC (= 1/CI) contains information inherent in CI but in a form directly interpretable in terms of character change on a tree. It also lacks the pretense of being scaled between fixed limits. When data contain no homoplasy, SC = 1.0. As homoplasy increases, SC increases (essentially) without bounds. In an examination of 28 different data sets from the literature, Archie (1989a, b, 1996) found SC ranged from 1.06–4.71. In the present study, SC = 1.436. HC (= SC – 1), on the other hand, has a minimum value of 0.0. In our study, the average homoplasy per character = 0.436. Both statistics indicate that our data contain little homoplasy.

We also recognize the controversy with regard to use of bootstrap proportions as a measure of phylogenetic strength (Sanderson, 1995), yet their properties are better known than other similar measures. For instance, the bootstrap proportion is recognized as a biased indicator in that it underestimates the true proportion for nodes strongly supported (Felsenstein and Kishino, 1993; Hillis and Bull, 1993; Nei, 1996). In this regard, Hillis and Bull (1993) found that when four operational taxonomic units (OTUs) were differentiated, a bootstrap probability (P) $\geq$ 70% corresponds to a probability $\geq$ 95% that the node is real. Given this, bootstrap support for clades in Figure 1a is actually quite good in that all major divisions are supported at $\geq$ 70%. It is important to verify the robustness of nodes within a tree because this, in turn,

substantiates the accuracy of the entire phylogeny. This is important because, once verified, molecular phylogenies can be used as a basis to infer patterns of evolution among morphological, physiological, ontogenetic, and life history traits.

Rattlesnake Phylogeny

Molecular (mtDNA) approaches to reconstruct a rattlesnake phylogeny have been performed since Quinn (1987). Knight et al. (1993) used sequence data from 12S and 16S mtDNA ribosomal RNA genes to demonstrate the monophyly of rattlesnakes and proposed their origin as mid-Cenozoic. Further, these researchers concluded that *Crotalus* and *Sistrurus* are each monophyletic. In contrast, Parkinson (1999) showed that *Crotalus* is paraphyletic with respect to a monophyletic *Sistrurus*, and that *Agkistrodon* is sister to rattlesnakes. Kraus et al. (1996) previously found *Agkistrodon* as sister to rattlesnakes in several of their analyses. Murphy et al. (this volume) demonstrated that *Sistrurus* is paraphyletic, and recommended placement of *S. ravus* as a member of *Crotalus* (= *C. ravus*).

Pook et al. (2000) identified 37 unique haplotypes from 68 inividuals of the *C. viridis* complex. A single monophyletic species was supported but with two major clades, the first with *C. v. viridis* and *C. v. nuntius*, and the second with all remaining taxa west of the Rocky Mountains. These results are in close agreement with those of Quinn (1987). Pook et al. (2000) further identified three distinct branches within the western clade: southwestern United States group (*C. v. cerberus*), Great Basin group (*C. v. abyssus* and *C. v. lutosus*), and a Pacific Coast and inland group (*C. v. caliginis, C. v. concolor, C. v. helleri,* and *C. v. oreganus*). They indicated, however, that recognition of the southwestern clade as an isolate and its position as the basal member of the western clade were in need of further study.

Distribution of mtDNA haplotypes in the analysis of Pook et al. (2000) was not fully congruent with the subspecies recognized by Klauber (1972). Whereas some subspecies in their study formed distinct clades, others were positioned deep within other subspecies (i.e*., C. v. caliginis* within *C. v. helleri*, and *C. v. abyssus* within *C. v. lutosus*). The grouping of *C. v. concolor* with the Pacific Coast group, although somewhat difficult to comprehend from a geographic viewpoint, was deemed logical based on studies of venom composition. Pook et al. (2000) identified six of the nine subspecies as either paraphyletic or insignificant local variants within other clades. Furthermore, they argued that recognition of nine *C. viridis* subspecies as categories of equal rank masked the phylogenetic signal in their data.

The results of Ashton and de Queiroz (2001) differ from those of Pook et al. (2000) primarily in how these authors depicted relationships among members of the western clade. These differences might have been due to sampling procedures and choice of markers (see Taxonomic Conclusions and Recommendations).

Our study of the *C. viridis* complex reflects a genetic architecture with both deep and relatively shallow components. At the deepest levels of differentiation, our results support those of Pook et al. (2000) and reaffirm the monophyly of the entire complex (bootstrap support = 100%). We also agree with Quinn, (1987) and Ashton and de Queiroz (2001) that the complex is divided into eastern and western clades (94% support in our analyses).

The remaining seven Western Rattlesnake subspecies comprise the western clade. We differ from Pook et al. (2000) and Ashton and de Queiroz (2001), however, in the allocation of the remaining and more recently evolved members of the western clade. Our differences primarily reflect the evolutionary rates of the mtDNA markers being used, and our intensive sampling of *C. viridis* in the Colorado Plateau region, which we emphasize is important to understanding the evolution of this group. In this sense, the above researchers used slower-evolving markers and had few samples from the Colorado Plateau, whereas we utilized genes with faster evolutionary rates and sampled extensively from this region (Figs. 1–2). In the sections below, we evaluate our results with respect to previous studies.

Prairie Rattlesnake (*Crotalus v. viridis*) and Hopi Rattlesnake (*C. v. nuntius*)

Klauber (1935) was hesitant to designate *C. v. nuntius* as a subspecies because the head and body pattern, edges of the dorsal blotches, characteristic arrangement of markings on the head, and form of the tail rings all demonstrated a close relationship with *C. v. viridis*. Klauber felt, however, that the conspicuous size and color differences between the two and significant differences in scale counts warranted their separation. Klauber (1935:83) noted, for example, that the smallest (out of 149 individuals) pregnant *C. v. viridis* from Colorado was 588 mm total length (TL), whereas the smallest pregnant *C. v. nuntius* (out of 6 individuals) was 395 mm TL.

Klauber (1935) considered those rattlesnakes north of the Little Colorado River Basin (Fig. 2) as taxonomically problematic. This situation was exacerbated by the fact that Hopi Indians often brought in rattlesnakes for their religious ceremonies from areas distant to the reservation. Klauber (1935:86) observed both subspecies in the Snake Dance (a ceremony for which the subspecific epithet "*nuntius*" or messenger, was coined). Despite this activity, Klauber indicated that individuals from the San Juan drainage area in northeastern Arizona were indeed *C. v. viridis*. Klauber (1930;126) stated that specimens typical of *confluentus* (= *C. v. viridis*) were captured "…as far westward as the Santa Fe's branch line to the Grand Canyon" (which would be at Williams, Arizona, ca. 52 km west of Flagstaff).

Our results (e.g., Fig. 5a) indicate that the eastern clade of the *C. viridis* complex comprises at least five subdivisions, four of which comprise *C. v. viridis* and one that represents *C. v. nuntius*. Boostrap support for these subdivisions is reasonable, ranging from 59–68%. Interestingly, *C. v. nuntius* projects the lowest boostrap support. The percent sequence divergence between *C. v. nuntius* and *C. v. viridis* is 0.4% (Table 1). Our results also indicate that *C. v. nuntius* is essentially devoid of detectable mtDNA variation with respect to ATPase 8 and 6. The within-group *p*-distance for *C. v. nuntius* (N = 23) was 0.0 ± 0.0% (Table 2).

Morphological analyses by Quinn (1987) separated *C. v. viridis* into two groups on the east and west sides of the Rocky Mountains. Our molecular results, however, do not support this east-west generalization, as we identified individuals from clades V1 and V2 on both sides of the Continental Divide. We currently recognize the eastern clade as a single species, *C. viridis,* but this might change as we increase geographic sampling.

Arizona Black Rattlesnake (*Crotalus v. cerberus*)

Klauber was equivocal with regard to the status of *C. v. cerberus*. Klauber (1949:65) noted that *C. v. oreganus* was a single subspecies ranging along the Pacific slope from southern British Columbia into northern Baja California. At that time, *C. v. cerberus* was considered to be an isolated population of *C. v. oreganus* in the mountains of central Arizona (Klauber, 1930, 1936). Later, Klauber (1949:66) recognized the "…added weight of a complete territorial separation" with regard to the status of *C. v. cerberus*, yet noted "…the *helleri-cerberus* differences are somewhat less consistent"…than those separating *helleri* from *oreganus.* The latter he judged (p.66) "…as important and consistent as those between any other subspecies of *C. viridis*, such as *lutosus* and *viridis*." Klauber (1949:88) concluded that the relationship of *C. v. cerberus* with "…*helleri*, which is a member of the uninterrupted *viridis* chain, is so close and obvious that (the former) should not be considered a separate species, regardless of its present isolation."

Klauber (1949:87) also indicated considerable variation in color and pattern within *C. v. cerberus*, especially with respect to northern vs southern populations in Arizona. He determined *C. v. cerberus* to be most closely related to southern specimens of *C. v. helleri*, particularly those found at higher elevations in Baja California Norte. Our analyses show that *C. v. cerberus* (Plate 12e) is the most variable taxon of our major clades, exhibiting 1.5% within-group sequence-divergence (Table 2). It is noteworthy that this average spans only 10 individuals.

We concur with Pook et al. (2000) that *C. v. cerberus* is a unique lineage (supported at 87% in our study). Our results place it as the basal-most member of the western clade. The remainder of the western clade is supported at 66%, and this group is arranged into five separate subdivisions which are: *C. v. abyssus*, a paraphletic *C. v. lutosus*, *C. v. concolor*; a paraphyletic *C. v. oreganus*, and *C. v. caliginis*-*C. v. helleri*. Each of these subdivisions is well resolved with bootstrap values ranging from 72–92%. Although relationships of the clades are unclear in our bootstrap MP and weighted MP analyses (Figs. 5a, 6a), the MP strict consensus tree (Fig. 5b) is better resolved, but with one division of *C. v. oreganus* (O1) forming an unresolved polytomy. The weighted MP consensus is presented in Figure 5b, except that the second subdivision of *C. v. oreganus* (O2) forms an unresolved polytomy. Overall, bootstrap analyses depict the majority of the western clade (i.e., four of seven subspecies) as unresolved, and the strict consensus (weighted and unweighted) provided slightly more resolution.

Northern Pacific Rattlesnake (*Crotalus v. oreganus*)

Klauber (1949:72) noted that *C. v. oreganus* is variable in ventral scale counts (San Francisco Bay area vs central Sierra Nevada), and that color pattern differences are also characteristic of, and consistent for, several local areas within its distribution (e.g., northwestern California, southwestern San Joaquin

Valley (Plate 14c), higher elevations of the southern Sierra Nevadas, and possibly the Trinity Mountains). He also noted that *C. v. oreganus* intergrades with other subspecies whose distributions it abuts (*C. v. viridis*, *C. v. lutosus*, and *C. v. helleri*). Klauber (1930:127) stated, "…altogether, I consider intergradation between *confluentus* [= *viridis*] and *oreganus* most definitely proven through *abyssus* and *lutosus,* and it is upon this link that I base the retention of the name *confluentus oreganus* for the coast form." We discuss Klauber's perspectives on intergradation in a subsequent section.

Klauber (1949) expressed that *C. v. oreganus* was most closely related to *C. v. lutosus* and distantly related to *C. v. viridis*. He was equivocal regarding the relationship between *C. v. oreganus* and *C. v. helleri*, suggesting the latter could be a derivative of *C. v. oreganus*, but also suggesting that (p. 73) *C. v. helleri* "…may represent an independent invader from an ancestral form in southern Arizona, with a subsequent meeting and re-amalgamation in the Central California area where *oreganus* and *helleri* now intergrade." Brattstrom (1964), on the other hand, suggested a close relationship between *C. v. oreganus* and *C. v. helleri*, based on osteological data. Results stemming from venom electrophoresis (Foote and MacMahon, 1977) were in agreement with those of Brattstrom (1964). Schneider (1986) used morphological evidence to suggest that *C. v. oreganus* and *C. v. helleri* intergrade broadly in California where their respective ranges abut.

Our results juxtapose with those of Klauber and indicate that *C. v. oreganus* is paraphyletic. The MP strict consensus tree (Fig. 5b) has the two subdivisions of *C. v. oreganus* as separate clades, and the weighted MP strict consensus tree (Fig. 6b) is similar. Based upon geographic location of the type specimen, we would recognize the clade O2 as *C. oreganus* (see Plate 14a), and clade O1 represents an undescribed *C. oreganus*-like form, and further sampling and analysis will be required before it can be formally described.

Southern Pacific Rattlesnake (*Crotalus v. helleri*)

This subspecies was recognized by Klauber (1949:81–82) as phenotypically homogeneous, an attribute associated with its reduced and ecologically more uniform habitat, particularly when compared to either *C. v. oreganus* or *C. v. cerberus*. Klauber (1949) noted, nevertheless, that individuals from desert foothill areas were uniformly lighter colored, whereas those from areas higher in elevation were darker, often nearly black, and thus reminiscent of *C. v. cerberus* (see Plate 13a). He also found phenotypic differences in *C. v. helleri,* particularly with regard to specimens from the southern part of the range, but his sample sizes from Baja California were too small for statistical analyses. Klauber proposed that *C. v. helleri* was closely related to *C. v. oreganus* and *C. v. cerberus*. He concluded that *C. v. helleri* and *C. v. cerberus* were alike in characters that separate *C. v. helleri* and *C. v. oreganus*, suggesting a close relationship between the former pair (Klauber, 1949).

Our data suggest that *C. v. helleri* is distinct but situated between two subdivisions of *C. v. oreganus* (unweighted MP consensus; see Fig. 5b), or in an unresolved polytomy with *C. v. concolor* (weighted MP consensus; see Fig. 6b). It is also in a larger, unresolved polytomy in the boostrapped versions of the unweighted and weighted MP analyses (Figs. 5a and 6a, respectively). It should be noted that the clades are distinct in the bootstrap analyses, but their relationships to one another are unresolved.

Coronado Island Rattlesnake (*Crotalus v. caliginis*)

Klauber (1949:94) described *C. v. caliginis*, a taxon endemic to Isla Sur of the Isla de los Coronados located off the west coast of Baja California Norte (Fig. 1). South Coronado is the largest (ca. 2.82 km in length, 205 m in elevation) and most southern and eastern of the four islands in the group, and is ca. 13 km from Tijuana. The island is rocky with precipitous cliffs and the beaches are few and limited in their extent. In addition, the channel separating the island from the mainland is deep and cold and the wind blows almost continuously toward the mainland. Klauber stated, "...*caliginis* is obviously derived from *helleri* of the nearby mainland, yet it has evidently been separated from the mainland population for a considerable time." But he also recognized, "…while the evidence points to a long isolation of *caliginis*, I am unable to find any consistent differences between it and *helleri* in squamation and pattern." The major difference, Klauber concluded, is one of stunting, with *C. v. caliginis* being much smaller than *C. v. helleri.* Klauber (1949:95) showed that pregnant females (N = 4) from the *C. v. caliginis* type series averaged 570 mm TL, whereas the smallest pregnant *C. v. helleri* measured by Klauber was 596 mm TL. The size difference is thus real.

Klauber (1949) also reported that *C. v. caliginis* appears to feed almost exclusively on lizards, in spite of the fact that small mammals are present on South Coronado Island. This stands in contrast to the diet of small mammals that typify the prey of juvenile and adult *C. v. helleri* on the mainland. He speculated that this may reflect a diurnal rather than nocturnal activity pattern for *C. v. caliginis*, which may in turn indicate a potential response to the cold and foggy climate of the island. As noted by Holycross et al. (this volume), a shift in the diet of *C. willardi* from lizards and invertebrates to primarily mammals is associated with age and body size. Consequently, an alternative hypothesis is that *C. v. caliginis* preys upon lizards (and is thus diurnal) due to gape-limitations.

Klauber was reluctant to identify insular populations of rattlesnakes as new taxa unless the data were compelling. Although he was unable to find consistent differences in squamation or pattern between *C. v. helleri* and *C. v caliginis*, (Klauber, 1949) considered that long isolation from the mainland and lack of opportunity for gene flow, as well as differences in body size and natural history, warranted subspecific designation for the latter. Grismer (2001, 2002) agreed with Klauber's rationale, but he considered those differences sufficient to elevate *C. v. caliginis* to specific status.

Our results and those of Pook et al. (2000) indicate, however, that *C. v. caliginis* is imbedded within the *C. v. helleri* clade. If gene flow from the peninsula does not occur (as per Klauber's reasoning), then possibly separation of the island from the mainland is more recent than Klauber originally thought. In addition, there are characters other than those based on mtDNA genes that can serve as synapomorphies to distinguish species (discussed below).

Midget Faded Rattlesnake (*Crotalus v. concolor*)

Klauber (1930:111–112) identified *C. v. concolor* as a subspecies of *C. v. viridis* (as *C. confluentus decolor*) "…differing from all other subspecies in coloration and size, being the lightest as well as the smallest of the several subspecies." Klauber (1935:83) also indicated, "…just as *nuntius* is a stunted form of *confluentus* [= *C. viridis*], so *concolor* seems to be a stunted form of *lutosus*; *concolor* is superficially more like *nuntius* than any other of the *confluentus* subspecies, although it is doubted whether the relationship is a direct one. In any case, they differ in color, pattern, and head scales, especially the number of scales before and between the supraoculars." With reference to intergradation between *C. v. concolor* and *C. v. viridis*, Klauber (1930:126) noted, "…such is rather to be assumed both from the territory and the characteristics of the subspecies, but available material permits no definite conclusions." Klauber (1936:242), however, indicated intergradation between *C. v. concolor* and *C. v. viridis* north of the valley of the San Juan River in Utah.

Based on morphological data, Hammerson (1981, 1999) considered populations of *C. viridis* in the four corners region (Arizona, Colorado, New Mexico, and Utah) to be intergrades between *C. v. viridis* and *C. v. concolor*. The mtDNA results of Quinn (1987) for specimens from this region, however, did not support this view, as no *C. v. concolor* haplotypes were revealed. Our results (Fig. 5a) corroborate those of Quinn (1987). In addition, in analyzing patterns of morphological diversity, Aird (1984) and Quinn (1987) found *C. v. concolor* widely separated from all other subspecies of *C. viridis* (see Plate 14d–f, Plate 15).

With regard to venom, Glenn and Straight (1977) determined that *C. v. concolor* appears to possess one of the most lethal crotaline venoms in the New World, and considered the venom 10–30 times more lethal than all other *C. viridis* subspecies (all subspecies were tested except for *C. v. abyssus*). Further, in view of their venom analysis, Glenn and Straight (1977) raised questions about the phylogenetic relationship of *C. v. concolor* and stated, "If venom lethality were considered instead of other morphological criteria such as squamation, *C. v. concolor* could be considered a separate species, not a subspecies of *C. viridis*." Subsequently, Pool and Bieber (1981) demonstrated the occurrence of a potent presynaptic neurotoxin in *C. v. concolor* venom. From a taxonomic perspective, Aird (1985) argued that the venom of *C. v. concolor* is more similar to that of *C. v. viridis* than to *C. v. lutosus*. Unless convergence is operating, our mtDNA results would suggest otherwise because *C. v. viridis* is a member of the eastern clade, whereas *C. v. concolor* and *C. v. lutosus* are members of the western clade. The average net percent sequence divergence between the two clades is 10.5 (± 2.7)%. In contrast to Aird (1985), based on isozyme analyses Quinn (1987) showed that *C. v. concolor* was more similar to *C. v. lutosus* than to *C. v. viridis*.

In comparison to *C. v. viridis*, and perhaps other members of the *C. viridis* complex, *C. v. concolor* exhibits differences in behavior. Ashton (1999), for

example, described shedding aggregations in *C. v. concolor* at or near dens in June and July, which often consisted of multiple males in the absence of females. This type of shedding aggregation has not been described for *C. v. viridis* (e.g., Duvall et al., 1985; King and Duvall, 1990). Unlike *C. v. viridis* in central Wyoming, which was not observed to make movements during cold evenings, *C. v. concolor* in southwestern Wyoming was observed to make movements at cold temperatures (ca. 5°C) during the evening in late September (see Porras, 2000). In contrast to the movement patterns of *C. v. lutosus*, *C. v. oreganus*, and *C. v. viridis* (see Duval et. al, 1985; Macartney et al., 1988; King and Duvall, 1990; Cobb, 1994), which travel long distances during the active season (up to 11 km), Ashton (1999) found that *C. v. concolor* in southwestern Wyoming did not travel far from den sites and showed no seasonal migrations. Based on our observations of *C. v. concolor* in southwestern Wyoming, we concur with Ashton (1999); furthermore, we have encountered *C. v. concolor* at dens in this region throughout the active season (April to October; L. Porras and G. Schuett, unpublished). This level of seasonal den site fidelity has not been observed in other members of the *C. viridis* complex.

We show herein that *C. v. concolor* is not merely a stunted *C. v. lutosus,* as discussed by Klauber. In both unweighted and weighted MP consensus trees (Figs. 5b and 6b, respectively), *C. v. concolor* shares an unresolved polytomy with one (of two) subdivisions of *C. v. oreganus*. Bootstrap evaluations of these MP trees place *C. v. concolor* within a larger polytomy that contains both subdivisions of *C. v. oreganus,* as well as *C. v. helleri* and *C. v. caliginis.*

One interesting finding of our research is that while *C. v. concolor* displays a moderate within-group *p*-distance (0.7 ± 0.1%; Table 2), molecular variation within this clade is completely unstructured. That is to say, there is no geographic differentiation within *C. v. concolor* with respect to the mtDNA genes we inspected (Figs. 5–8), in spite of the fact that 23 individuals were evaluated from southwestern Wyoming and western Colorado, as well as northeastern and southeastern Utah.

Grand Canyon Rattlesnake (*Crotalus v. abyssus*)

In Figures 5 and 6, *C. v. abyssus*-*C. v. lutosus* was partitioned into a well-defined (at 88% support) *C. v. abyssus* and paraphyletic *C. v. lutosus*. These three subdivisions represent the most recently evolved lineages of the western clade. Furthermore, we found evidence of additional subdivisions within each clade.

Klauber (1930:114) identified *C. v. abyssus* as "…a peculiar phase of *Crotalus confluentus* [= *C. viridis*] distinguished by its vermilion or salmon coloration and an almost complete absence of markings in the adult." He further noted (p. 115–116) that it has been taken "…only in the Grand Canyon of the Colorado…but on both sides of the river and at least to the rim of the Canyon." Young and Miller (1980) echoed the above conclusions relating to color and geographic location. Klauber (1930:117) concluded that *C. v. abyssus* was closely related to *C. v. lutosus* primarily "…in character of body markings, width of postocular stripe, scales before and between supraoculars, and in tail rings. In color it more nearly resembles *confluentus* [= *C. v. viridis*], especially the stunted red form [= *C. v. nuntius*] found in the vicinity of Winslow, Arizona. The latter, however, is a darker, richer red with typical *confluentus* markings and scutellation. This may be a case of parallel development or intergradation down the Little Colorado River." Our data support some of Klauber's conjecture. Percent sequence divergence between *C. v. lutosus* and *C. v. abyssus* is 1.0 ± 0.3. Young et al. (1980) found *C. v. abyssus* to be more closely related to *C. v. lutosus* than to either *C. v. nuntius* or *C. v. concolor* based on venom profiles.

There are, however, two areas where our observations do not agree with those of Klauber (1930:114). Most *C. v. abyssus* we have encountered were not vermilion or salmon in coloration, but yellowish-tan, pale gray, pale brown, or buff. More importantly, we have found haplotypes of *C. v. abyssus* external to the Grand Canyon. We have also examined museum specimens that we determined to be *C. v. abyssus* based on morphological characters (L. Porras et al., unpublished). In Utah, individuals of *C. v. abyssus* were found in the Grand Escalante-Staircase National Monument (Kane Co.) from near the Paria River (Plate 16a), and from the Kaiparowitz Plateau and adjacent Straight Cliffs and Fifty-Mile Bench. The eastern Kaiparowitz Plateau and the Straight Cliffs/Fifty-Mile Bench escarpment drain into Lake Powell by way of southward-running ephemeral creeks and arroyos.

The Kaiparowitz Plateau is a rugged region that opens northwestward from Lake Powell at the upstream terminus of the Grand Canyon. In the southern Kaiparowitz (an area lower in elevation), individuals

phenotypically resemble *C. v. abyssus* from the Grand Canyon, whereas those from the more northern areas of the Kaiparowitz region are somewhat atypical (Plate 16b–d). Yet, from the standpoint of our molecular markers, all are members of the *C. v. abyssus* clade (M. Douglas et al., unpublished).

The ecology of *C. v. abyssus* is scarcely known. In the most extensive ecological evaluation of this taxon, Reed and Douglas (2002) used radiotelemetry to evaluate movement behavior, activity range size, and habitat use of nine individuals in the Little Colorado River gorge, ca. 3 km above its confluence with the Colorado River in Marble Canyon (see Fig. 2). On average, snakes moved 26 m/day and 45 m/movement, with males moving greater distances than females (although movement frequency was equal between sexes). In contrast to *C. v. viridis* (King and Duvall, 1990), individuals in their study exhibited low directionality of movements. Activity size varied from < 4 to > 30 ha among individuals, but these ranges were elongated due to the corridor-like nature of the canyon bottom. Individual snakes appeared to be dietary opportunists, eating a variety of rodent and lizard prey. They also preferentially used riparian areas and avoided floodplains, using talus and upland mesquite habitats in rough proportion to their availabilities.

Great Basin Rattlesnake (*Crotalus v. lutosus*)

This subspecies has a range that extends from the Sierra Nevadas in California, across the Great Basin to the Rocky Mountains in central Utah, and southward to the Grand Canyon (see Plate 13b–e). Klauber (1930) considered that *C. v. lutosus* intergraded with other *C. viridis* subspecies along much of its boundary, but these relationships did not emerge until subsequent publications, specifically when Klauber defined new (or redefined previous) subspecies. Quinn (1987), however, indicated that gene flow was not known to occur between *C. v. lutosus* and *C. v. concolor*. Along the eastern boundary, we have not detected intergradation between *C. v. lutosus* and *C. v. viridis*, or between *C. v. lutosus* and *C. v. concolor.* Interestingly, *C. v. concolor* and *C. v. lutosus* are syntopic near Arcadia (Duchesene Co., Utah; J. Glenn and R. Nohavec, pers. comm.). Also, we are aware of contact zones between *C. v. lutosus* and *C. v. abyssus*, and *C. v. lutosus* and *C. v. oreganus*, but this information will be presented elsewhere (M. Douglas et al., unpublished).

Our data show that *C. v. lutosus* is paraphyletic and shows a closer relationship to *C. v. abyssus* than to *C. v. concolor.* Based on our geographic sampling (e.g., location of the type locality), we designate the clades L1 and L2 as *C. v. lutosus* (Fig. 5a), and the clade L3 represents an undescribed *lutosus*-like population. Additional specimens of *C. v. lutosus* from throughout the range will be required before we can resolve the taxonomic allocation of the L3 clade.

Our confirmation that *C. v. lutosus* penetrates into the Grand Canyon (Fig. 2) is important. Individuals with *C. v. lutosus* haplotypes were found at side canyons in the following reaches of the Grand Canyon (as defined by Schmidt and Graf, 1990): Redwall Gorge (at South Canyon), the Middle Granite Gorge (at Randy's Rock, Stone Creek, and Deer Creek), Muav Gorge (at Kanab Creek), and Lower Canyon (at Tuckup Canyon). All of these sites are on the north side of the Colorado River. We are not surprised that *C. v. lutosus* is found within the Grand Canyon, because this taxon occupies a variety of habitats in a broad elevational range, and thus can be characterized as an efficient colonizer. Fowlie (1965:157–158) recorded it on the north rim of the Grand Canyon at Toroweap Valley and Point Sublime (west and east of Kanab Creek, respectively). Jett (1972:12) observed a single specimen of *C. v. lutosus* in Kanab Creek Canyon approximately 12.7 km above its confluence with the Colorado River (also see Miller et al., 1982). Many tributaries and deep canyons drain southward from the north rim of the Kaibab Plateau. Rattlesnakes clearly follow riparian corridors and also canyons with declining topography. The presence of *C. v. lutosus* in the Grand Canyon, particularly at confluence of tributaries and side canyons with the Colorado River, is a logical outcome of its life history.

In conclusion, the mtDNA haplotypes uncovered in our analyses support seven of the nine subspecific lineages recognized by Klauber (1972). Klauber's perspectives were derived more than a half-century ago, and the resolution gained from a molecular approach has considerably improved those earlier perspectives. We feel that despite certain shortcomings, Klauber's lineages of *C. viridis* are robust; nonetheless, several (*C. v. viridis, C. v. oreganus, C. v. lutosus*) reflect additional cryptic variation that may render them paraphyletic. From a phenotypic standpoint this paraphyly may eventually be resolved once appropriate and sufficient samples are acquired and analyzed.

Klauber's View on Intergradation in *C. viridis*

While there is no doubt that Klauber had a good eye for detecting intraspecific differentiation, he often interpreted phenotypic evidence for intergradation in a non-quantitative fashion. Often, he did not quantify those characteristics among individuals he suspected were intergrades. This is unusual, simply because Klauber was among the first among herpetologists to incorporate statistical analysis on problems of phenotypic variation. We attribute this to the fact that issues of intergradation were more "gestalt" to Klauber rather than truly quantitative. While he made efforts to classify single or groups of individuals with regard to membership with neighboring subspecies (e.g., Klauber, 1943), most often those efforts were to demonstrate statistical and graphical approaches rather than to evaluate potential intergradation. We suggest, instead, that Klauber viewed aberrant phenotypic variance (i.e., variation not falling within predetermined "types") as prima facia evidence for intergradation. In that sense, Klauber used intergradation and hybridization as convenient hooks upon which to hang discordant variation (see Douglas et al., 1999a). Klauber (1930:127) expressed that *C. v. concolor* showed "Intergradation with either *confluentus* [= *C. v. viridis*] to the east or north, or *lutosus* to the west, or both." Also, he indicated (Klauber, 1930) that *C. v. abyssus* is believed to intergrade with *C. v. lutosus* and *C. v. viridis*, and that *C. v. lutosus* intergrades with *C. v. oreganus* to the west and *C. mitchellii stephensi* (as *C. confluentus stephensi*) to the southwest. Klauber (1936:253) provided a map depicting *C. v. abyssus* as intergrading with *C. v. nuntius* along the south rim of the Grand Canyon, and probably with *C. v. lutosus* along Kanab Creek in the Grand Canyon. He similarly depicted *C. v. nuntius* as intergrading with *C. v. viridis* in northeastern Arizona, and the latter with *C. v. concolor* in southeastern Utah.

In essence, Klauber appears to have perceived *C. viridis* as a species linked via gene flow from its eastern to its western terminus. Klauber (1935:86) commented, "…the most certain intergradation (as known today) of the two terminal forms, *oreganus* and *confluentus* [= *viridis*], is that via the detour *confluentus*, *nuntius*, *abyssus*, *lutosus*, *oreganus*, and this is not as certain as is desirable." Today such an interpretation is difficult for us to unequivocally accept because it forces the premise that phenotypic variability among clades is environmentally rather than genetically induced. In other words, selection overwhelms gene flow. How else could those subspecific patterns recognized by Klauber sustain themselves? Examples exist where selection has indeed been paramount (e.g., natural selection in *Nerodia sipedon*, Camin and Ehrlich, 1958; industrial melanism in *Biston betularia*, Kettlewell, 1973), yet most biologists today would agree these are exceptions rather than the rule. We suggest that the levels of gene flow envisioned by Klauber would induce cohesion and homogeneity among subspecies rather than the diversification we see. Consequently, although Klauber observed great differentiation among *C. viridis* throughout its range, he concomitantly visualized a mixing of these distinct traits among forms not only at zones of subspecific contact (which would be expected), but also in those areas where the phenotype of a particular rattlesnake did not conform to the typology of the resident clade.

Klauber's perspectives on intergradation within the *C. viridis* complex strongly influenced viewpoints of subsequent researchers, particularly those working in the four-corners region (i.e., Arizona, Colorado, New Mexico, Utah). Woodbury et al. (1958), for example, stated that Glen Canyon (now inundated by Lake Powell, Arizona and Utah) "…is a region of subspecific intergradation between the yellow rattlesnake of the Escalante and Henry Mountain region (*C. v. concolor*), the reddish-colored Grand Canyon rattlesnake (*C. v. abyssus*), the little pink rattlesnake of the Little Colorado River basin (*C. v. nuntius*), and possibly the typical greenish form of the western rattlesnake from northwestern New Mexico and northeastern Arizona (*C. v. viridis*)." Similarly, Woodbury (1961) further noted in a reconnaissance of the Navajo Reservoir basin "…three specimens (Archuleta Co., Colorado) are probably intergrades of *C. v. viridis* and either *C. v. nuntius* or *C. v. concolor*. Our specimens did not show clear characters of any of the above subspecies so they will be regarded as intergrades until further collecting and comparison enables a more precise allocation." Hammerson (1999:385-386) likewise stated, "Moffatt, Routt, and Rio Blanco counties apparently constitute an area of intergradation between *concolor* and *viridis*" (see Plate 14e). He also considered "…rattlesnakes from southwestern Colorado (north to Montrose County and east of La Plata County, or perhaps Archeluta County) should be regarded as *viridis-concolor* intergrades...." Comparably, Graham (1991) noted, "…populations of rattlesnakes at Natural Bridges (southeastern Utah) appear to be intergrades between two subspecies of *C.*

viridis: the midget faded (*C. v. concolor*) and the prairie rattlesnake (*C. v. viridis*)" (but, see Plate 14f). Similar perspectives can be found elsewhere (e.g., Douglas, 1966; Miller et al., 1982; Stebbins, 1985; Lowe et al., 1986; Bartlett and Tenant, 2000).

How much of this speculation is simply honest confusion invoked by phenotypic plasticity in a polytypic species of rattlesnake? Does this represent an echo of Klauber's perspectives? We suggest the latter is paramount and base our perspectives upon the innately human desire to tell (and hear) a good story (Gould, 1980), coupled with the traditional approach of field biologists to build upon published observations of other researchers. With regard to the latter, Klauber was prolific in publishing taxonomic viewpoints (Klauber, 1930, 1936, 1949), whether substantiated or speculative. In addition, biologists are often unduly influenced by the philosophical milieu of their time. European researchers of the early 1940s were captivated by the concept of ecophenotypy, and subsequently interpreted the diversity of whitefish (*Coregonus*) inhabiting Swiss lakes in light of this favored hypothesis. These erroneous interpretations stand today as taxonomic "stumbling blocks" for the recognition of biodiversity in those lakes (M. R. Douglas and P. Brunner, unpublished).

Similarly, we posit that Klauber must have been influenced by the concept of "Rassenkreis" (i.e., ring of races), which was in vogue in the 1930s and early 1940s, and was a central topic in numerous publications (i.e., Miller, 1931, 1941; Fitch, 1940; Huxley, 1942; Mayr, 1942). The term was first coined in a smaller systematic publication (Rensch, 1926), then subsequently defined (Rensch, 1929:13) as "…a complex of geographic races that have diverged from each other, are geographic substitutes for each other, and are capable of unlimited reproduction with their neighboring geographic races." Furthermore, Rensch noted that a Rassenkreis was named according to the rules of nomenclature after the first described (i.e., nominal) race (e.g., *Crotalus viridis viridis*). He further stated that the term was synonymous with what some researchers considered as "Art" (species), "Formenkreis" (ring of forms), or "Rassenkette" (chain of races), but that the new term was preferred in that it is unequivocal. The difference was that geographic races were reproductively isolated from all other geographic races of the same Rassenkreis except for the neighboring races. Mayr (1982) noted that use of an internationally more suitable term, polytypic species, originally introduced by Julian Huxley (1938:255), might have been a response to the negative connotations of "race" being promulgated at that time by Adolph Hitler. Additionally, Rensch (1929) proposed to officially recognize not only groups of geographically representative subspecies (i.e., "Rassenkreis"), but groups of geographically representative species which he termed "Artenkreis," which Mayr later renamed as superspecies. Huxley (1938:255) instead suggested the term "geographical subgenus" which would translate in today's lexicon as a species complex.

Gene flow was expected among components of a "Rassenkreis" as a phenomenon synonymous with its definition. Huxley (1942:180) stated, "…numerous examples are to be found of Rassenkreise whose extreme subspecies are so distinct that they would rightly be classified as separate species if the intergrading, connecting types were not known." In our view, Klauber fully expected the subspecies of *C. viridis* to exchange genes in such a manner that individual components of the ring were interminably linked. In fact, Klauber went out of his way to press this interpretation on numerous occasions, and occasionally to employ anecdotal perspectives and non-synthetic data as reinforcement. This approach, while contemporary in Klauber's era, is now considered an impediment to understanding the evolution of so-called polytypic species such as *C. viridis*, just as the outdated concept of ecophenotypy is an impediment to understanding evolution of *Coregonus* in the Swiss lakes.

The maternal inheritance of the genetic markers used in this study does not allow us to unequivocally reject hypotheses of hybridization. A similar perspective was noted by Chow and Takeyama (2000), who found mtDNA heterogeneity among samples of swordfish (*Xiphias gladius*) but were unable to determine if these were subsamples from a panmictic unit or represented population admixture. Intergradation between taxa of the *C. viridis* complex has been documented based strictly on phenotypic analysis (e.g., *C. v. helleri* x *C. v. oreganus*; Schneider, 1986), but we have no evidence at this time that its occurrence is widespread. The fact that we find reciprocal monophyly among the clades in our study, however, argues against the view of rampant intergradation as presented by Klauber. Further, *C. viridis* is known to hybridize with other species of rattlesnakes outside of the complex (e.g., Perkins, 1951; Cook, 1955; Klauber, 1972; Murphy and Crabtree, 1988; Glenn and Straight, 1990;

Rubio, 1998). We conclude that it would be surprising if occasional intergrades or hybrids were not found.

Biogeography and Divergence Times

Just as mtDNA is often less than optimal as a marker to document intergradation and hybridization among clades, it likewise has difficulty in distinguishing the post-Pleistocene divergence of populations or species. In the former situation, the problem stems from the maternal inheritance of the molecule, whereas in the latter it stems from the fact that mtDNA does not accumulate mutations rapidly enough to adequately reflect this relatively recent divergence (as per Brunner et al., 1998). In addition, much of the observed post-Pleistocene divergence within clades often follows a severe reduction in population sizes and the genetic bottlenecking it produces. In essence, those species that have gone through such a reduction in population size and/or distribution have recalibrated their molecular clock such that phylogeographic evolution is reinitiated. Baker et al. (1994), for example, found using mtDNA that lineage variation in Arctic-breeding shorebirds (Red Knot, *Calidris canutus*) was minimal, and thus relationships among geographic populations could not be determined. These birds were apparently bottlenecked through a small population in the late Pleistocene and only expanded into their current broad distribution within the last 10,000 years (Baker and Marshall, 1997). Similarly, Walker et al. (1998) found only a single mtDNA control region haplotype in 66 Snapping Turtles (*Chelydra serpentina*) collected from 10 different states in the southeastern United States. These researchers noted how unusual it was to recognize only a single evolutionarily significant unit (ESU) in an otherwise phylogeographically rich region, and particularly one that had previously demonstrated considerable genetic diversity in other turtle species (Walker and Avise, 1998). As with the Red Knot, the most parsimonious explanation for these results is a severe post-Pleistocene bottleneck in *C. serpentina* across the southeastern United States, followed by a relatively recent range expansion.

Avise et al. (1998) summarized divergence times for intraspecific phylogroups of non-avian vertebrates. Among 189 species surveyed for mtDNA population structure across major portions of their respective ranges, 103 (54%) displayed a Category 1 phylogeographic pattern (i.e., a deep gene tree with major allopatric lineages). In mammals, 52 of 72 inferred phylogroup separations (72%) date to the Pleistocene, while most of the remainder date to the Pliocene. These numbers are similar to those for avian taxa. Interpretations for other vertebrates are complicated by suspected slower mtDNA clock calibrations (Avise et al., 1992). Under assumptions of a standard clock in amphibians and reptiles, 27 of 47 phylogroup separations (57%) date to the Pleistocene. In fishes, 19 of 26 (73%) date to the Pleistocene. These percentages drop to 15% and 31%, respectively, under a 4-fold slower clock.

Crotalus viridis shows a Category 1 phylogeographic pattern, and if we apply the above-mentioned rationale to evaluate divergence of *C. viridis* phylogroups and use only mean *p*-distances and a standard clock, we find that 57% of the separations in this species date to the Pleistocene and the remainder (43%) to the Pliocene. This is the same percentage derived by Avise et al. (1998) for amphibians and reptiles in general. If we perform the same calculations for *C. viridis* as above but use the fish clock, we find 29% of the separations date to the Pleistocene and the remainder (71%) to the Pliocene. Finally, if the slow clock is employed in our calculations, only 4% of the separations are Pleistocene, whereas the remainder (96%) are Pliocene.

The earliest fossil record of *C. viridis* is from Late Miocene (Driftwood Creek, Hitchcock Co., Nebraska; Brattstrom, 1967). Holman (2000) suggested, however, that the specific identification of this fossil, as well as the age of the site, need to be confirmed. Middle and late Pliocene fossils of *C. viridis* are additionally recorded from Kansas, but most records date from the late Pleistocene and are recorded from California, Colorado, Idaho, Kansas, and Nevada (Holman, 2000:231). In this sense, the *C. viridis* complex appears more comparable to Song Sparrows (Zink and Dittman, 1993), freshwater turtles (Walker and Avise, 1998), Tiger Salamanders (Shaffer and McKnight, 1996), Tassel-eared Squirrels (Lamb et al., 1997), North American Puma (Culver et al., 2000), and other taxa influenced primarily by the Pleistocene history of North America. This is not to say that all speciation events within the *C. viridis* complex necessarily occurred during the Pleistocene. Much like Avise and Walker (1998), we see speciation as an extended temporal process rather than a point event, and we suspect that pre-Pleistocene conditions had an active role both in initiating and completing widespread separation of populations of the *C. viridis* complex.

Although phylogeographic patterns can be revealed within a species complex, explaining those patterns in a cogent manner is much more difficult. Are the patterns we show for *C. viridis* the result of clades diversifying within isolated and deep-historical refugia, or are they the result of post-Pleistocene gene flow? Is it possible to detect directional migrations in this complex? The challenge is to achieve an objective rather than ad hoc explanations for the observed patterns.

Three biogeographic hypotheses have been forwarded for the origins of disjunct populations of Tassel-eared Squirrels (*Sciurus aberti*) in the American southwest (Lamb et al., 1997). They are: (1) vicariant relicts from an ancestral form widely distributed across the continuous coniferous forests of the Pleistocene; (2) post-Pleistocene dispersal across unsuitable non-montane habitats; and (3) early vicariant events from which dispersal ensued at a later time. Molecular evidence was consistent with the last hypothesis and suggested an early Pleistocene vicariant event followed by Quaternary dispersal in conjunction with a documented northward range expansion of Ponderosa Pine. Although it is too early in our study to make biogeographic pronouncements (Pook et al., 2000; but see Ashton and de Queiroz, 2001), our molecular data suggest that Pleistocene events were influential with regard to differentiation of taxa of the western clade of *C. viridis*.

Species and Intraspecific Diversity

The formal definition of a species has been (and will likely remain) a long-standing debate (Davis, 1996). While primarily philosophical, this debate also has numerous pragmatic applications of which the most important is the manner by which biologists taxonomically partition nature (Cracraft, 1997). This question must be answered before an exact quantification of biodiversity can be accomplished. Its answer also brings into focus the general concept of subspecies (see Introduction) and, more specifically, how patterns of variation should be dealt with within and among populations.

The demarcation between true species and subspecies can be enormously vague. Huxley (1939:105), for example, acknowledged that a subspecies involved the following points: "(1) geographical replacement; (2) frequently but not always partial discontinuity.... A subspecies is, therefore, a natural or 'real' taxonomic unit in the sense that it is a self-reproducing group with a characteristic geographical distribution, distinguished from other similar groups by measurable character-differences which can be determined on any reasonably-sized series. Where genetic analysis is possible, they are interfertile with adjacent subspecies of the same polytypic species or Formenkreis (although not necessarily with remote members of the same Formenkreis). This implies that it will often be in genetic interchange with adjacent subspecies."

Formal concepts and definitions of species have ramifications beyond the arcane and philosophical. Of the numerous species concepts (reviewed in Mayden, 1997; Howard and Berlocher, 1998), which is most appropriate? While this question is still debated, a general consensus is developing among biologists that the Biological Species Concept (BSC) has limited utility, and that an Evolutionary Species Concept (ESP) or Phylogenetic Species Concept (PSC) better capture the current view of species in a wide range of taxa. Rosen (1978:175; 1979:275–278) convincingly argued that the BSC is of little use in phylogenetic reconstruction because its primary definer (i.e., reproductive compatibility) is a plesiomorphic character within a lineage (see Kottelat 1997:14, 17). The BSC, therefore, has little capacity to specify relationships within a genealogical framework. With that in mind, various formulations of evolutionary and phylogenetic species concepts have been proposed (Cracraft, 1983; Donoghue, 1985; Frost and Hillis, 1990; Avise, 1994, 2000), but each emphasizes descent as prima facia evidence rather than interbreeding or its potential (e.g., reproductive characteristics). A phylogenetic species (as per Cracraft, 1983) constitutes "…the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent…." with diagnosis based strictly upon one (or more) shared-derived characters that diagnose a monophyletic assemblage of individuals. One difficulty with this rationale is the criteria by which clades are to be recognized at microevolutionary scales. Critics have argued that an approach promulgating clade diagnosis on the basis of synapomorphies at one or a few genes makes little sense, leading Avise and Wollenberg (1997) to suggest that some fusion of the PSC and BSC should be attempted.

It is difficult to imagine how an amalgam of the BSC and PSC will be acceptable to a broad range of biologists. Instead, we support the concept of phylogenetic species in that they are "…basal, diagnosably distinct taxa…comprised of one or more populations

that share a combination of characters that distinguish them from other such units" (Cracraft, 1983). We also support arguments by Cracraft et al. (1998:148) that fixed differences in mtDNA can be evolutionary markers for delineating taxa. Finally, as stated by Cracraft et al. (1998), we recognize that the absence of such markers cannot be taken as evidence that variation in other (unsampled) diagnostic characters does not exist.

With regard to intraspecific taxa, recent studies suggest that distinct, allopatric lineages are best regarded as phylogenetic species, and that significant diversity may be hidden by more traditional taxonomic practices (Wilson and Brown, 1953; Frost et al., 1992; Sites and Crandall, 1997; Parkinson et al., 2000). On the other hand, there are indications that traditional taxonomy, at least at the infraspecific level, has often seriously overestimated levels of biodiversity in numerous species. This has been the case in species of snakes, where several studies have demonstrated conflicts among and between nominate subspecies (reviewed by Rodríguez-Robles and De Jesús-Escobar, 2000). Rodriquez-Robles et al. (1999) evaluated the phylogeography of the California Mountain Kingsnake (*Lampropeltis zonata*) and found that the seven currently recognized subspecies collapsed into two large clades, one of which was divided into two further subclades. Similarly, Rodríguez-Robles and De Jesús-Escobar (2000) proposed that the *Pituophis melanoleucus* species complex (i.e., New World bull-, gopher-, and pinesnakes) be reduced from 15 subspecies to three species. Finally, Burbrink et al. (2000) found that none of the eight subspecies of the *Elaphe obsoleta* complex (Eastern Ratsnake) represented a distinct evolutionary lineage.

Biologists studying other groups of vertebrates have found similar problems. The Leopard (*Panthera pardus*), for example, is a geographically widespread carnivore with 27 currently recognized subspecies. Molecular analyses coupled with morphological data suggested that it should be consolidated into eight (30% of the total) subspecies (Miththapala et al., 1996). In the majority of cases, newly designated Leopard subspecies conform to recognized geographical barriers that facilitated allopatric divergence. Similarly, Culver et al. (2000) evaluated 32 subspecies of Puma (*Puma concolor*), and recommended a reduction to six phylogeographic groups (an 82% decrease). Furthermore, these researchers noted that in spite of the continent-wide distribution of the North American subspecies, they demonstrated a marked reduction in mtDNA and microsatellite variation. These finding were congruent with a founder event involving a small number of individuals that migrated northward from South America approximately 10–12,000 years before present (ybp), subsequent to the abrupt extinction of large North American mammal species in the late Pleistocene and early Holocene (Pielou, 1991:251).

The Tiger (*Panthera tigris*) is another large carnivore consisting of five extant (and at least three extinct) subspecies determined primarily by pelage color, body size, and distribution. One extant form is insular (Sumatran Tiger), whereas other populations are distributed on mainland Asia. Using mtDNA genes, Cracraft et al. (1998) found that only the Sumatran Tiger was diagnosably distinct. The question was how to treat this variation in a taxonomic manner? Researchers using the BSC previously classified *P. tigris* as a single species with multiple subspecies, yet this nomenclature does not accurately represent the historical (and inherent) patterns of variation or the diagnostic status of the different subspecies. Within the framework of the BSC, a solution would be to recognize a single species with two subspecies, but this conclusion would be contrary to the data at hand. Furthermore, Cracraft et al. (1998) argued that phylogenetic species "...cannot be subdivided into other diagnosable units…" because they are basal or terminal taxa. Under the PSC, subspecies are logically dispensable because if they were distinct they would be recognized as independent phylogenetic species. If they were not, then they should be clustered with similar populations within a single phylogenetic species. With regard to *P. tigris*, the PSC recognizes two taxa at the species level, *P. sumatrae* and *P. tigris*. In this sense, the PSC is a scientific hypothesis that recognizes a population (or group of populations) as a phylogenetic species based on explicit data.

We are, however, acutely aware that all species concepts have both theoretical and operational complications when applied to a wide range of organisms. As concepts change and techniques improve, conclusions pertaining to recognition of species will also change. Such hypothesis development and testing is a normal process of science.

Implications for Conservation and Management

Just as species concepts have ramifications with regard to systematics and biodiversity, they also impinge at various levels on conservation biology.

Table 3. A revised taxonomy for the *Crotalus viridis* complex. Standard English names follow Crother et al. (2001).

Taxon (binomial name)	Standard English name
Crotalus viridis (Rafinesque 1818)	Prairie Rattlesnake
Crotalus oreganus Holbrook 1840	Northern Pacific Rattlesnake
Crotalus cerberus (Coues 1875)	Arizona Black Rattlesnake
Crotalus helleri Meek 1905	Southern Pacific Rattlesnake
Crotalus concolor Woodbury 1929	Midget Faded Rattlesnake
Crotalus lutosus Klauber 1930	Great Basin Rattlesnake
Crotalus abyssus Klauber 1930	Grand Canyon Rattlesnake

Most obvious is the basic need to define diagnostic limits regarding geographic patterns of variation (i.e., to recognize and manage the biodiversity we observe). As in taxonomic procedures, it is imperative to identify populations (or groups of populations) that display independent evolutionary histories. But the overall practicality of conservation-related issues often drives the requirement more toward the immediate. The question of OTU identity, for example, affects which organisms are to be utilized in captive breeding programs of zoos, similar institutions, and game preserves (Avise, 1994). Additionally, it directly affects which of several dwindling populations should be protected under the U. S. Endangered Species Act. Finally, there is the question of OTU identity and its application to the burgeoning trade in wildlife, whether it is with living organisms or parts thereof (Cracraft et al., 1998). As above, the monolithic nature of formal taxonomy is often viewed as an impediment to these conservation tasks. As a result, there is a recognized movement to avoid species-level questions and apply a different standard to such problems, one that is considered more relevant to conservation action.

These difficulties are most pronounced in polytypic species, such as in *C. viridis*, not only due to the potential the latter engender for a priori designation of subspecies (as above), but also because polytypic species often contain populations isolated as a result of sharp habitat demarcations. These may be terrestrial ecotones (Smith et al., 1997; Schneider et al., 1999) or even lake-specific effects (Douglas et al., 1999b). To add to the confusion, populations often evolve independently from a molecular perspective but show convergence (homoplasy) at a morphological level (M. R. Douglas and P. Brunner, unpublished). Thus, the rapidity of clade-identification using a molecular approach has been wedded to the immediate demand of conservation needs such that the concepts of evolutionarily significant unit (ESU) and management unit (MU) has now been formalized (Moritz, 1994, 1995) following the work of Ryder (1986), Waples (1991), and Dizon et al., (1992). As per Avise (1998:379), ESUs are relatively deep and historical subdivisions in populations that center on four aspects of genealogical concordance: (1) across multiple sequence characters within a non-recombining segment of DNA; (2) across multiple independent loci; (3) geographically in gene tree partitions across multiple co-distributed species; and (4) beween gene tree partitions and traditional biogeographic evidence.

In genetic analysis, the concept underlying an MU is as follows (Avise 2000:267): "Any population that exchanges so few migrants with others as to be genetically distinct from them, will normally be demographically independent in the present time." MUs are referred to as "stocks" in commercial fisheries, for which harvesting quotas and other management plans are directed. Mitochondrial haplotypes are especially powerful for identifying MUs because of their typical fourfold smaller effective population size compared to haplotypes at nuclear autosomal loci.

Cracraft et al. (1998) correctly note that ESUs have no taxonomic recognition, and it is the latter that is influential within a legal framework. Furthermore, objectives underlying the new terminology are met by several formal species concepts, and in particular by the PSC. This is the underlying objective that ESUs are meant to emulate (Cracraft et al., 1998:148). The question is why should we bother to utilize an informal taxonomic status (ESU) when a formal definition (PSC) is available?

In the following section we utilize the above arguments and the results of our molecular analyses to address the confusion concerning the taxonomy of the Western Rattlesnake (*C. viridis*).

Taxonomic Conclusions and Recommendations

We recognize seven of the nine subspecies of *C. viridis* reviewed and discussed by Klauber (1972) as phylogenetic species, and our molecular results (Fig.

5a) revealed two undescribed clades (L3: *lutosus*-like, and O1: *oreganus*-like) that will require additional sampling and analysis before formal definition is possible. We present a formal taxonomic nomenclature of these species in Table 3, and base our conclusions on the following four points:

(1) The nine currently recognized subspecies of *C. viridis* were originally described as species or subspecies. Interestingly, Klauber (1972:164) was prescient to recognize, "…some of the newer methods of blood and venom studies may eventually indicate that the forms which we now consider *viridis* subspecies may really belong to two or more different species…." Klauber realized that the weight of evidence differentiating these organisms was considerable, and in spite of his philosophical position at the time he understood that future studies of a biochemical nature might result in these forms being re-classified as species. We suggest that Klauber did not complete the classification himself because the definition of a rassenkreis, complete with its expectations of intergradation among forms, was a philosophical bridge too difficult for him to cross.

(2) Our research (and that of others) shows that there are two highly divergent lineages of *C. viridis* that have been designated as eastern and western groups. The eastern group of the complex should be recognized as *C. viridis*. Due to low sequence divergence values, *C. v. nuntius* should be synonymized into *C. viridis* (as per Quinn, 1987, Pook et al., 2000). Nonetheless, we will revisit this issue as we increase sample size and analyses from varying geographic localities. There may be, for instance, cryptic species within the *C. viridis* clade (as per Figs. 5–8).

With the elevation of the eastern group to full species status, the taxonomy of the western group must therefore undergo its own revision. Our results revealed that the western clade is composed of multiple, well-defined lineages (high sequence divergence values) that are in large part congruent with the taxonomic designations presented by Klauber (1972). Furthermore, these lineages occur in relatively discrete geographic regions, and appear to show little to no intergradation or hybridization. Thus, elevation of the taxa *abyssus*, *cerberus*, *concolor*, *helleri*, *lutosus*, and *oreganus* from subspecific status to full species is warranted. Grismer (2001) argued that *C. v. caliginis* should be elevated to specific status based on its insular distribution (and thus lack of gene flow to the mainland), but our mtDNA results and the results of others who used different mtDNA genes (Pook et al., 2000; Ashton and de Queiroz, 2001) show that *caliginis* is not sufficiently diverged from *helleri* to warrant specific elevation. Based on these concordant molecular findings, we recommend that *caliginis* should be recognized as an insular population of *C. helleri.*

Although the interrelationships of the western group are not resolved equally by methods of analysis used in this study (MP, ML), expansion of geographic sampling and increased sample sizes, as well as additional use of other methods of phylogenetic reconstruction (e.g., Bayesian; Yang and Rannala, 1997), will likely resolve regions of the tree that are less robust. In our analyses, for example, there is strong support for *C. cerberus* as the basal-most taxon of the western group, and those taxa with the most derived molecular characters are *C. abyssus* and *C. lutosus*.

(3) Based on our data, we reject the taxonomic decision made by Ashton and de Queiroz (2001) to designate the western clade as a single species (*C. oreganus*) with multiple subspecies (as presented above and including *caliginis*). In their molecular analysis of the *C. viridis* complex, Ashton and de Queiroz (2001) based their taxonomic recommendations on only 25 individuals (23 localities) representing nine clades. Many (68%) of the localities matched those of Pook et al. (2000), and five of their samples (~ 20%) came from two localities. Despite the fact that sample size was small, it was sufficient to resolve *C. viridis* into eastern and western clades (as per Quinn, 1987; Pook et al., 2000), and a certain level of resolution was attained with respect to *C. v. nuntius*, *C. v. viridis*, as well as some members of the western group (*C. v. cerberus* and *C. v. concolor*). Their results, however, did not validate the taxa *C. v. abyssus*, *C. v. caliginis, C. v. helleri*, *C. v. lutsosus*, and *C. v. oreganus*. Despite these shortcomings, Ashton and de Queiroz (2001) provided taxonomic recommendations for the eastern (*C. viridis*, with two subspecies) and western (*C. oreganus*, with seven subspecies) clades. Given that no new insights to the phylogeny of the *C. viridis* complex were revealed (see Quinn, 1987; Pook et al., 2000), we contend that their taxonomic changes were essentially gratuitous.

(4) As already discussed, we view the PSC as superior to the BSC and ESC. Importantly, although the PSC and ESC both embrace ancestor-descendant histories as important to understanding (i.e., diagnosing) species, the PSC also explicitly avoids the pitfalls of recognizing and naming subspecies (Wilson and

Brown, 1953; Frost and Hillis, 1990; Frost et al., 1992). Unlike the BSC and ESC, a species under the PSC is a terminal taxon that cannot be subdivided into other diagnosable units (Craycraft et al., 1998). Hence, none of the species in our taxonomic arrangement (Table 3) bear subspecific epithets. Nonetheless, despite the choice of species concepts, we contend that based on molecular, morphological, geographical, and natural historical evidence, seven of the nine subspecies of *C. viridis* are diagnosable as good species, and thus on separate evolutionary trajectories. The taxonomy proposed by Ashton and de Queiroz (2001) masks what we argue is the reality of the diversity of this group (i.e., species status). Finally, our present mtDNA analysis of the *C. viridis* complex has important ramifications that extend beyond understanding evolutionary relationships and geographic variation. Based on our work, past research on other aspects of the biology of this group (e.g., venom analysis, reproductive cycles, geographic distribution, morphology, and conservation measures) will require rigorous reexamination and reformulation of conclusions.

Acknowledgments.—Numerous individuals assisted us in this and related projects by providing specimens, tissues, and/or various other invaluable courtesies. They are: Scott Binhgam, Breck Bartholomew, Patrick Brunner, Rob Bryson, Dan Carpenter, Roger Cogan, Dia Couttouw, Bruce Christman, Mike Demlong, Dale DeNardo, Bill Falvey, D. Fogell, Jack Foquette, Steve Goldberg, Lee Grismer, Bryan Hamilton, Jessica Haskell, Ken Kardong, Travis LaDuc, Rick Langley, Bob Larson, Elaine Leslie, Steve Mackessy, Cody Meachum, Gian Mercurio, Chris Nickel, Larry Nienaber, Regis Opferman, John Perez, Dennis Pope, Dave Prival, Dan Richards, Dennis Sargent, Ryan Sawby, Jeff Servoss, Tara Sprankle, H. Staker, Luke Thirkill, Steve Thompson, Ross Timmons, Chuck Vincent, Bill White, Rick Wilson, and Steve Zimmerman. We are especially indebted to the following individuals for their companionship in the field, and/or dedication to this and related projects: Randy Babb, Ed Cassano, Mike Cardwell, Geoff Carpenter, Andy Didiuk, Marty Feldner, the late Jim Glenn, Ron Gonzales, Bob Hansen, Bill Hayes, Tim Hoffnagle, Hans Koenig, Fred Kraus, Bill Lamar, Karl Larsen, Bob Mackin, Bill Montgomery, Bob Nohavec, Erika Nowak, Jack O'Leile, Charlie Painter, Trevor Persons, Bob Reed, Roger Repp, Matt Spille, Bryan Starrett, Emily Taylor, Blake Thomason, Larry Valentine, and Patrick Viehoever. We owe a special debt of gratitude to the late Jim Glen and to Bob Nohavec (VA Medical Center in Salt Lake City, Utah), not only for providing numerous specimens, but for field companionship and countless hours of stimulating dialogue. We also thank Jack Sites (Monte L. Bean Museum, Brigham Young University) for the loan of museum material and other courtesies. Ron Gonzales, Bob Hansen, and two anonymous referees provided valuable comments on an earlier draft of this manuscript. We acknowledge various state, tribal, and federal agencies for graciously providing collecting permits: Arizona Game and Fish Department, Colorado Division of Wildlife, Nevada Division of Wildlife, Utah Division of Wildlife Resources, The Hualapai Tribe, The Navajo Natio, Grand Canyon National Park/Glen Canyon National Recreational Area, Natural Bridges National Monument (Southeast Utah Group), and the Escalante-Grand Staircase National Monument.

Literature Cited

Adams, C. 1901. Baseleveling and its faunal significance, with illustrations from southeastern United States. Am. Nat. 35:839–852.

Adams, D. K., and A. C. Comrie. 1997. The North American monsoon. Bull. Am. Meteorol. Soc. 78:2197–2214.

Aird, S. D. 1984. Morphological and Biochemical Differentiation of the Western Rattlesnake in Colorado, Wyoming and Utah. Unpublished Ph.D. dissertation, Colorado State University, Fort Collins.

———. 1985. A quantitative assessment of variation in venom constituents within and between three nominate rattlesnake subspecies. Toxicon 23:1000–1004.

———, C. S. Seebart, and I. I. Kaiser. 1988. Preliminary fractionation and characterization of the venom of the Great Basin rattlesnake (*Crotalus viridis lutosus*). Herpetologica 44:71–85.

Anderson, R. S., J. L. Betancourt, J. I. Mead, R. H. Hevly, and D. P. Adams. 2000. Middle- and late-Wisconsin paleobotanic and paleoclimatic records from the southern Colorado Plateau, U.S.A. Palaeogeogr. Palaeoclimatol. Palaeoecol. 155:31–57.

Archie, J. W. 1989a. A randomization test for phylogenetic information in systematic data. Syst. Zool. 38:219–252.

———. 1989b. Homoplasy excess ratios: new indices for measuring levels of homoplasy in phylogenetic systematics and a critique of the consistency index. Syst. Zool. 38:235–269.

———. 1996. Measures of homoplasy. Pp. 153–188 *In* M. J. Sanderson and L. Hufford (Eds.), Homoplasy: The Recurrence of Similarity in Evolution. Academic Press, New York.

Ashton, K. G. 1999. Shedding aggregations of *Crotalus viridis concolor*. Herpetol. Rev. 30:211–213.

———, and A. de Queiroz. 2001. Molecular systematics of the western rattlesnake, *Crotalus viridis* (Viperidae), with comments on the utility of the D-loop in phylogenetic studies of snakes. Mol. Phylogenet. Evol. 21:176–189.

Avise, J. C. 1994. Molecular Markers, Natural History and Evolution. Chapman & Hall, New York.

———. 1998. Conservation genetics in the marine realm. J. Heredity 89:377–382.

———. 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge.

———, J. Arnold, R. M. Ball, Jr., E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Ann. Rev. Ecol. Syst. 18:489–522.

———, B. W. Bowen, T. Lamb, A. B. Meylan, and E. Bermingham. 1992. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the Testudines. Mol. Biol. Evol. 9:457–473.

———, and D. Walker. 1998. Pleistocene phylogenetic effects on avian populations and the speciation process. Proc. R. Soc. London B 265:457–463.

——— , and ———. 1999. Species realities and numbers in sexual vertebrates: perspectives from an asexually transmitted genome. Proc. Natl. Acad. Sci. U.S.A. 96:992–995.

———, ———, and G. C. Johns. 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. Proc. R. Soc. London B 265:1707-1712.

———, and K. Wollenberg. 1997. Phylogenetics and the origin of species. Proc. Nat. Acad. Sci. U.S.A. 77:7748–7755.

Baker, A. J., and D. Marshall. 1997. Mitochondrial control region sequences as tools for understanding evolution. Pp. 51–82 *In* D. P. Mindell (Ed.), Avian Molecular Systematics and Evolution. Academic Press, New York.

———, T. Piersma, and R. Rosenmeier. 1994. Unravelling the intraspecific phylogeography of knots *Calidris canutus*: a progress report on the search for genetic markers. J. Ornithol. 135:599–608.

Baker, R. H., X. Yu, and R. DeSalle. 1998. Assessing the relative contribution of molelcular and morphological characters in simultaneous analysis trees. Mol. Phylogenet. Evol. 9:427–436.

Barker, F. K., and S. M. Lanyon. 2000. The impact of parsimony weighting schemes on inferred relationships among toucans and Neotropical barbets (Aves: Piciformes). Mol. Phylogenet. Evol. 15:215–234.

Bartlett, R. D., and A. Tennant. 2000. Snakes of North America: Western Region. Gulf Publishing, Houston.

Benson, L. D., and R. A. Darrow. 1981. Trees and Shrubs of the Southwestern Deserts. University of Arizona Press, Tucson.

Bermingham, E., and A. P. Martin. 1998. Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. Mol. Ecol. 7:499–517.

———, S. S. McCafferty, and A. P. Martin. 1997 Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. Pp. 113–128 *In* T. D. Kocher and C. A. Stepien (Eds.), Molecular Systematics of Fishes. Academic Press, New York.

Betancourt, J. L. 1990. Late Quaternary biogeography of the Colorado Plateau. Pp. 259–293 *In* J. L. Betancourt, T. R. Van Devender, and P. S. Martin (Eds.), Packrat Middens: The Last 40,000 Years of Biotic Change. University of Arizona Press, Tucson.

Bjorklund, M. 1999. Are third positions really that bad? A test using vertebrate cytochrome *b*. Cladistics 15:191–197.

Brattstrom, B. H. 1964. Evolution of the pit vipers. Trans. San Diego Soc. Nat. Hist. 13:185–268.

———. 1967. A succession of Pliocene and Pleistocene snake faunas from the High Plains of the United States. Copeia 1967:188–202.

Brown, W. M., M. George, Jr., and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Nat. Acad. Sci. U.S.A. 76:1967–1971.

Brunner, P. C., M. R. Douglas, and L. Bernatchez. 1998. Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic charr *Salvelinus alpinus* (Teleostei: Salmonidae) from central Alpine lakes. Mol. Ecol. 7:209–223.

———, ———, A. Osinov, C. C. Wilson, and L. Bernatchez. 2001. Holarctic phylogeography of artic charr (*Salvelinus alpinus* L.) inferred from mitochondrial DNA sequences. Evolution 55:573–586

Burbrink, F. T. 2001. Systematics of the eastern ratsnake complex (*Elaphe obsoleta*). Herpetol. Monogr. 15:1–53.

———, R. Lawson, and J. B. Slowinski. 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution 54:2107–2118.

Camin, J. H., and P. R. Ehrlich. 1958. Natural selection in water snakes (*Natrix sipedon* L.) on islands in Lake Erie. Evolution 12:504–511.

Chow, S., and H. Takeyama. 2000. Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units in the swordfish. J. Fish Biol. 56:1087–1098.

Cobb, V. A. 1994. The ecology of pregnancy in free-ranging Great Basin rattlesnakes (*Crotalus viridis lutosus*). Unpublished Ph.D. dissertation, Idaho State University, Pocatello.

Cook, S. F., Jr. 1955. Rattlesnake hybrids: *Crotalus viridis* x *Crotalus scutulatus*. Copeia 1955:139–141.

Cox, D. C. and W. W. Tanner. 1995. Snakes of Utah. Brigham Young University, Provo.

Coues, E. 1875. Synopsis of the reptiles and batrachians of Arizona. Rep. Geog. Geol. Expl. Surv. W. 100 Merid. (Wheeler Report) 5:585–633.

Cracraft, J. 1983. Species concepts and speciation analysis. Curr. Ornithol. 1:159–187.

———. 1997. Species concepts in systematics and conservation biology - an ornithological viewpoint. Pp. 325–339 *In* M. F. Claridge, H. A. Dawah, and M. R. Wilson (Eds.), Species: The Units of Biodiversity. Chapman & Hall, London.

———, J. Feinstein, J. Vaughn, and K. Helm-Bychowski. 1998. Sorting out tigers (*Panthera tigris*): mitochondrial sequences, nuclear inserts, systematics, and conservation genetics. Anim. Conserv. 1998:139–150.

Crother, B. I., J. Boundy, K. de Queiroz, and D. Frost. 2001. Scientific and Standard English Names of Amphibians and reptiles of North America North of Mexico, with Comments Regarding Confidence in our Understanding. Society for the Study of Amphibians and Reptiles. Herpetol. Circ. 29.

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

Darwin, C. 1859. On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life. John Murray, London (facsimile of the 1st ed. published in 1964 by Harvard University Press, Cambridge, Massachusetts).

Davis, J. I. 1996. Phylogenetics, molecular variation, and species concepts. BioScience 46:502–511.

DeFilippis, V. R., and W. S. Moore. 2000. Resolution of phylogenetic relationships among recently evolved species as a function of amount of DNA sequence: an empirical study based on woodpeckers (Aves: Picidae). Mol. Phylogenet. Evol. 16:143–160.

Dizon, A. E., C. Lockyer, W. F. Perrin, D. P. Demaster, and J. Sison. 1992. Rethinking the stock concept-a phylogeographic approach. Conserv. Biol. 6:24–36.

Donoghue, M. J. 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. Bryologist 88:172–181.

Douglas, C. L. 1966. Amphibians and reptiles of Mesa Verde National Park, Colorado. Univ. Kansas Mus. Nat Hist. 15:711–744.

Douglas, M. E., W. L. Minckley, and B. D. DeMarais. 1999a. Did vicariance mold phenotypes of western North American fishes? Evidence from Gila River cyprinids. Evolution 53:238–246.

Douglas, M. R., P. C. Brunner, and L. Bernatchez. 1999b. Do assemblages of *Coregonus* (Teleostei: Salmoniformes) in the Central Alpine region of Europe represent species flocks? Mol. Ecol. 8:589–603.

DUVALL, D., M. B. KING, AND K. J. GUTZWILER. 1985. Behavioral ecology and ethology of the prairie rattlesnake. Natl. Geogr. Res. 1:80–111.

ENDLER, J. A. 1977. Geographic Variation, Speciation, and Clines. Monographs in Population Biology 10. Princeton University Press, Princeton, New Jersey.

FELSENSTEIN, J., AND H. KISHINO. 1993. Is there something wrong with the bootstrap on phylogenies? A reply to Hillis and Bull. Syst. Biol. 42:193–200.

FITCH, H. S. 1940. A biogeographical study of the *ordinoides* Artenkreis of garter snakes (genus *Thamnophis*). Univ. California Publ. Zool. 44:1–150.

FOOTE, R., AND J. A. MCMAHON. 1977. Electrophoretic studies of rattlesnakes (*Crotalus* and *Sistrurus*) venom: taxonomic implications. Comp. Biochem. Physiol. 57B:235–241.

FORSTNER, M. R. J., S. K. DAVIS, AND E. AREVALO. 1995. Support for the hypothesis of anguimorph ancestry for the suborder Serpentes from phylogenetic analysis of mitochondrial DNA sequences. Mol. Phylogenet. Evol. 4:93–102.

FOWLIE, J. A. 1965. Snakes of Arizona. Azul Quinta Press, Fallbrook, California.

FRANKHAM, R. 1995. Effective population size/adult population size ratios in wildlife: a review. Genet. Res. 66:95–107.

FROST, D. R., AND D. M. HILLIS. 1990. Species in concept and practice: herpetological applications. Herpetologica 46:87–104.

———, A. G. KLUGE, AND D. M. HILLIS. 1992. Species in contemporary herpetology: comments on phylogenetic inference and taxonomy. Herpetol. Rev. 23:46–54.

FU, J. 2000. Toward the phylogeny of the family Lacertidae-why 4708 base pairs of mtDNA sequences cannot draw the picture. Biol. J. Linn. Soc. 71:203–217

GLENN, J. L., AND R. STRAIGHT. 1977. The midget faded rattlesnake (*Crotalus viridis concolor*) venom: lethal toxicity and individual variability. Toxicon 15:129–133.

———, AND ———. 1982. The rattlesnakes and their venom yield and lethal toxicity. *In* A. T. Tu (Ed.), Rattlesnake Venoms. Marcel Dekker, New York and Basel.

———, AND ———. 1990. Venom characteristics as an indicator of hybridization between *Crotalus viridis viridis* and *Crotalus scutulatus* in New Mexico. Toxicon 28:857–862.

GLOYD, H. K. 1940. The Rattlesnakes, Genera *Sistrurus* and *Crotalus*. The Chicago Academy of Sciences, Chicago.

GOULD, S. J. 1980. Sociobiology and the theory of natural selection. Pp. 257–269 *In* G. W. Barlow and J. Silverberg (Eds.), Sociobiology: Beyond Nature/Nuture? Amer. Assoc. Adv. Sci., Selected Symposium No. 35. Westview, Colorado.

———, AND R. F. JOHNSTON. 1972. Geographic variation. Ann. Rev. Ecol. Syst. 3:457–498.

GRAHAM, T. B. 1991. Western rattlesnake ecology at Natural Bridges N. M., Utah. Park Sci. 11:10–11.

GRAUR, D., AND W.-H. LI. 2000. Fundamentals of Molecular Evolution, 2nd ed. Sinauer Associates, Sunderland, Massachusetts.

GRIFFITHS, C. S. 1997. Correlation of functional domains and rates of nucleotide substitution in cytochrome *b*. Mol. Phylogenet. Evol. 7:352–365.

GRISMER, L. L. 2001. An evolutionary classification and checklist of amphibians and reptiles on the Pacific islands of Baja California, Mexico. Bull. So. California Acad. Sci.100:12–23.

HAMMERSON, G. A. 1981. An ecographic Analysis of the Herpetofauna of Colorado. Unpublished Ph.D. dissertation, University of Colorado, Boulder.

———. 1999. Amphibians and Reptiles in Colorado. 2nd ed. University Press of Colorado, Niwot.

HIGGINS, R. W., Y. YAO, AND X. L. WANG. 1997. Influence of the North American monsoon system on the United States summer precipitation regime. J. Climatol. 10:2600–2622.

HILLIS, D. M., AND J. J. BULL. 1993. An empirical test of bootstrapping as a method of assessisng confidence in phylogenetic analysis. Syst. Biol. 42:182–192.

HOLBROOK, J. E. 1840. North American Herpetology (Philadelphia, Pennsylvania) 4:1–126.

HOLMAN, J. A. 2000. Fossil Snakes of North America: Origin, Evolution, Distribution, Paleoecology. Indiana University Press, Bloomington.

HOWARD, D. J., AND S. H. BERLOCHER (Eds.). 1998. Endless Forms: Species and Speciation. Oxford University Press, Oxford.

HUNT, C. B. 1967. Physiography of the United States. The Colorado Plateau. W. H. Freeman, San Francisco.

Huxley, J. S. 1938. Species formation and geographical isolation. Proc. Linn. Soc. Lond. 150:253–264.
———. 1939. Subspecies and varieties. Proc. Linn. Soc. Lond. 151:105–114.
———. 1942. Evolution: The Modern Synthesis. George Allen and Unwin Ltd., London.
Jett, S. C. 1972. Biogeographical notes on the Kanab Canyon system, Mohave and Coconino Counties, Arizona. Plateau 45:1–16.
Källersjõ, M., J. S. Farris, A. G. Kluge, and C. Bult. 1992. Skewness and permutations. Cladistics 8:275–287.
Kettlewell, H. B. D. 1973. The Evolution of Melanism. Clarendon Press, Oxford.
King, M. B., and Duvall, D. 1990. Prairie rattlesnake seasonal migrations: episodes of movement, vernal foraging, and sex differences. Anim. Behav. 39:924–935.
Klauber, L. M. 1930. New and renamed subspecies of *Crotalus confluentus* Say, with remarks on related species. Trans. San Diego Soc. Nat. Hist. 6:95–144.
———. 1935. A new subspecies of *Crotalus confluentus*, the prairie rattlesnake. Trans. San Diego Soc. Nat. Hist. 8:75–90.
———. 1936. Key to the rattlesnakes with summary of characteristics. Trans. San Diego Soc. Nat. Hist. 8:185–276.
———. 1943. A graphic method of showing relationships. Bull. Zool. Soc. San Diego 18:61–76.
———. 1949. Some new and revived subspecies of rattlesnakes. Trans. San Diego Soc. Nat. Hist. 11:61–116.
———. 1956. Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind. 2 Vols. University of California Press, Berkeley and Los Angeles
———. 1972. Rattlesnakes: their Habits, Life Histories, and Influence on Mankind. 2 Vols., 2nd ed. University of California Press, Berkeley and Los Angeles.
Klicka, J., and R. M. Zink. 1997. The importance of recent Ice Age in speciation: a failed paradigm. Science 277:1666–1669.
Knight, A., D. Styer, S. Pelikan, J. A. Campbell, L. D. Densmore III, and D. P. Mindell. 1993. Choosing among hypotheses of rattlesnake phylogeny: a best-fit rate test for DNA sequence data. Syst. Biol. 42:356–367.
Kocher, T. D., and K. L. Carleton. 1997. Base substitutions in fish mitochondrial DNA: patterns and rates. Pp. 13–24 *In* T. D. Kocher and C. A. Stepien (Eds.), Molecular Systematics of Fishes. Academic Press, New York.
Kottelat, M. 1997. European freshwater fishes. Biologia 52 (suppl.) 5:1–271.
Kraus, F., D. G. Mink, and W. M. Brown. 1996. Crotaline intergeneric relationships based on mitochondrial DNA sequence data. Copeia 1996:763–773.
Kumar, S. 1996. Patterns of nucleotide substitution in mitochondrial protein coding genes of vertebrates. Genetics 143:537–548.
Kumazawa, Y., H. Ota, M. Nishida, and T. Ozawa. 1998. The complete nucleotide sequence of a snake (*Dinodon semicarinatus*) mitochondrial genome with two identical control regions. Genetics 150:313–329.
Lamb, T., T. R. Jones, and P. J. Wettstein. 1997. Evolutionary genetics and phylogeography of tassel-eared squirrels (*Sciurus aberti*). J. Mammal. 78:117–133.
Lowe, C. H., C. R. Schwalbe, and T. B. Johnson. 1986. The Venomous Reptiles of Arizona. Arizona Game and Fish Department, Phoenix.
Macartney, J. M., P. T. Gregory, and K. W. Larsen. 1988. A tabular survey on data of movements and home ranges of snakes. J. Herpetol. 22:61–73.
Maddison, D. R., and W. P. Maddison. 2001. MacClade4: Analysis of Phylogeny and Character Evolution, ver. 4.03. Sinauer Associates, Sunderland, Massachusetts.
Mayden, R. L. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. Pp. 381–424 *In* M. F. Claridge, H. A. Dawah, and M. R. Wilson (Eds.), Species: The Units of Biodiversity. Chapman & Hall, London.
Mayr, E. 1942. Systematics and the Origin of Species From the Viewpoint of a Zoologist. Columbia University Press, New York.
———. 1982. The Growth of Biological Thought: Diversity, Evolution and Inheritance. Harvard University Press, Cambridge, Massachusetts.
Merriam, C. H. 1890. Results of a biological survey of the San Francisco Mountain region and desert of the Little Colorado in Arizona. North American Fauna 3:1–136.

MEEK, S. E. 1905. An annotated list of a collection of reptiles from Southern California and Northern Lower California. Field Mus. Publ. Zool. 7:3–19.

MEYER, A. 1994. Shortcomings of the cytochrome *b* gene as a molecular marker. Trends Ecol. Evol. 9:278–280.

MILLER, A. H. 1931. Systematic revision and natural history of the American shrikes (*Lanius*). Univ. California Publ. Zool. 38:11–242.

———. 1941. Speciation in the avian genus *Junco*. Univ. California Publ. Zool. 44:173–434.

MILLER, D. M., R. A. YOUNG, T. W. GATLIN, AND J. A. RICHARDSON. 1982. Amphibians and Reptiles of the Grand Canyon National Park. Grand Canyon Natural History Association Monograph 4. Classic Printers, Prescott, Arizona.

MINDELL, D. P., AND C. E. THACKER. 1996. Rates of molecular evolution: phylogenetic issues and applications. Ann. Rev. Ecol. Syst. 27:279–303.

MITHTHAPALA, S., J. SEIDENSTICKER, AND S. J. O'BRIEN. 1996. Phylogeographic subspecies recognition in leopards (*Panthera pardus*): molecular genetic variation. Conserv. Biol. 10:1115–1132.

MIYAMOTO, M. 1996. A congruence study of the molecular and morphological data for eutherian mammals. Mol. Phylogenet. Evol. 6:373–390.

MOCK, C. J. 1996. Climate controls and spatial variations of precipitation in the western United States. J. Climatol. 9:1111–1125.

MOORE, W. S. 1995. Inferring phylogenies from mtDNA variation: Mitochondrial gene trees versus nuclear gene trees. Evolution 51:627–629.

———, AND V. R. DEFILIPPIS. 1997. The window of taxonomic resolution for avian phylogenies based on mitochondrial cytochrome *b* DNA sequences. Pp. 83–119 *In* D. P. Mindell (Ed.), Avian Molecular Evolution and Systematics. Academic Press, San Diego.

MORITZ, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Mol. Ecol. 3:401–411.

———. 1995. Use of molecular phylogenies for conservation. Phil. Trans. R. Soc. London 349:113–118.

MURPHY, R. W., AND C. B. CRABTREE. 1988. Genetic identification of a natural hybrid rattlesnake: *Crotalus scutulatus scutulatus* x *C. viridis viridis*. Herpetologica 44:119–123.

NEI, M. 1996. Phylogenetic analysis in molecular evolutionary genetics. Ann. Rev. Ecol. Syst. 30:371–403

PARKER, P. G., A. A. SNOW, M. D. SCHUG, G. C. BOOTON, AND P. A. FUERST. 1998. What molecules can tell us about populations: choosing and using a molecular marker. Ecology 79:361–382.

PARKINSON, C. L. 1999. Molecular systematics and biogeographical history of pitvipers as determined by mitochondrial ribosomal DNA sequences. Copeia 1999:576–586.

———, K. M. ZAMUDIO, AND H. W. GREENE. 2000. Phylogeography of the pitviper clade *Agkistrodon*: historical ecology, species status, and conservation of cantils. Mol. Ecol. 9:411–420.

PERKINS, C. B. 1951. Hybrid rattlesnakes. Herpetologica 7:156

PIELOU, E. C. 1991. After the Ice Age: The Return of Life to Glaciated North America. The University of Chicago Press, Chicago.

POOK, C. E., W. WUSTER, AND R. S. THORPE. 2000. Historical biogeography of the western rattlesnake (Serpentes: Viperidae: *Crotalus viridis*), inferred from mitochondrial DNA sequence information. Mol. Phylogenet. Evol. 15:269–282.

POOL, W. R., AND A. L. BIEBER . 1981. Fractionation of midget-faded rattlesnake (*Crotalus viridis concolor*) venom: lethal fractions and enzymatic activities. Toxicon 19:517–527.

PORRAS. L. W. 2000. In a world of its own: the midget-faded rattlesnake (*Crotalus viridis concolor*) Reptiles 8(9):28–36.

POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818.

QUINN, H. R. 1987. Morphology, Isozymes, and Mitochondrial DNA as Systematic Indicators in *Crotalus*. Unpublished Ph.D. dissertation, University of Houston, University Park.

RAFINESQUE, C. S. 1818. Farther account of discoveries in natural history in the Western states. Amer. Monthly Mag. Crit. Rev. 4:39–42.

REED, R. N., AND M. E. DOUGLAS. 2002. Ecology of the Grand Canyon rattlesnake (*Crotalus viridis abyssus*) in the Little Colorado River Canyon, Arizona. Southwest. Nat. 47:30–39.

Reeder, T. W. 1995. Phylogenetic relationships among phrynosomatid lizards as inferred from mitochondrial ribosomal DNA sequences: substitional bias and information content of transitions relative to transversions. Mol. Phylogenet. Evol. 4:203–222.

Rensch, B. 1926. Rassenkreisstudien bei Mollusken I. Der Rassenkreis der Felsenschnecke *Campylaea zonata* Studer. Zoologischer Anzeiger, Band 67:253–263.

———. 1929. Das Prinzip Geographischer Rassenkreise und das Problem der Artbildung. Borntraeger Publishers, Berlin.

Rodrígues-Robles, J. A., and J. M. De Jesús-Escobar. 2000. Molecular systematics of New World gopher, bull, and pinesnakes (*Pituophis*: Colubridae), a transcontinental species complex. Mol. Phylogenet. Evol. 14:35–50.

———, D. F. DeNardo, and R. E. Staub. 1999. Phylogeography of the California mountain king snake, *Lampropeltis zonata* (Colubridae). Mol. Ecol. 8:1923–1934.

Rosen, D. E. 1978. Vicariant patterns and historical explanation in biogeography. Syst. Zool. 27:159–188.

———. 1979. Fishes from the uplands and intermontaine basins of Guatemala: revisionary studies and comparative geography. Bull. Amer. Mus. Nat. Hist. 162:267–376.

Rubio, M. 1998. Rattlesnake: Portrait of a Predator. Smithsonian Institution Press. Washington and London.

Ryder, O. A. 1986. Species conservation and systematics: the dilemma of subspecies. Trends Ecol. Evol. 1:9–10.

Sanderson, M. J. 1995. Objections to bootstrapping phylogenies: a critique. Syst. Biol. 44:299–320.

Schmidt, J. C., and J. B. Graf. 1990. Aggradation and degradation of alluvial sand deposits, 1965 to 1986, Colorado River, Grand Canyon National Park, Arizona. U.S. Geological Survey Professional Paper 1493:1–74.

Schneider, C. J., T. B. Smith, B. Larison, and C. Moritz. 1999. A test of alternative models of diversification in tropical rainforests: ecological gradients vs rainforest refugia. Proc. Nat. Acad. Sci. U.S.A. 96:13869–13873.

Schneider, G. E. 1986. Geographic variation in the contact zone of two subspecies of the Pacific rattlesnake, *Crotalus viridis oreganus* and *Crotalus viridis helleri.* Unpublished M.S. thesis, University of California, Santa Barbara.

Shaffer, H. B., J. M. Clark, and F. Kraus. 1991. When molecules and morphology clash: a phylogenetic analysis of the North American ambystomatid (Caudata: Ambystomatidae) salamanders. Syst. Zool. 40:284–303.

———, and M. L. McKnight. 1996. The polytypic species revisited: genetic differentiation and molecular phylogenetics of the tiger salamander *Ambystoma tigrinum* (Amphibia: Caudata) complex. Evolution 50:417–433.

Sites, J. W. Jr., and K. A. Crandall. 1997. Testing species boundaries in biodiversity studies. Conserv. Biol. 11:128–1297.

Smith, T. B., R. K. Wayne, D. J. Girman, and M. W. Bruford. 1997. A role for ecotones in generating rainforest biodiversity. Science 276:1855–1857.

Stebbins, R. C. 1985. A Field Guide to Western Reptiles and Amphibians, 2nd ed. Houghton Mifflin, Boston.

Steel, M., and D. Penny. 2000. Parsimony, likelihood, and the role of models in molecular phylogenetics. Mol. Biol. Evol. 17:839–850.

Swofford, D. L. 2002. PAUP* - Phylogenetic Analysis Using Parsimony (*And Other Methods). Beta Version 4.0b8. Sinauer Associates, Sunderland, Massachusetts.

———, G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogenetic inference. Pp. 407–514 *In* D. M. Hillis, C. Moritz, and B. K. Mable (Eds.), Molecular Systematics, 2nd ed. Sinauer Associates, Sunderland, Massachusetts.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10:512–526.

Templeton, A. R. 2001. Using phylogeographic analysis of gene trees to test species status and processes. Mol. Ecol. 10:779–791.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673–4680.

WALKER, D., AND J. C. AVISE. 1998. Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. Ann. Rev. Ecol. Syst. 29:23–58.

———, P. E. MOLER, K. A. BUHLMANN, AND J. C. AVISE. 1998 Phylogeographic uniformity in mitochondrial DNA of the snapping turtle (*Chelydra serpentina*). Anim. Conserv. 1998: 55–60.

WAPLES, R. S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. Marine Fish. Rev. 52:11–22.

WIENS, J. J., AND B. D. HOLLINGSWORTH. 2000. War of the iguanas: conflicting molecular and morphological phylogenies, and long branch attraction in iguanid lizards. Syst. Biol. 49:143–159.

WILSON, E. O., AND W. L. BROWN, JR. 1953. The subspecies concept and its taxonomic application. Syst. Zool. 2:97–111.

WOODBURY, A. M. 1929. A new rattlesnake from Utah. Bull. Univ. Utah 20:1–2.

———. 1958. Preliminary report on biological resources of the Glen Canyon Reservoir. University of Utah Anthropological Papers 31 (Glen Canyon Series No. 2).

———. 1961. Ecological studies of the flora and fauna of Navajo Reservoir Basin, Colorado and New Mexico. University of Utah Anthropological Papers 55 (Upper Colorado Series No. 5).

WRIGHT, A. H., AND WRIGHT, A. A. 1957. Handbook of Snakes of the United States and Canada. 2 Vols. Cornell University Press, Ithaca, New York.

WU, C.-I. 1991. Inferences of species phylogeny in relation to segregation of ancient polymorphisms. Genetics 127:429–435.

XIA, X. 1998. The rate heterogeneity of nonsynonymous substitutions in mammalian mitochondrial genes. Mol. Biol. Evol. 15:336–344.

YANG, Z. 1996. Among-site rate variation and its impact on phylogenetic analyses. Trends Ecol. Evol. 11:367–372.

———, AND B. RANNALA. 1997. Bayesian phylogenetic inference using DNA sequences: a Markov chain Monte Carlo method. Mol. Biol. Evol. 14:717–724.

YOUNG, R. A., AND D. M. MILLER. 1980. Notes on the natural history of the Grand Canyon rattlesnake, *Crotalus viridis abyssus* Klauber. Bull. Chicago Herpetol. Soc. 15:1–5.

———, ———, AND D. C. OCHSNER. 1980. The Grand Canyon rattlesnake (*Crotalus viridis abyssus*): comparison of venom profiles with other *viridis* subspecies. Comp. Biochem. Physiol. 66B:601–603.

ZAMUDIO, K. R., K. B. JONES, AND R. H. WARD. 1997. Molecular systematics of short-horned lizards: biogeography and taxonomy of a widespread species complex. Syst. Biol. 46:284–305.

ZINK, R. M., AND D. L. DITTMAN. 1993. Gene flow, refugia, and the evolution of geographic variation in the song sparrow (*Melospiza melodia*). Evolution 47:717–729.

APPENDIX I

Haplotype designation (= HP) for the 149 *Crotalus viridis* in this study, with state, county, and general location of capture. RM = river mile (Colorado River, Grand Canyon). NM = National Monument. GCNP = Grand Canyon National Park. NRA = National Recreation Area.

HP	Country/State	County	General Location of Capture
A1	USA: Arizona	Coconino	Nankoweap Canyon (RM 51.7N)
A1	USA: Arizona	Coconino	Kwagunt Creek (RM 56.1N)
A1	USA: Arizona	Coconino	Espejo Creek (RM 66.7S)
A1	USA: Arizona	Coconino	Above Tanner Camp (RM 67.7N)
A1	USA: Arizona	Coconino	Hermit Creek (RM 95S)
A1	USA: Arizona	Coconino	Hermit Creek (RM 95S)
A1	USA: Arizona	Coconino	Bass Camp (RM 108N)
A1	USA: Arizona	Coconino	Indian Gardens (Bright Angel Trail), GCNP
A1	USA: Arizona	Coconino	Cardenas Creek Swamp (RM 71S)

A1	USA: Arizona	Coconino	Lava-Chuar Creek (RM 65.5N)
A1	USA: Arizona	Coconino	Tanner Camp (RM 68.4N)
A1	USA: Arizona	Coconino	Little Colorado River confluence (RM 61.5S)
A1	USA: Arizona	Coconino	Little Colorado River confluence (RM 61.5S)
A1	USA: Arizona	Coconino	Little Colorado River confluence (RM 61.5S)
A1	USA: Arizona	Coconino	Little Colorado River confluence (RM 61.5S)
A1	USA: Arizona	Coconino	4 km above Little Colorado River confluence
A1	USA: Arizona	Coconino	3 km above Little Colorado River confluence
A1	USA: Arizona	Coconino	4 km above Little Colorado River confluence
A1	USA: Arizona	Coconino	3 km above Little Colorado River confluence
A1	USA: Arizona	Coconino	Kanab Creek (RM 143.5N)
A1	USA: Arizona	Coconino	Lava Canyon (RM 65.5N)
A1	USA: Utah	Kane	Hwy. 89, 3.6 km N of Arizona state line
A2	USA: Arizona	Coconino	Lake Powell, at Wahweap Marina
A2	USA: Arizona	Coconino	Carbon Creek (RM 64.1N)
A2	USA: Arizona	Coconino	Carbon Creek (RM 64.1N)
A2	USA: Arizona	Coconini	Little Colorado River confluence
A2	USA: Arizona	Coconino	Lake Powell at Wahweap Marina
A2	USA: Arizona	Coconino	Ferry Swale
A2	USA: Arizona	Coconino	Soap Creek (RM 11N)
C1	USA: Arizona	Gila	Mount Ord, Mazatzal Mountains
C1	USA: Arizona	Coconino	S of Flagstaff
C1	USA: Arizona	Coconino	S of Flagstaff
C1	USA: Arizona	Coconino	S of Flagstaff
C1	USA: Arizona	Coconino	S of Flagstaff
C1	USA: Arizona	Gila	Reynold's Creek, Sierra Anchas
C1	USA: Arizona	Gila	Turkey Creek, Sierra Anchas
C1	USA: Arizona	Greenlee	Honeymoon, near Eagle Creek
C1	USA: Arizona	Maricopa	Sycamore Creek
C1	USA: Arizona	Yavapai	S of Verde River
H1	USA: California	Los Angeles	Hacienda Heights
H1	USA: California	Santa Barbara	Sierra Madre Mountains
H1	USA: California	Ventura	Junction of Hwy. 33 and Lockwood Valley Road
H1	Mexico: Baja California Norte		Isla Sur, Islas de los Coronados
K1	USA: Colorado	Delta	Escalante Canyon
K1	USA: Colorado	Delta	Escalante Canyon
K1	USA: Colorado	Delta	Escalante Canyon
K1	USA: Colorado	Delta	19.2 km NW of Delta
K1	USA: Colorado	Delta	Escalante Canyon
K1	USA: Utah	Emery	Green River
K1	USA: Utah	Wayne	38.4 km NNE confluence of Green and Colorado rivers
K1	USA: Utah	Wayne	38.4 km NNE confluence of Green and Colorado rivers
K1	USA: Utah	Wayne	38.4 km NNE confluence of Green and Colorado rivers
K1	USA: Utah	Garfield	Hite Marina
K1	USA: Utah	Grand	18 km S of exit 173 on I-70
K1	USA: Utah	San Juan	27.8 km E of Halls Crossing Marina
K1	USA: Utah	Uintah	Arcadia
K1	USA: Utah	Wayne	Route 95, 22.2 km SSE of Hanksville
K1	USA: Wyoming	Sweetwater	6.2 km S Hwy. 191, E of Flaming Gorge NRA
K1	USA: Wyoming	Sweetwater	6.2 km S Hwy. 191, E of Flaming Gorge NRA

K1	USA: Wyoming	Sweetwater	Hwy. 530, W of Flaming Gorge NRA
K1	USA: Wyoming	Sweetwater	Hwy. 530, W of Flaming Gorge NRA
K1	USA: Wyoming	Sweetwater	Sage Creek, E of Flaming Gorge NRA
K1	USA: Wyoming	Sweetwater	Sage Creek, E of Flaming Gorge NRA
K1	USA: Wyoming	Sweetwater	Sage Creek, E of Flaming Gorge NRA
K2	USA: Colorado	Mesa	DeBeque cut-off
K2	USA: Colorado	Mesa	DeBeque cut-off
L1	USA: Arizona	Coconino	Stone Creek (RM 132N)
L1	USA: Arizona	Coconino	Below Deer Creek (RM 136N)
L1	USA: Arizona	Coconino	Tuckup Canyon (RM 164.5N)
L1	USA: Arizona	Coconino	Jacob's Lake
L1	USA: Arizona	Coconino	Mile marker 302, E Fredonia on Hwy 89A
L1	USA: Arizona	Coconino	Kanab Creek (RM 143.5N)
L1	USA: Arizona	Coconino	Kanab Creek, 0.8 km above confluence (RM 143.5N)
L1	USA: Arizona	Coconino	2 km above confluence of Kanab Creek
L1	USA: Arizona	Coconino	Kanab Creek, 3.2 km S of junction Jump-up Canyon
L1	USA: Arizona	Mohave	6.4 km W of Fredonia on Hwy. 309
L1	USA: Utah	Iron	18 km W of Parowan
L1	USA: Utah	Iron	32 km SSE of Cedar City
L1	USA: Utah	Utah	Lake Mountains
L2	USA: Arizona	Coconino	South Canyon (RM 31.6N)
L2	USA: Utah	Kane	10 km S of Cannonville
L3	USA: Arizona	Coconino	Randy's Rock (RM 126.3 N)
L3	USA: Nevada	Nye	25.9 km E of Hwy. 376
L3	USA: Nevada	Nye	25.9 km E of Hwy. 376
L3	USA: Nevada	Nye	25.9 km E of Hwy. 376
L3	USA: Nevada	Nye	25.9 km E of Hwy. 376
L3	USA: Nevada	White Pine	16 km SSE of Lund
L3	USA: Utah	Washington	1.6 km S of Gunlock Reservoir
N1	USA: Arizona	Coconino	Meteor Crater Road
N1	USA: Arizona	Coconino	Meteor Crater Road
N1	USA: Arizona	Coconino	Tonalea
N1	USA: Arizona	Coconino	Tonalea
N1	USA: Arizona	Coconino	22 km E of junction Hwy. 89 and FR 545
N1	USA: Arizona	Coconino	22 km E of junction Hwy. 89 and FR 545
N1	USA: Arizona	Coconino	22 km E of junction Hwy. 89 and FR 545
N1	USA: Arizona	Coconino	22 km E of junction Hwy. 89 and FR 545
N1	USA: Arizona	Coconino	22 km E of junction Hwy. 89 and FR 545
N1	USA: Arizona	Coconino	Wupatki NM
N1	USA: Arizona	Coconino	S of Wupatki NM
N1	USA: Arizona	Coconino	20 km E of junction Hwy. 89 and FR 545
N1	USA: Arizona	Coconino	Wupatki NM
N1	USA: Arizona	Coconino	Wupatki NM
N1	USA: Arizona	Coconino	22 km E of junction Hwy. 89 and FR 545
N1	USA: Arizona	Coconino	S of Wupatki NM
N1	USA: Arizona	Coconino	Buffalo Ranch Road
N1	USA: Arizona	Coconino	Buffalo Ranch Road
N1	USA: Arizona	Coconino	Meteor Crater Road
N1	USA: Arizona	Coconino	Meteor Crater Road
N1	USA: Arizona	Coconino	Meteor Crater Road

N1	USA: Arizona	Coconino	Meteor Crater Road
N1	USA: Arizona	Coconino	Meteor Crater Road
O1	USA: California	Fresno	Foothills NE of Fresno
O1	USA: California	Kings	Hwy. 198, near Hanford
O1	USA: California	Santa Cruz	Loch Lomond Reservoir
O1	USA: California	Tulare	Southfork Kaweah River Drainage
O1	USA: California	Tulare	4.8 km WSW of Earlimart
O2	USA: Washington	Grant	3.2 km S of George
V1	USA: Arizona	Cochise	Portal Road, 0.4 km W of New Mexico state line
V1	USA: Colorado	El Paso	Colorado Springs
V1	USA: Colorado	Moffat	34.4 km N of Craig
V1	USA: Colorado	Moffat	34.4 km N of Craig
V1	USA: Colorado	Moffat	34.4 km N of Craig
V1	USA: Colorado	Moffat	34.4 km N of Craig
V1	USA: Colorado	Moffat	34.4 km N of Craig
V1	USA: Colorado	Moffat	34.4 km N of Craig
V1	USA: New Mexico	Eddy	Carlsbad
V1	USA: New Mexico	Eddy	Carlsbad
V1	USA: New Mexico	Eddy	Carlsbad
V1	USA: New Mexico	Eddy	Roswell
V1	USA: Wyoming	Carbon	Sinclair
V2	USA: New Mexico	Grant	19.2 km N of Hachita
V2	USA: New Mexico	Valencia	NM 47, 20.8 km NW of US 60
V2	USA: New Mexico	Luna	Florida Mountains, SSE of Deming
V3	USA: New Mexico	Hidalgo	No specific location
V3	USA: New Mexico	Socorro	Hwy. 60, 10.1 km W of FR 235
V3	USA: New Mexico	Valencia	27.4 km NW of junction US 60 and NM 47
V3	USA: New Mexico	Valencia	I-25, 46.1 km S of I-40
V4	USA: Colorado	Montezuma	Hwy. 41 0.65 km N of mile marker 3
V4	USA: Colorado	Montrose	Hwy. 90 near Paradox
V4	USA: Colorado	San Miguel	Hwy. 141, 61.4 km S of Montrose County line.
V4	USA: Colorado	San Miguel	Hwy. 141, 38.4 km S of Montrose County line
V4	USA: New Mexico	San Juan	Waterflow, E of Shiprock on Route 64
V4	USA: Utah	San Juan	Intersection of Hwy. 191 and road to Lisbon Valley
V4	USA: Utah	San Juan	Intersection of Hwy. 191 and Route 211
V4	USA: Utah	San Juan	Casa Colorado Rock
V4	USA: Utah	San Juan	40 km S of Moab
V4	USA: Utah	San Juan	40 km S of Moab
V4	USA: Utah	San Juan	40 km S of Moab
V4	USA: Utah	San Juan	Natural Bridges NM