PHYLOGEOGRAPHY OF FOUR SPECIES OF BOREAL-ADAPTED RODENTS IN THE CENTRAL ROCKY MOUNTAIN REGION

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CHAPTER 1

INTRODUCTION: INTRASPECIFIC PHYLOGEOGRAPHY, MOLECULAR MARKERS, AND BOREAL-ADAPTED MAMMALS

The study of intraspecific phylogeography examines principles and processes that govern the distribution of gene genealogies across geographic landscapes (Avise et al. 1987; Avise 1994, 2000). Introduced by John Avise and colleagues in the mid-1980s (Avise et al. 1987), intraspecific phylogeography remains an active field of study, combining a diverse array of disciplines, including ecology, molecular genetics, biogeography, population genetics, phylogenetic systematics, and historic geography. The concept was introduced to bring together principles of population genetics and phylogenetic systematics. Population geneticists address microevolutionary changes within and among populations but seldom use terms and concepts relating to systematics when interpreting data. Likewise, traditional systematists describe variation in behavior or morphologic and phenotypic characters, but do not identify the genetic basis for variation that is detected. Based on the hundreds of papers on intraspecific phylogeography published in peer-reviewed journals during the past 14 years, it is obvious that Avise et al. (1987) were successful in stimulating researchers to bridge the gap

between traditional systematics and population genetics (Avise 1998).

One of the most significant contributions to studies of ecology and evolution made possible by the use of molecular techniques is the assessment of population structure over broad geographic areas (Avise 1994, 2000). Such studies can reveal aspects of population history and processes not readily apparent from direct ecological studies. There are several advantages for using DNA when addressing population genetic and phylogeographic questions. Most molecular data have a genetic basis, whereas phenotypic data may be influenced by intrinsic and extrinsic factors, thereby masking true phylogenetic relationships among individuals, populations, or species (Airoldi and Hoffman 1984; Anderson 1959; Moritz and Hillis 1996). In addition, depending on the type of questions being addressed, specific sequences or segments of DNA can be selected *a priori* based on knowledge of evolutionary rates and mode of inheritance (Dowling et al. 1996; Moritz and Hillis 1996). For example, when addressing questions at the intraspecific level, it is essential to include a molecular marker that evolves at a rapid rate to identify population substructure. Conversely, if questions are posed at higher level categories (i.e., species, genera, families, etc.) a marker that evolves at a

much slower rate is more appropriate than a rapidly evolving molecular marker.

Analysis of mitochondrial DNA (mtDNA) in phylogeographic and population genetic studies has proven invaluable because it is maternally inherited, does not undergo genetic recombination during sexual reproduction, has a rapid mutation rate when compared to nuclear DNA, and has varying mutation rates among different regions of the mtDNA genome (Avise 1989, 1995; Mitton 1994; Moritz 1994; Rand 1994). The mitochondrial genome is composed of the same set of 37 homologous genes among all taxa examined, including 22 tRNA genes, 2 rDNA genes, 13 peptide coding genes, and a control region (Lewin 2000).

The control region is the only non-coding segment of the animal mtDNA genome. In mammals it is located between the conserved phenylalanine- and proline-tRNA genes and is approximately 0.8 to 2.0 kilobases long (Bibb et al. 1981; Brown 1985; Gadaleta et al. 1989). Base substitution rates and accumulation of duplications, deletions, and insertions in the control region are 5 to 10 times more rapid than estimated rates for most single-copy nuclear genes (Brown 1985; Brown et al. 1993; Brown and Simpson 1982; Viliglant et al. 1989), making it the most variable segment of the mtDNA genome (Cann et al. 1984; Saccone et al. 1991). Segments within the peripheral (i.e., right and left)

domains of the control region have been shown to evolve 4 to 5 times more rapidly than the more conserved central domain (Lopez et al. 1997; Saccone et al. 1991; Wilkinson and Chapman 1991). Because of the elevated rates of molecular evolution, DNA sequence data from the variable portions of the non-coding control region have provided good resolution for identifying population substructure in most vertebrates thus far examined (Avise 2000).

The ND5 and ND6 subunits of NADH (reduced form of nictinamide adenine dinucleotide) dehydrogenase complex are located between the glutamic acid- and leucine-tRNA regions. This fragment, which is approximately 2.4 kilobases long, acts as a coenzyme in electron-transfer reactions involved with cellular respiration. Analysis of mtDNA restriction-site data for NADH-dehydrogenase segments is an effective marker because it evolves at a rate appropriate to detect population substructure and has been useful in reconstructing population histories in African elephants (*Loxodonta africana*---Georgiadis 1996), Atlantic walrus (*Odobenus rosmarus*---Andersen et al. 1998) and a number of non-mammalian organisms, including *Daphnia* (Weider and Hobaek 1994), chum salmon (*Oncorhynchus keta*---Parker et al. 1993), Arctic charr (*Salvelinus alpinus*---Brunner et al. 1998), and rainbow smelt (*Osmerus mordax*---Bernatchez 1997).

Most previous studies of intraspecific phylogeography that included data derived from DNA have relied on construction of a phylogenetic tree using methods developed for comparisons of higher taxonomic levels (e.g., Arbogast et al. 2001; Avise et al. 1987; Avise 1994). As a result of a limited amount of divergence between individuals or populations, phylogenetic trees often provide poor resolution due to short branch lengths. In addition, it is often difficult to identify an appropriate individual, population, or species to root an intraspecific phylogenetic tree (Crandall et al. 1994). To overcome these problems, Templeton et al. (1987) introduced a cladistic approach for constructing a genealogy of haplotypes to test for nonrandom associations between genealogy of haplotypes and geography that are due to contemporary (i.e., restricted gene flow via isolation by distance), historic (fragmentation, range expansion, or colonization), or a combination of both factors.

Contemporary distributions of taxa are governed by abiotic and biotic factors. As such, the discontinuous distribution of cool, mesic habitats (i.e., boreal) in the central Rocky Mountain region provides an excellent opportunity to investigate several questions relating to historic and contemporary aspects of intraspecific phylogeography. For example, have contemporary

distributions of boreal habitats and geologic history of the central Rocky Mountain region influenced population genetic and phylogeographic structure of boreal-adapted mammals? Do species with more specialized requirements (i.e., physiologic constraints, habitat, and diet) exhibit more pronounced spatial genetic structure than species with less specialized requirements? Do codistributed species exhibit similar geographic substructuring due to similar historic, geologic, and environmental components at the community level? To address these questions it would be ideal to select a suite of species that are sympatric over a broad geographic area.

Several small, non-volant, boreal-adapted mammals are discontinuously distributed in the central Rocky Mountain region (Armstrong 1972; Long 1965; Turner 1974). It has been hypothesized that inhospitable habitat in the form of shortgrass prairie, intermontane basins, and arid river valleys apparently serves as an ecologic obstacle to gene flow as a result of alterations of habitat during and following the Pleistocene. For example, Findley and Anderson (1956) and Kirkland (1981) hypothesized that the Green River Canyon and Wyoming Basin, which formed at the end of the Pleistocene, serve as barriers or filters to gene flow for several boreal-adapted mammals in Colorado and western Wyoming, eastern Idaho, and eastern Utah. The

historic break up and contemporary distribution of the boreal habitats in eastern Wyoming have been hypothesized as a plausible explanation for the patterns of observed morphologic and phenotypic characters in species of boreal-adapted mammals in the Black Hills of South Dakota and Wyoming and neighboring mountains to the west (Long 1965; Turner 1974). In addition, formation of montane glaciers in central Colorado has been hypothesized to have contributed to deep phylogeographic breaks as a result of populations residing in northern and southern refugia for extended periods of time during the Pleistocene (Armstrong 1977; Arbogast et al. 2001). The yellow-bellied marmot (*Marmota flaviventris*), red squirrel (*Tamiasciurus hudsonicus*), red-backed vole (*Clethrionomys gapperi*), and montane vole (*Microtus montanus*) were selected as study organisms because each species exhibits varying degrees of tolerance to biotic and abiotic factors which may govern their ability to disperse (i.e., vagility) across the heterogeneous landscape of the central Rocky Mountain region. With the exception of *M. montanus*, which does not occur in the Black Hills in South Dakota and Wyoming, all 4 species are sympatric throughout the region.

Marmota flaviventris is a semi-fossorial species that occurs in alpine tundra and subalpine and montane meadows. This species can be found in a wide range of altitudes in

canyons and foothills where succulent vegetation and rocky outcrops are found (Kirkland 1981; Skaggs and Boecklen 1996). *Tamiasciurus hudsonicus* occurs in subalpine and montane forests and is thought to have coevolved with coniferous forests (Benkman 1999; Smith 1970). *Clethrionomys gapperi* inhabits mesic forests in montane and subalpine woodlots where mature forests of spruce, pine, and aspen produce abundant seed crops and surface litter (Kirkland 1977; Merritt and Merritt 1978; Nordyke and Buskirk 1991; Wywialowski and Smith 1988). Physiologically, this species requires more water than other small mammals of comparable size (Getz 1968; McManus 1974) and, consequently, is not common in more xeric environments. *Microtus montanus* occurs in alpine meadows where lush grasses and abundant forbs provide sufficient cover, although some individuals have been collected in more xeric environments, including dry grasslands with forbs and sagebrush (Armstrong 1977). Because of natural history attributes, I hypothesize that *T. hudsonicus* should exhibit the greatest amount of genetic structure, *C. gapperi* and *M. flaviventris* should exhibit an intermediate amount of genetic structure, and *M. montanus* should exhibit the least amount of genetic structure across the heterogeneous landscape of the central Rocky Mountain region.

The following chapters incorporate concepts and theory of phylogeography with the goal of better understanding historic and contemporary processes that explain the patterns of genetic variability for boreal-adapted mammals in the central Rocky Mountain region. Incorporation of molecular methods will allow me to perform rigorous tests of earlier hypotheses regarding how historic, geologic, and ecological factors (Arbogast et al. 2001; Findley and Anderson 1956; Kirkland 1981; Long 1965; Turner 1974) have contributed to the contemporary relationships of populations for several species in the central Rocky Mountain region. Chapter 2 focuses on intraspecific phylogeography of *T. hudsonicus*. It has been hypothesized that climatic oscillations, such as those that occurred during and following the Pleistocene, may have greatly influenced population genetic and phylogeographic structure of *T. hudsonicus* in the central Rocky Mountain region (Arbogast et al. 2001). Because *T. hudsonicus* may have coevolved with coniferous habitats (Benkman 1999; Smith 1970) and is not known to undertake long-distance excursions (>600 m; Berteaux and Boutin 2000; Larsen and Boutin 1994), it should be a good model to test hypotheses regarding effects of restriction of contemporary gene flow on genetic structure over a heterogeneous landscape. Chapter 3 uses a comparative phylogeographic approach to assess whether

multiple, codistributed species share a common history, or alternatively, if species reacted in an individualistic manner to climatic oscillations during and following the Pleistocene. Chapters 2 and 3 are written in the style required by the *Journal of Mammalogy*, to which they will be submitted for publication.

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INTRASPECIFIC PHYLOGEOGRAPHY OF RED SQUIRRELS (*TAMIASCIURUS HUDSONICUS*) IN THE CENTRAL ROCKY MOUNTAIN REGION

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We used variation in a portion of the mitochondrial DNA control region to examine the phylogeography of *Tamiasciurus hudsonicus*, a boreal-adapted small mammal in the central Rocky Mountain region. AMOVA revealed that 65.66% of the genetic diversity was attributable to variation within populations, 16.93% to variation among populations on different mountain ranges, and 17.41% to variation among populations within mountain ranges. Nested clade analysis revealed two major clades that likely diverged in allopatry during the Pleistocene: a southern clade from southern Colorado and a northern clade from northern Colorado,

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Wyoming, eastern Utah, and eastern Idaho. Historically restricted gene flow as a result of geographic barriers was indicated between populations on opposite sides of the Green River Canyon and Wyoming Basin and among populations in eastern Wyoming. In some instances genetic structure indicated isolation by distance. Results were generally discordant with current subspecific designations of *T. hudsonicus* in this region.
Key words: intraspecific phylogeography, Pleistocene, red squirrel, Rocky Mountains, *Tamiasciurus hudsonicus*

Recent reviews address how phylogeographic patterns of flora and fauna relate to historical and contemporary environmental changes, such as those that occurred during and following the Pleistocene (Avise and Walker 1998, 1999; Comes and Kadereit 1998; Hewitt 1996, 1999; Taberlet et al. 1998). Although continental glaciers were absent in the Rocky Mountain region in the conterminous United States, the region was influenced by repeated advances and retreats of cordilleran, or alpine glaciers (Armstrong 1972; Hafner and Sullivan 1995; Knight 1994; Long 1965). Advancing alpine glaciers forced vegetational zones of boreal habitat to ice-free areas at lower elevations (Beiswenger 1991; Martin et al. 1979; Patterson 1984; Porter et al. 1983; Van Devender and Spaulding 1979; Whitlock 1993). As the climate warmed

during interglacial episodes, boreal-adapted organisms recolonized areas previously glaciated. As a result, distribution of organisms with boreal affinities fluctuated widely during climatic oscillations of the Pleistocene which resulted in genetic divergence at the intraspecific level (Armstrong 1972; Long 1965; Turner 1974).

The red squirrel, *Tamiasciurus hudsonicus*, is one of the most common and widespread arboreal species inhabiting subalpine and montane forests in North America. The distribution of *T. hudsonicus*, which is more fragmented than depicted on most range maps (Steele 1998), closely resembles the fragmented distribution of boreal communities throughout the Rocky Mountain region. Boreal habitat in mountainous regions of western United States is separated by more xeric plant communities comprised of short and mixed-grass prairie and shrublands in intermontane basins (Beauvais 2000; Knight 1994). Except for riparian habitats, these intermontane basins probably serve as ecological obstacles to dispersal of *T. hudsonicus* between patches of boreal habitat.

Effects of Pleistocene climatic oscillations are evident in patterns of geographic variation in *T. hudsonicus*. Based on morphology, Findley and Anderson (1956), Kelson (1951), and Kirkland (1981) recognized separate subspecies of *T. hudsonicus* on the 2 sides of the Wyoming Basin and Green River Canyon. They concluded that

the Wyoming Basin and the late Pleistocene formation of the Green River Canyon resulted in extrinsic barriers or filters to gene flow in *T. hudsonicus*. Turner (1974), also relying on morphological variation, concluded that *T. hudsonicus* from the Black Hills in South Dakota and Wyoming is a relict of the Wisconsin glaciation. A recent study of mitochondrial DNA (mtDNA) variation in *T. hudsonicus* revealed a pronounced phylogeographic discontinuity that suggested dispersal from northern and southern Pleistocene refugia in Colorado (Arbogast et al. 2001).

Analysis of mitochondrial DNA (mtDNA) in phylogeographic and population genetic studies has proven invaluable because characteristics of this genome provide meaningful resolution at the intraspecific level (Eizirk et al. 2001; Wilson et al. 2000). Some of the most significant contributions that molecular techniques have made to studies of ecology and evolution are the assessment, over broad geographic areas, of population structure at the intraspecific level (Avise 2000). However, previous studies of intraspecific phylogeography typically have constructed phylogenetic trees with methods developed for comparison of higher-level taxa (e.g., Arbogast et al. 2001; Avise et al. 1987; Avise 1994). These trees often provide poor resolution due to short branch lengths that result from a limited amount of divergence between individuals or

populations. Furthermore, it is often difficult to identify an appropriate individual, population, or species to root an intraspecific phylogenetic tree (Crandall et al. 1994).

An alternative to traditional phylogeny reconstruction is nested clade analysis (Templeton et al. 1987). This analytical method builds a haplotype genealogy by using a cladistic approach and can be used to infer (with contingency analysis) explanations for proposed associations between haplotype genealogy and geography. This method tests for nonrandom associations between haplotype genealogy and geography that are due to contemporary (i.e., restricted gene flow via isolation by distance) or historical factors (fragmentation, range expansion, or colonization) or a combination of both factors (Templeton et al. 1987). Nested clade analysis is superior to techniques implemented in traditional phylogeographic studies because it overcomes the problems of incorporating inappropriate methods and simply overlaying haplotype networks or phylogenetic trees on geography to subjectively infer historical and contemporary gene flow (Avise 1994; Avise et al. 1987; Templeton et al. 1995; Templeton 1998).

Our goal was to use mitochondrial DNA variation to examine effects of contemporary and historical climatic factors on genetic structure of *T. hudsonicus* over a heterogeneous landscape in the central Rocky Mountain

region. We hypothesized that present-day xeric plant communities act as ecological obstacles to gene flow and that climatic oscillations during and following the Pleistocene resulted in deep phylogenetic breaks among populations of *T. hudsonicus* in eastern Wyoming (i.e., Black Hills), populations on either side of the Green River Canyon and Wyoming Basin, and among populations in central and southern Colorado. In addition, we wanted to incorporate molecular data to test the subspecies taxonomy of *T. hudsonicus*.

Materials and Methods

Specimens (n = 153) were collected from 17 mountain ranges throughout the central portion of the Rocky Mountains (Fig. 1; Table 1). Voucher specimens (skin, skull, or skeleton) are housed in the Sternberg Museum of Natural History (MHP), Fort Hays State University. Whole genomic DNA was extracted from spleen or muscle tissue following the protocol of Longmire et al. (1997). Following extraction, DNA was stored in 1X TE at 4°C. Tissues or extracted DNA are housed in the Oklahoma State University Collection of Vertebrates (OSU). The entire control region of 5 *T. hudsonicus* from widely separated collecting localities was amplified via the polymerase chain reaction (PCR) using primers for the non-coding control region (H00651 and L15926; Kocher et al. 1989). Amplification reactions were

performed in 50 μl volumes. Reaction mixes consisted of 50-200 ng DNA, 5 μl of 10X buffer, 1 mM of each dNTP, 0.5 mM of each primer, 2.5 mM $MgCl_2$, and 1.25 units of *Taq* DNA polymerase (Promega, Madison, Wisconsin). PCR cycles were as follows: 94°C for 3 min, followed by 35 cycles of 94°C for 45 sec, 54°C for 30 sec, and 72°C for 1.0 min with a final elongation period of 72°C for 30 min. PCR products (i.e., 5 μl) were electrophoresed through a 1% agarose gel, stained with ethidium bromide, and visualized via ultraviolet light. Successful amplicons were purified using the Wizard PCR Prep DNA Purification System (Promega, Madison, Wisconsin). Primer H00651 (Kocher et al. 1989) was used to sequence a portion of the right domain of the control region via cycle sequencing according to the manufacturer's instructions (BigDye™; Perkin Elmer, Foster City, California). Cycling conditions were as follows: 25 cycles at 96°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min. Sequence product was electrophoresed on an Applied Biosystems Inc. (Foster City, California) 377 automated DNA sequencer.

After sequences were aligned using the Clustal W option of the MacVector v6.5 computer program (Oxford Molecular 1998), internal primers (OSU 5020L, 5'-CCTTTAGCTGGCATAGGTA-3'; OSU 5021H, 5'-CATTATATGGAGTGGAGAAGG-3') were developed within conserved sequences of the right domain of the

control region. Single-stranded conformation polymorphism (SSCP) analysis (Dean and Gerrard 1991; Orita 1989; Sunnucks et al. 2000) was used to identify mtDNA haplotypes of all individuals. Amplification reactions for SSCP were performed in 10 μl volumes. Reaction mixes consisted of 50-200 ng DNA, 1 μl of 10X buffer, 0.5 mM of each primer, 2.5 mM $MgCl_2$, 1 μCi α^{32}P-dCTP, and 1.0 units of *Taq* DNA polymerase (Promega). PCR cycles were as follows: 94°C for 3 min; 35 cycles of 94°C for 45 sec, 54°C for 30 sec, and 72°C for 1.0 min; followed by 72°C for 30 min. Amplified PCR products were electrophoresed through a non-denaturing 6% acrylamide gel (69:1 acrylamide:bis-acrylamide). SSCP gels were run at room temperature for 24 h at 300 Volts, 3 Watts, and 10 mAMPS. Each individual was assigned a haplotype based on banding patterns visualized via autoradiography. A representative of each unique haplotype was included on all subsequent gels. A minimum of 3 individuals of each haplotype from separate populations were sequenced to verify that individuals with the same apparent haplotype had the same DNA sequence (Friesen et al. 1997).

Genetic divergence among haplotypes was estimated using the distance method of Tamura and Nei (1993) in the computer program MEGA v1.01 (Kumar et al. 1993). The computer program Puzzle v4.0.2 (Strimmer and von Haeseler 1996) was used to estimate the gamma correction (α = 0.22) for using

the Tamura and Nei (1993) model of nucleotide evolution. Nucleotide diversity (π) and haplotype diversity (h) were calculated using version 2.0 of the computer program Arlequin (Schneider et al. 1997). The AMOVA procedure (Excoffier et al. 1992) implemented in Arlequin was used to analyze nucleotide diversity (Φ-statistics, an analogue to Wright's F_{st}) at different hierarchical levels (i.e., among mountain ranges, among populations within each mountain range, and within populations of each mountain range). Significance levels associated with Φ_{st} values were calculated using 1000 permutations in Arlequin. The effective number of dispersing females (Takahata and Palumbi 1985) was estimated from the approximation $N_f m = ((1/\Phi_{st}) - 1)/2$.

The program TCS (Version 1.06; Clement et al. 2000) was used to generate an unrooted haplotype cladogram (i.e., haplotype genealogy) following the algorithm of Templeton et al. (1992). The network then was nested by hand with haplotypes nested into 1-step clades, 1-step clades nested into 2-step clades, and so on, following the procedure outlined by Templeton et al. (1987). The program GeoDis (Posada et al. 1999) and a key provided by the authors (http://bioag.byu.edu/zoology/crandall_lab/geodis.htm) was used to make inferences regarding historical and

contemporary processes. Interpretations of the significance of clade distance, $D_c(X)$ [average distance of clade *X* from the geographical center of that clade], nested clade distance, $D_n(X)$ [average distance of clade *X* individuals from the geographical center of the higher level clade in which clade *X* is nested], and average distance between tip (clades that are not interior nodes in the haplotype tree) and interior clades within the nested group, $(I\text{-}T)_c$, and tip to interior distance for the nested clade, $(I\text{-}T)_n$, were used to infer processes that gave rise to observed geographic patterns of mtDNA haplotypes by use of the procedures of Templeton et al. (1995).

Results

Sequences generated in this study have been deposited in GenBank (accession numbers XXXXX-XXXXX). Alignment of 273-275 base pairs of control region sequences resulted in 36 variable sites (28 transitions, 6 transversions, and 2 insertion/deletion events), defining 35 haplotypes in a sample of 153 *T. hudsonicus* (Fig. 2). Only 2 nucleotide positions (31 and 225) had more than 2 nucleotides detected. The largest number of substitutions (14) was observed between haplotype A from the Black Hills and haplotypes N and R, both from the Sangre de Cristo Mountains in southern

Colorado. Percent sequence difference among haplotypes ranged from 0.00% to 10.62%, with a mean of 3.20%.

The number of haplotypes found within mountain ranges varied from 1 to 7, with the majority of mountain ranges possessing 3 or 4 haplotypes (Table 1). All mountain ranges shared at least 1 haplotype with at least 1 other mountain range. Haplotype variation formed 3 geographic groups: 1) southern Colorado, 2) eastern and central Wyoming and northern Colorado, and 3) western Wyoming, eastern Utah, and eastern Idaho (Table 1). Only 2 haplotypes (A and D) occurred on both sides of the Green River Canyon and Wyoming Basin. The most frequent haplotype (A) was found in 44 individuals, most of which were from 7 mountain ranges east of the Green River Canyon and Wyoming Basin. The 2nd most common haplotype (B) was found in individuals from mountain ranges west of the Green River Canyon and Wyoming Basin. Except for a single individual from the Rampart Range, the 3rd and 4th most common haplotypes (C and D, respectively) occurred only in southern Colorado (Sangre de Cristo, Wet, and Culebra mountains). Haplotype F, the 5th most common haplotype, occurred in the Bighorn Mountains in north central Wyoming, the Wind River and Gros Ventre mountains in northwestern Wyoming, and the Bear River Mountains in eastern Idaho. Sixteen of the 35 haplotypes (46%) were restricted to single individuals.

Excluding the Wasatch and Centennial mountains, which were each represented by a single individual, within-population haplotype diversity (h) was generally high (0.00 to 0.929, mean 0.646); however, nucleotide diversity (π) was low (0.000 to 0.022, mean 0.007), indicating the presence of many closely related haplotypes. Geographic distribution of π values (Table 1) showed relatively high values in formerly glaciated localities in northwest Wyoming (Gros Ventre, Wind River, and Beartooth mountains), eastern Idaho (Bear River and Caribou mountains), north central Wyoming (Bighorn Mountains), and central Colorado (Rampart Mountains).

Hierarchical analysis of genetic variation revealed that 65.66% of genetic variability was attributable to variation among individuals within populations, 17.41% to differences among populations within mountain ranges, and 16.93% was attributed to differences among populations on different mountain ranges. Φ_{st} was 0.34 indicating significant population substructure (P = 0.001) and several pair-wise comparisons of Φ_{st} values among mountain ranges also were significant ($P < 0.05$; Table 2). Except for the Centennial and Bear River mountains, the southern Colorado localities (Culebra, Sangre de Cristo, and Wet mountains) were significantly different with the sequential Bonferroni correction from all other mountain ranges and showed low

levels of divergence when compared amongst themselves. The Black Hills and Laramie Mountains were significantly divergent from several localities (9 of 17 and 11 of 17 comparisons, respectively), especially mountain ranges in western Wyoming, eastern Idaho, eastern Utah, and southern Colorado.

Nested clade analysis revealed 2 highly divergent clades (Fig. 3). Haplotypes separated by 7 or fewer mutational events (base pair differences) could be connected with 95% confidence. As a result, clades 3-1 and 3-2 could not be connected with 95% confidence. Haplotypes from clade 2-2 occurred primarily in mountain ranges east of the Green River and Wyoming Basin in northern Colorado and eastern Wyoming. Haplotypes from clades 2-3, 2-4, and 2-5 were found in mountain ranges throughout western Wyoming, eastern Utah, and eastern Idaho. Except for 1 individual from the Rampart Range, haplotypes in clade 2-1 occurred only in mountain ranges in southern Colorado (Wet, Culebra, and Sangre de Cristo).

Geographic structure was significant at the 1-, 2-, and 3-step clade levels and for the entire cladogram (Table 3). Significant association of clades and geography was revealed in haplotypes nested within clades 1-3, 1-9, and 1-13 (Fig. 4); these significant associations are the result of gene flow restricted via isolation by distance (Table 4).

Significant association of haplotypes nested in clade 1-4, 1-step clades nested in clade 2-2, 2-step clades nested in clade 3-1, and the entire cladogram are the result of allopatric fragmentation (Fig. 4; Table 4).

Discussion

Because of its close association with boreal communities, *T. hudsonicus* is a sensitive indicator of biogeographical processes. Gene flow among populations of *T. hudsonicus* is not equal across all landscapes in the central Rocky Mountain region. Inferred processes that explain observed patterns of mtDNA haplotypes indicated that both historical and contemporary factors contribute to the biogeography of *T. hudsonicus* in the central Rocky Mountain region.

The isolation-by-distance model of gene flow predicts that geographically adjacent localities will be more similar genetically to one another as a result of contemporary gene flow; however, the greater the distance between populations, the more differentiated populations become as a result of lower amounts of contemporary gene flow. Restricted gene flow via isolation by distance seems to apply in two major areas within our study area, one in contiguous boreal habitat in northwestern Wyoming, eastern Idaho, and eastern Utah and the other in southern Colorado. Correspondingly,

female philopatry with only short-distance dispersal (<600 m) of individuals from their natal site has been demonstrated for *T. hudsonicus* in Canada and Minnesota (Berteaux and Boutin 2000; Larsen and Boutin 1994; and Sun 1997). With such philopatry, it takes many generations for novel mtDNA lineages to disperse over a broad geographic area (Avise 1994; Templeton 1998).

Our results from nested clade analysis support the hypothesis of Findley and Anderson (1956), Kelson (1951), and Kirkland (1981) that the Wyoming Basin and subsequent formation of the Green River Canyon during the late Pleistocene acted as barriers to gene flow for *T. hudsonicus*. In general, mtDNA haplotypes are not shared among populations on opposite sides of this barrier. Another region of inferred fragmentation includes mountain ranges in the eastern half of Wyoming and western South Dakota (i.e., Black Hills, Bighorn, Laramie, Sierra Madre, and Medicine Bow mountains).

The extent to which species are organized into subpopulations can be inferred from Φ_{st}, an analog to Wright's (1965) F_{st}. Assuming populations are in selection-mutation equilibrium, the island model of gene flow (Wright 1965) predicts that only a single immigrant per generation is needed to overcome the influence of genetic drift (Mills and Allendorf 1996). In a stepping-stone model of gene

flow, 2 to 4 immigrants per generation are needed to overcome influences of genetic drift (Crow and Aoki 1982). Based on Φ_{st} and nested clade results, the Black Hills population of *T. hudsonicus* is more closely related to populations from eastern Wyoming and northern and central Colorado than to those west of the Green River Canyon and Wyoming Basin and those in southern Colorado. *Tamiasciurus hudsonicus* from the Laramie Range appear to be as divergent as the Black Hills population when compared with other populations in the central Rocky Mountain region; however, haplotypes in the Laramie Range (i.e., clade 1-4, Fig. 3) differ by only a single substitution from haplotypes present in the Black Hills. It appears that contemporary gene flow between populations that show significant divergence, such as the Black Hills or the Laramie Range with other mountain ranges in the region, are not sufficient to overcome the effects of genetic drift. The unique haplotypes distributed to the Black Hills and Laramie Mountains probably arose *in situ*. Haplotypes in the Bighorn Mountains also are closely related to haplotypes in the Black Hills with a single exception; haplotype F, which may represent post-Pleistocene dispersal of *T. hudsonicus*. Haplotype F is more closely related to haplotypes in western Wyoming, eastern Idaho, and eastern Utah than to haplotypes in eastern Wyoming. *Tamiasciurus hudsonicus* may be using the Owl Creek

Mountains, which connect the Bighorn with the Absaroka and Beartooth mountain ranges in northwest Wyoming, as a corridor for gene flow. Individuals also may be using boreal habitats in southern Montana as avenues for gene flow. A more adequate sampling regime (i.e., increased sample sizes and inclusion of individuals from areas that lack representation) may reveal additional haplotypes that are distributed in the Bighorn Mountains and mountain ranges in western Wyoming and eastern Idaho and eastern Utah.

Turner (1974) stated that the Black Hills, Bighorn, and Laramie mountains probably were connected until at least 10,500 to 9,650 years ago. Corridors for gene flow may have remained along escarpments and other topographic breaks well after communities with boreal affinities retreated northward and upslope following the Pleistocene (Elliot-Fisk et al. 1983; Mears 1981). Jones et al. (1983) and Turner (1974) speculated that *T. hudsonicus* probably became isolated in the Black Hills approximately 8,450 to 4,680 years ago, thus representing a biogeographic relict. Our findings confirm this conclusion. Our findings also support the notion that, when comparing boreal-adapted species, the Black Hills have closer affinities with the Bighorn Mountains than with the Laramie Mountains to the south.

Allopatric fragmentation also was inferred in central and southern Colorado. Glacial periods may have impacted

the phylogeography of *T. hudsonicus* by separating this species into at least 2 physically isolated, refugial populations that existed for extended periods of time (Fig. 3). Populations in the Wet, Culebra, and Sangre de Cristo mountains in southern Colorado are divergent from those in all mountain ranges except the Centennial and Bear River mountains (eastern Utah and eastern Idaho, respectively). We are thus left with the task of explaining this pattern when apparently suitable habitat exists for gene flow among populations of *T. hudsonicus*. Although this pattern may be an artifact of small sample size, nested clade analysis, which helps to determine if sampling is inadequate, did not indicate this. Alternatively, our identification of a strong genetic discontinuity between populations from central and southern Colorado agree with Arbogast et al. (2001). In addition, Aagaard et al. (1995) found that Douglas-fir (*Pseudotsuga menziesii*) showed a pronounced phylogeographic discontinuity in the southwestern United States.

The Sawatch Mountains contain the 4 highest peaks in Colorado (Armstrong 1972). Therefore, alpine glaciers that occurred in the Sawatch Mountains, and in the Front Range to the north and San Juan Mountains to the west, may have acted as barriers for gene flow for extended periods during the Pleistocene (Armstrong 1972; Hafner and Sullivan 1995;

Porter et al. 1983) and forced populations of *T. hudsonicus* farther south than their contemporary distribution (Harris 1990). As the climate warmed and glaciers receded at the close of the Wisconsin (i.e., Pinedale glacial period in the Rocky Mountains) approximately 14,000 to 9,000 years ago (Porter et al. 1983), the Colorado and Arkansas river drainages, including the Royal Gorge which separates the Wet Mountains from ranges to the north, may have continued to restrict gene flow between northern and southern refugial populations. These river drainages and associated geologic formations may still be restricting movement of individuals. There does appear to be some post-Pleistocene immigration because 1 haplotype (D) with close affinities to the southern mtDNA lineages was collected from the Rampart Range to the north of the Arkansas and Colorado river drainages. Contemporary plant communities in the Arkansas Hills, immediately north of the Arkansas River, are dominated by stands of piñon-juniper, whereas areas to the west of the Sawatch Mountains are dominated by sagebrush plant communities (Armstrong 1972). Hafner and Sullivan (1995) found a shallow genetic break for populations of pika (*Ochotona princeps*) in this region. However, a more pronounced phylogeogeographic discontinuity in *O. princeps* occurs farther north, between populations in southern Wyoming and northern Colorado (Hafner and Sullivan 1995).

Results from our study suggest a pronounced phylogeographic discontinuity for *T. hudsonicus* on either side of the headwaters of the Colorado and Arkansas river drainages. Although Smith (1970) detected little difference in vocal patterns within a single population of *T. hudsonicus* from British Columbia, maintenance of a contact zone between northern and southern mtDNA lineages may result from a behavioral mechanism, such as variation in vocal dialects and olfactory cues, which are important in courtship and maintenance of territories in *T. hudsonicus* (Gurnell 1984; Lair 1990; Smith 1968; 1978).

The unexpectedly high π values for several mountain ranges may be a result of admixture (i.e., during glacial/interglacial cycles) of evolutionary lineages of *T. hudsonicus* that resided in separate refugial populations during the Pleistocene. Glaciation occurred only on high mountain ranges, with as many as 5 glacial epochs throughout the Pleistocene in the Wind River Mountains in western Wyoming (Long 1965). These glacial events coincided with advances and retreats of continental glaciers (Beiswenger 1991; Porter et al. 1983). The Bighorn, Rampart, Beartooth, Gros Ventre, Wind River, Bear River, and Caribou were glaciated and all possess high π values. The exception is the Medicine Bow Mountains, which also were glaciated repeatedly during the Pleistocene (Long 1965), but because

of low sample size (n = 4), interpretation regarding genetic variability in *T. hudsonicus* in the Medicine Bow Mountains would be speculative.

It is a long-held belief that glaciation during the Pleistocene did not lead to speciation events, but did contribute to intraspecific population divergence (Avise and Walker 1998; Findley and Anderson 1956; Findley 1969; Kelson 1951; Kirkland 1981; Patterson 1984). The current subspecific designations of *T. hudsonicus* are based on morphologic differences between populations (Armstrong 1972; Durrant and Hansen 1954; Findley 1961; Hardy 1950; Kelson 1951; Long 1965) throughout the Rocky Mountain region. Several studies have noted considerable variability in color and cranial characters in *T. hudsonicus* in the region, causing difficulty in assigning individuals to lower taxonomic categories (Armstrong 1972; Durrant and Hanson 1954; Long 1965). There are 4 putative subspecies of *T. hudsonicus* that occur in the area of study - *T. h. dakotensis* in the Black Hills; *T. h. baileyi* throughout eastern Wyoming; *T. h. fremonti* throughout Colorado; and *T. h. ventorum* in western Wyoming, eastern Utah, and eastern Idaho (Fig. 1; Hall 1981). Although individuals from the Laramie and Bighorn mountains are referred to *T. h. baileyi*, pair-wise Φ_{st} values revealed that individuals in the Bighorn mountains exhibit less divergence from *T. h.*

ventorum in western Wyoming, eastern Idaho, and eastern Utah than to animals from the Laramie Range. Within the *T. h. fremonti* group in Colorado, mtDNA results reveal a deep phylogeographic separation between populations to the north and south of the Colorado and Arkansas river drainages. Populations north of the headwaters of the Colorado and Arkansas rivers clearly should not be included within the same subspecies as populations south of these river drainages. Nested clade analysis separated *T. h. dakotaensis* from populations to the west and *T. h. ventorum* from populations to the east. Lindsay (1987), who used multivariate analysis on cranial characters of *T. hudsonicus* from throughout the Rocky Mountain region, also reported divergence of *T. h. dakotaensis* from other populations. Our findings clearly imply that historic processes have contributed to taxonomic relationships of *T. hudsonicus* in the central Rocky Mountain region and additional investigations is warranted.

Ecological, historic, and evolutionary aspects of the central Rocky Mountain region clearly contributed to the current genetic structure of *T. hudsonicus*. Incorporating a comparative phylogeographic approach to see if other boreal-adapted species that occur over the same geographic area exhibit similar phylogeographic patterns will help determine if communities and landscapes share a common history or if

species respond in an individualistic manner to climatic oscillations (Avise 2000; Avise and Walker 1999; Bermingham and Moritz 1998; Hewitt 1999; Taberlet et al. 1998). It may also allow identification of recurrent colonization of species between areas that were previously considered to be isolated, as has been shown recently in several mountainous areas in the Great Basin (Brown 1971, 1978; Cooper 1987; Davis and Brown 1989; Grayson and Madsen 2000; Lawlor 1998). Finally, comparative phylogeographic analyses could have implications for conservation and management by identifying evolutionary isolated areas (Moritz and Faith 1998) and may have predictive value for future effects of global climate change.

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Table 1.---Frequency and distribution of mtDNA haplotypes of *Tamiasciurus hudsonicus* (n = 153), haplotype diversi (h) ± *SE*, and nucleotide diversity (π) ± *SE*, from 17 mountain ranges in the central Rocky Mountain region. Locality numbers refer to mountain ranges identified in Fig. 1.

	Haplotypes																								
Locality numbers/					A														A	A	A				A
Mountain range (n)	A	U	G	F	H	V	W	X	L	P	Y	M	K	T	B	Q	O	B	D	C	I	Z	A		
1 Black Hills (9)	4	1	4																						
2 Bighorn (13)	3			6	3	1																			
3 Laramie (15)	10						2	1	2																
4 Medicine Bow (4)	4																								
5 Sierra Madre (6)	4									1	1														
6 Gore (8)	5									1		2													
7 Rampart (12)	8												2	1											
14 Beartooth (8)	2														4	2									
10 Gros Ventre (8)				2											2		1	1	1	1					
11 Wind River (13)	3			1											4						3	1	1		
9 Bear River (6)				2											2										
12 Caribou (5)	1														3		1								
8 Wasatch (1)																									
13 Centennial (1)																									
15 Wet (11)																									
17 Culebra (12)																									
16 Sangre de Cristo (21)																									
Total	44	1	4	11	3	1	2	1	2	2	1	2	2	1	15	2	2	1	1	1	3	1	1		

Table 1.---Extended

Haploytpes																
A F	A G	A E	A J	C	D	H	E	J	N	S	R	*h*	*SE*	π	*SE*
												0.667	0.105	0.003	0.003
												0.731	0.088	0.015	0.009
												0.552	0.137	0.002	0.002
												0.000	0.000	0.000	0.000
												0.600	0.215	0.002	0.002
												0.607	0.164	0.003	0.002
					1							0.561	0.154	0.014	0.009
												0.714	0.123	0.011	0.007
												0.929	0.084	0.022	0.013
												0.846	0.065	0.011	0.007
1	1											0.867	0.129	0.012	0.008
												0.700	0.218	0.015	0.011
		1										1.000	0.000	0.000	0.000
			1									1.000	0.000	0.000	0.000
				1	8	2						0.473	0.162	0.003	0.003
				3			6	3				0.682	0.091	0.004	0.003
				10	3	2	2		2	1	1	0.757	0.086	0.005	0.004
1	1	1	1	14	12	4	8	3	2	1	1				

Table 2.---Numbers below the diagonal represent pair-wise ϕ_{st} values based on the sequential Bonferroni test (Rice 1989). Values marked with an asterisk are significant at table wide $\alpha = 0.05$. Values above the diagonal represent estimates of gene flow ($N_f m$) for all pair-wise comparisons of 17 mountain ranges in the central Rocky Mountain region from which *T. hudsonicus* was collected.

	Location					
Location	1	2	3	4	5	6
1. Black Hills		1.053	1.804	2.625	1.870	0.480
2. Bighorn	0.322		0.862	1.858	1.628	1.118
3. Laramie	0.217*	0.367*		-----	3.532	0.411
4. Medicine Bow	0.160	0.212	-0.088		Inf.	0.663
5. Sierra Madre	0.211	0.235	0.124	0.046		0.587
6. Wind River	0.510*	0.309*	0.549*	0.430*	0.460*	
7. Gros Ventre	0.470*	0.071	0.542*	0.355	0.383	0.153
8. Caribou	0.531*	0.169	0.599*	0.424	0.451	-0.093
9. Centennial	0.896	0.148	0.914*	1.000	0.879*	0.572
10. Bear River	0.881	0.458	0.901	1.000	0.867*	0.199
11. Wasatch	0.640*	0.131	0.699*	0.568*	0.574*	0.168
12. Beartooth	0.390*	0.249	0.424*	0.271	0.321	0.019
13. Gore	0.216	0.295	0.080	-0.024	0.030	0.489*
14. Rampart	0.075	0.208	0.037	-0.103	-0.021	0.365*
15. Wet	0.919*	0.760*	0.931*	0.926*	0.913*	0.826*
16. Culebra	0.933*	0.786*	0.941*	0.942*	0.929*	0.846*
17. Sangre de Cristo	0.895*	0.780*	0.907*	0.892*	0.888*	0.832*

Table 2.---Extended.

Location							
7	8	9	10	11	12	13	14
0.564	0.442	0.058	0.067	0.281	0.782	1.815	6.167
6.542	2.458	2.878	0.592	3.317	1.508	1.195	1.904
0.423	0.335	0.047	0.055	0.215	0.679	5.750	Inf.
0.908	0.679	0.000	0.000	0.380	1.345	-----	-----
0.805	0.609	0.069	0.077	0.371	1.058	Inf.	-----
2.768	-----	0.374	2.012	2.476	Inf.	0.522	0.870
	-----	-----	3.150	-----	2.293	0.649	0.966
-0.056		1.001	9.917	-----	-----	0.504	1.092
-0.128	0.333*		0.000	5.910	0.299	0.051	0.300
0.137	0.048	1.000		1.082	0.885	0.059	0.356
-0.144	-0.034	0.078	0.316		1.332	0.295	0.611
0.179	-0.017	0.626*	0.361*	0.273		0.912	2.077
0.435*	0.498	0.908*	0.894*	0.629*	0.354		Inf.
0.341	0.314	0.625	0.584	0.450*	0.194	0.006	
0.736*	0.824*	0.882*	0.915	0.806*	0.852*	0.922*	0.822*
0.768*	0.851*	0.911	0.935	0.836*	0.872*	0.936*	0.844*
0.766*	0.826*	0.848	0.890	0.809*	0.850*	0.896*	0.828*

Table 2.---Extended.

Location		
15	16	17
0.044	0.036	0.059
0.158	0.136	0.141
0.037	0.031	0.051
0.040	0.031	0.061
0.048	0.038	0.063
0.105	0.091	0.101
0.179	0.151	0.153
0.107	0.087	0.105
0.067	0.049	0.090
0.046	0.035	0.062
0.120	0.098	0.118
0.087	0.073	0.088
0.042	0.034	0.058
0.108	0.092	0.104
	0.409	1.286
0.550*		4.708
0.280	0.096	

Table 3.---Chi-square probabilities for geographical structure of clades identified in Fig. 3. Clades with $P < 0.05$ and marked with an asterisk suggest significant geographical structure. Clades with no genetic or geographical variation are excluded.

Clade	Permutational chi-square statistic	Probability
1-1	13.00	0.068
1-2	4.00	0.527
1-3	33.10	0.015*
1-4	259.83	0.001*
1-6	0.75	1.000
1-8	2.00	1.000
1-9	6.00	0.680
1-10	2.00	1.000
1-13	40.57	0.036*
2-1	41.87	0.047*
2-2	104.86	0.012*
2-4	21.58	0.205
2-5	16.00	0.473
3-1	135.15	0.002*
Entire cladogram	145.75	0.000*

Table 4.---Final inferences revealing processes that explain the distribution and phylogeny of haplotypes of *Tamiasciurus hudsonicus* from nested clade analysis depicted in Fig. 4. Final inference for each clade was obtained from the key available at (http://bioag.byu.edu/zoology/crandall_lab/geodis.htm). Only those clades that resulted in rejection of the null hypothesis (i.e., significantly small or large numbers) are included.

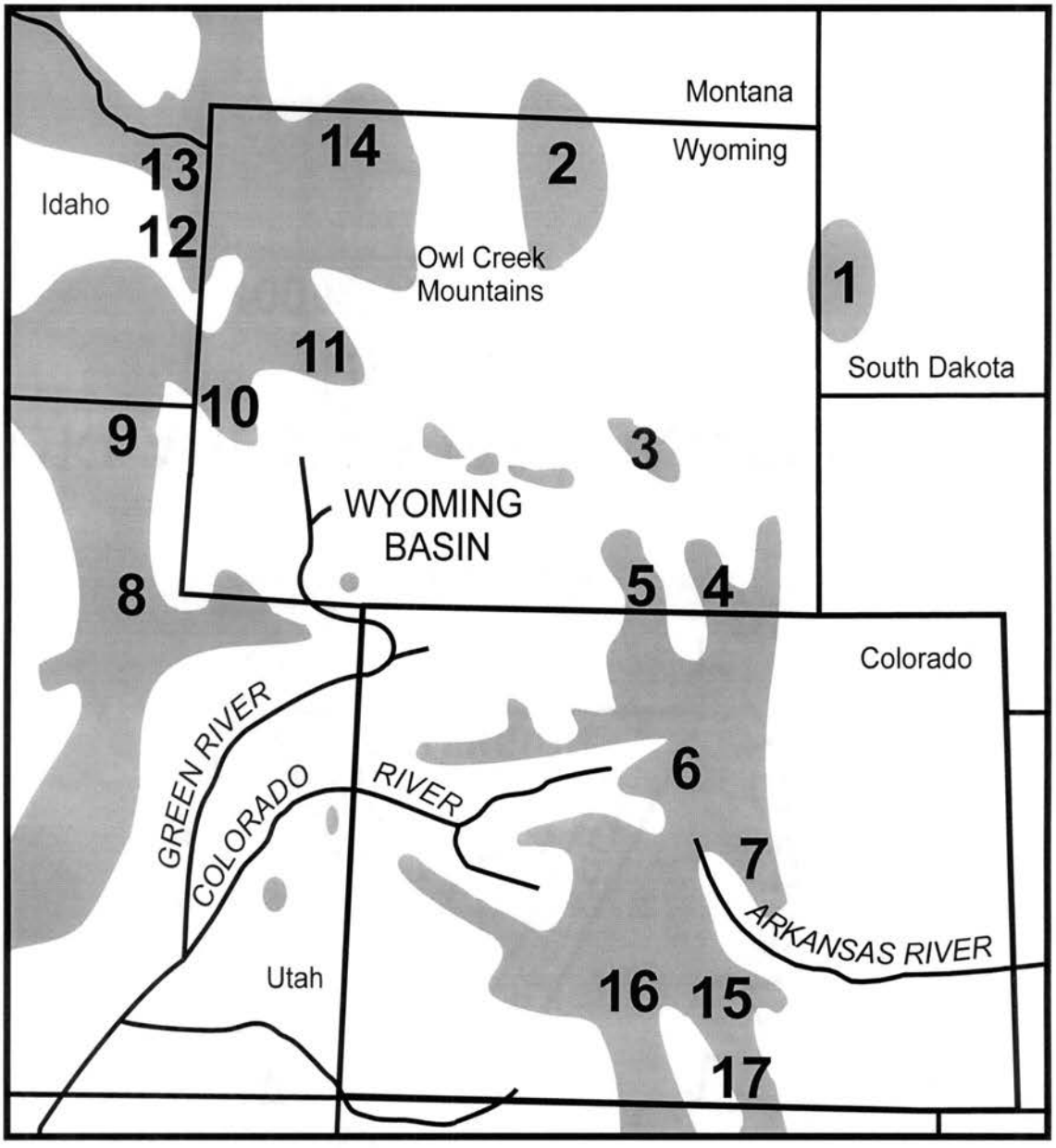
Nesting clades	Final inference
Haplotypes nested in 1-3	Isolation by distance
Haplotypes nested in 1-4	Allopatric fragmentation
Haplotypes nested in 1-9	Isolation by distance
Haplotypes nested in 1-13	Isolation by distance
1-step clades nested in 2-2	Allopatric fragmentation
2-step clades nested in 3-1	Allopatric fragmentation
Entire cladogram	Allopatric fragmentation

FIG. 1.---Distribution of boreal habitats in the central Rocky Mountain region modified from Findley and Anderson (1956). Numbers represent mountain ranges from which *Tamiasciurus hudsonicus* were collected, including 1) Black Hills, 2) Bighorn, 3) Laramie, 4) Medicine Bow, 5) Sierra Madre, 6) Gore, 7) Rampart, 8) Wasatch, 9) Bear River, 10) Gros Ventre, 11) Wind River, 12) Caribou, 13) Centennial, 14) Beartooth, 15) Wet, 16) Sangre de Cristo, and 17) Culebra. Subspecies designations (Armstrong 1972; Hall 1981; Long 1965) are as follows in relation to collecting localities (in parentheses): *Tamiasciurus hudsonicus dakotensis* (1); *T. h. baileyi* (2 and 3); *T. h. fremonti* (4 through 7, 15 through 17); and *T. h. ventorum* (8 through 14). Sample sizes are given in Table 1.

FIG. 2.---Thirty-six mutational sites of 35 haplotypes of the mtDNA control region in 153 *Tamiasciurus hudsonicus*. Insertion/deletion events are indicated with a dash, whereas dots indicate that the nucleotide is identical to that found in haplotype A.

FIG. 3.---Estimated 95% cladogram for *Tamiasciurus hudsonicus* haplotypes. Lines connecting haplotypes represent a single mutation event. Clades 3-1 and 3-2 could not be connected because parsimony was not supported at the 95% level. Small squares represent possible haplotypes that were not collected or became extinct after giving rise to a subsequent haplotype.

FIG. 4.---Results of nested clade analysis of geographic distance for mtDNA haplotypes of *Tamiasciurus hudsonicus*. Haplotypes are given at the top of the figure and grouped according to clade nesting depicted in Fig. 3. A superscript 'S' means that the distance measure was significantly small at the 5% level, and a superscript 'L' means that the distance measure was significantly large.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mtDNA haplo-types	3	4	6	10	18	19	31	38	61	68	79	84	87	89	95	124	125
A	C	C	T	A	T	A	A	A	T	T	A	__	T	C	T	A	G
B	T	.	G	G	.	.	.	.	.	.	.	.	.	.	.	.	.
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AB	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AC	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
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Figure 2.---Extended.

128	129	156	157	162	164	172	173	174	188	203	213	219	225	227	229	231	243	246
T	A	C	T	A	A	G	C	C	G	C	A	C	C	C	C	T	A	C
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.	.	.	C	.	.	A	T	.	A	T	.	.	.	.	T	C	.	.
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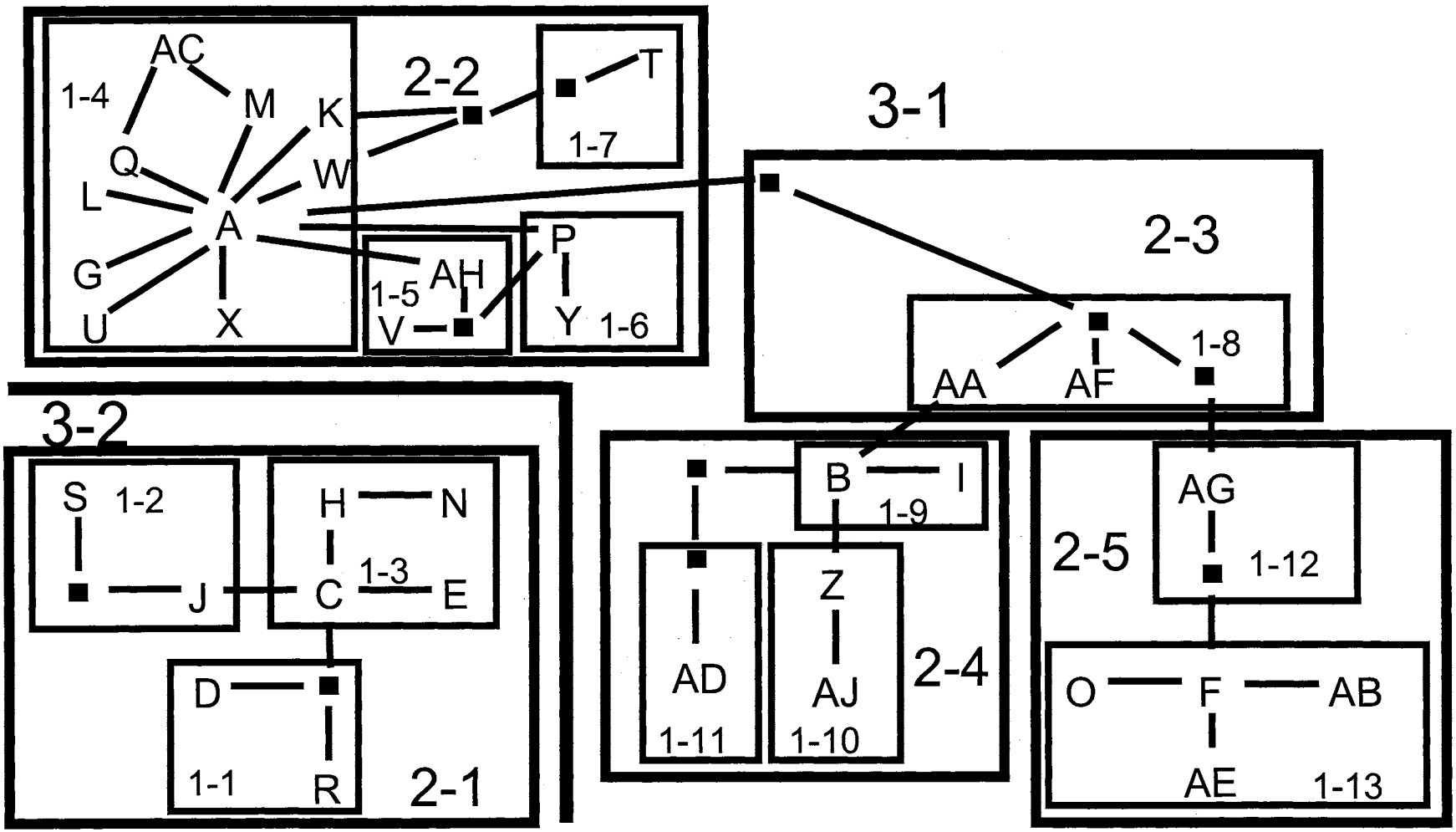
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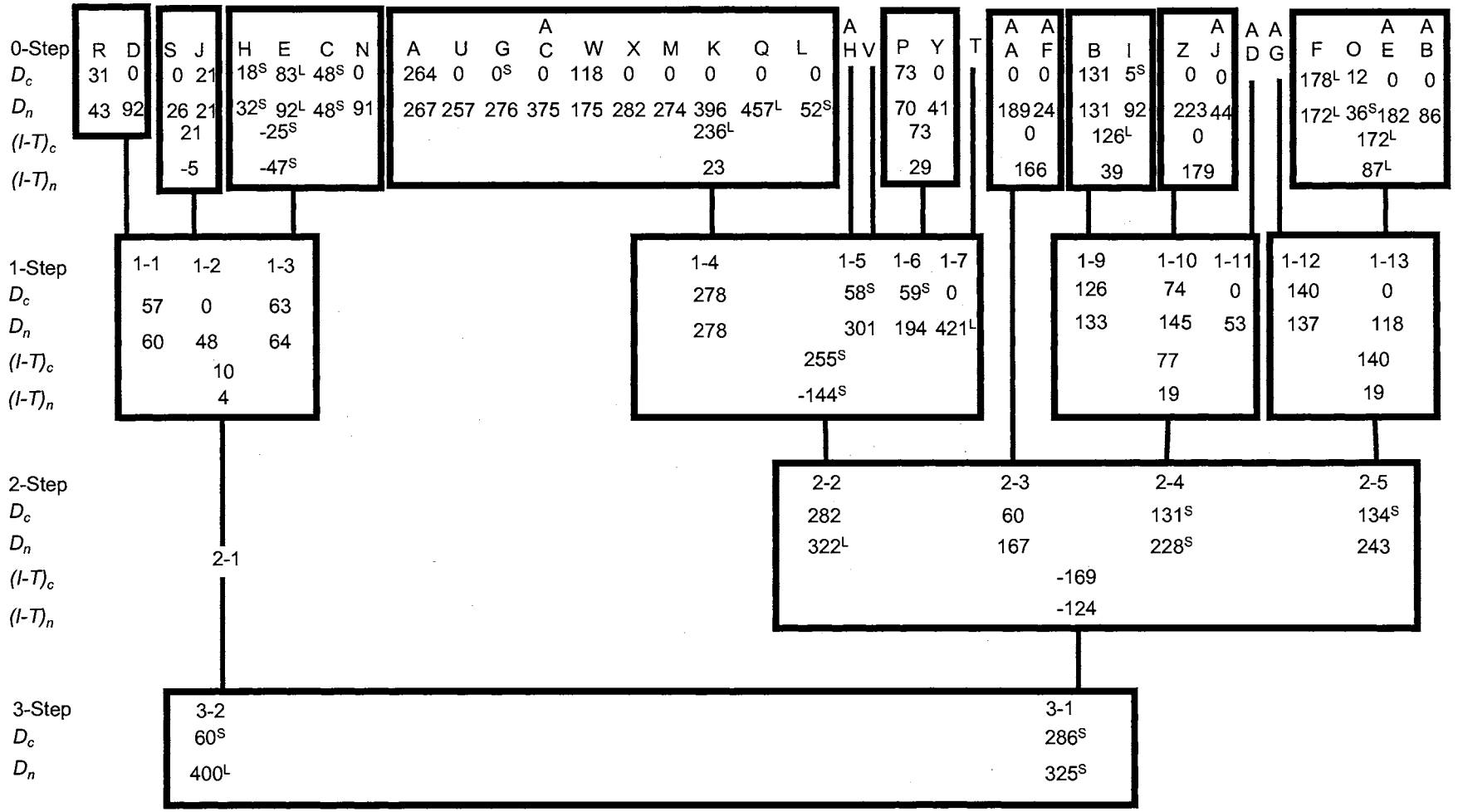
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223 44
0
179
A D
A G
F O A E A B
178L 12 0 0
172L 36S 182 86
172L
87L
1-Step
D_c
D_n
$(I\text{-}T)_c$
$(I\text{-}T)_n$
1-1 1-2 1-3
57 0 63
60 48 64
10
4
1-4 1-5 1-6 1-7
278 58S 59S 0
278 301 194 421L
255S
-144S
1-9 1-10 1-11
126 74 0
133 145 53
77
19
1-12 1-13
140 0
137 118
140
19
2-Step
D_c
D_n
$(I\text{-}T)_c$
$(I\text{-}T)_n$
2-1
2-2 2-3 2-4 2-5
282 60 131S 134S
322L 167 228S 243
-169
-124
3-Step
D_c
D_n
3-2
60S
400L
3-1
286S
325S

COMPARATIVE PHYLOGEOGRAPHY OF BOREAL-ADAPTED RODENTS IN THE CENTRAL ROCKY MOUNTAINS

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We used PCR-RFLP analysis of a 2.4-kb fragment encompassing the ND5 and ND6 subunits of the NADH dehydrogenase complex of the mitochondrial (mtDNA) genome to investigate genetic and phylogeographic structure in populations of *Marmota flaviventris*, *Tamiasciurus hudsonicus*, *Clethrionomys gapperi*, and *Microtus montanus* collected in boreal communities throughout the central Rocky Mountain region. Nested clade analysis revealed a combination of contemporary and historical factors to explain the observed distribution of mtDNA haplotypes. *Tamiasciurus hudsonicus* had the most population structure, followed by *C. gapperi*, *M. montanus*, and *M. flaviventris*. Phylogeographic structure was congruent for these 4 species on both sides of the Green River Canyon and Wyoming Basin but when present, was not

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congruent within eastern Wyoming. Species with specialized habitat and dietary requirements exhibited more depth of divergence than species with less specialized requirements. The strongest genetic discontinuity for *T. hudsonicus* occurred in central Colorado on either side of the headwaters of the Colorado and Arkansas river drainages, supporting the notion that the species was isolated in separate northern and southern refugia for extended periods of time during the Pleistocene.
Key words: *Clethrionomys gapperi*, comparative phylogeography, *Marmota flaviventris*, *Microtus montanus*, nested clade analysis, Pleistocene, *Tamiasciurus hudsonicus*

Phylogeographic theory has been used to assess processes governing geographic distributions of gene lineages for a diverse array of species (Avise 2000). The majority of such studies focused on intraspecific relationships for single species, usually over a broad geographic area. Relatively few studies have compared gene genealogies of multiple, codistributed species (Arctander et al. 1999; Avise 1994, 1998; da Silva and Patton 1998; Joseph et al. 1995; Schneider et al. 1998; Strange and Burr 1997; Sullivan et al. 2000; Taberlet et al. 1998; Templeton and Georgiadis 1996; Zink 1996). In these studies, a lack of concordance among species suggests differences in levels of

gene flow, responses to geographic barriers and climatic change, and other molecular, ecological, and demographic factors (Avise 1998). Thus, comparative phylogeography, together with knowledge of geological history and life-history differences among species, provides a rich base of information for interpretation of biogeography.

As summarized by Avise (1994, 2000), regional phylogeographic analysis for multiple species in the southeastern United States shows strong genealogic concordance of terrestrial and aquatic taxa from the Atlantic and Gulf coasts indicating that these species share a common history. Templeton and Georgiadis (1996) detected a concordant phylogeographic pattern between 2 species of African bovids and attributed the discordant phylogeographic pattern in a 3rd species to differences in habitat requirements, historic aspects, and vagility. In contrast, on a continental scale, Zink (1996) and Taberlet et al. (1998) reported a lack of congruence between multiple, codistributed species and concluded that species responded individually to environmental oscillations that occurred during and following the Pleistocene. Fossil evidence also suggests that the contemporary structure of communities formed during the past few thousand years and that species responded in a Gleasonian, or individualistic, manner to

climatic oscillations during and following the Pleistocene (Graham et al. 1996).

We used mitochondrial DNA (mtDNA) variation to examine phylogeographic pattern in 4 co-occurring, boreal-adapted species in the central Rocky Mountains of North America: yellow-bellied marmot (*Marmota flaviventris*), red squirrel (*Tamiasciurus hudsonicus*), red-backed vole (*Clethrionomys gapperi*), and montane vole (*Microtus montanus*). Although these species are all associated with mesic, boreal communities, each species represents a different level of tolerance to more xeric environments. These 4 species are sympatric throughout the central Rocky Mountain region, except for the Black Hills in eastern Wyoming and adjacent portions of South Dakota, where *M. montanus* does not occur. Because of their sympatric distribution and affinities to boreal habitat, we predicted that they should show similar phylogeographic patterns.

Environmental oscillations that occurred during and following the Pleistocene had a profound effect on the regional distribution of flora and fauna in the central Rocky Mountain region (Findley and Anderson 1956; Martin et al. 1979; Porter et al. 1983; Turner 1974; Whitlock 1993). During the Pleistocene, several alpine glaciers occurred on mountain peaks throughout the Rocky Mountains (Armstrong 1972; Porter et al. 1983). During the last glacial maximum

(approximately 18,000 years ago) boreal communities occurred at least 600 to 800 m lower than their current distribution (Whitlock 1993), thereby connecting boreal habitats that previously were disjunct. As the climate warmed, mesic plant communities were restricted to higher elevations, whereas more xeric plant communities became dominant in intermontane valleys (Beiswenger 1991; Whitlock 1993). Consequently, shortgrass prairie, intermontane basins, and arid river valleys that became established in the central Rocky Mountain region during and following the late Pleistocene may now serve as ecological obstacles to gene flow between conspecific populations of species adapted to boreal habitat.

To test whether *M. flaviventris*, *T. hudsonicus*, *C. gapperi*, and *M. montanus* share a common evolutionary history and the degree to which populations are substructured in the heterogeneous landscape of the central Rocky Mountains, we used the polymerase chain reaction combined with restriction fragment length polymorphism (PCR-RFLP) analysis for the ND5 and ND6 subunits of the NADH dehydrogenase complex of the mitochondrial (mtDNA) genome. We used nested clade analysis to evaluate statistically whether contemporary (i.e., restricted gene flow via isolation by distance), historic (fragmentation, range expansion, or colonization), or a combination of both factors produced the current spatial

arrangement of mtDNA lineages (Templeton et al. 1987; Templeton et al. 1992; Templeton et al. 1995; Templeton 1998).

Based on knowledge of life history characteristics for the 4 species we examined, we predicted that, although they might show similar geographic patterns of variation, there should be differences in levels of divergence among populations. *Tamiasciurus hudsonicus* exhibits a strong affinity to boreal conditions and may have coevolved with coniferous habitat (Benkman 1999; Smith 1970). Thus, it should show the most pronounced phylogeographic structure. The other 3 species should show lower levels of phylogeographic structure because they are less restricted to boreal habitat. *Clethrionomys gapperi* is an omnivorous, opportunistic feeder that occurs in a variety of habitats with abundant litter, rotting logs, stumps, and exposed roots (Merritt and Merritt 1978; Nordyke and Buskirk 1991; Wywialowski and Smith 1988). Individuals have been reported along major rivers between mountain ranges (Clark and Stromberg 1987). Due to physiological requirements, *C. gapperi* prefers moist environments (Getz 1968; McManus 1974). *Marmota flaviventris* occurs in a wide range of altitudes and frequently inhabits intermontane canyons and foothills where succulent vegetation and rocky outcrops are found (Frase and Armitage 1989; Kirkland 1981; Skaggs and

Boecklen 1996). Although *M. montanus* prefers moist habitats (Vaughan 1974), it has been found in more xeric environments than the other 3 species, including dry grasslands with forbs and sagebrush (Armstrong 1977).

These genetic data will provide a rigorous molecular test of previous hypotheses (Anderson 1959; Findley and Anderson 1956; Long 1965; Turner 1974) that inhospitable habitat in the form of shortgrass prairie, intermontane basins, and arid river valleys apparently serves as an ecological obstacle to gene flow as a result of alterations of habitat during and following the Pleistocene. For example, Findley and Anderson (1956) and Kirkland (1981) hypothesized that the Green River Canyon and Wyoming Basin, which formed at the end of the Pleistocene, serve as barriers or filters to gene flow for several boreal-adapted mammals in Colorado and western Wyoming, eastern Idaho, and eastern Utah. The historic break up and contemporary distribution of boreal habitats in eastern Wyoming have been hypothesized as a plausible explanation for the patterns of observed morphologic and phenotypic characters in species of boreal-adapted mammals in the Black Hills of South Dakota and Wyoming and neighboring mountains to the west (Long 1965; Turner 1974; Wilson et al. in litt.). In addition, formation of montane glaciers in central Colorado has been hypothesized for contributing to deep phylogeographic breaks

as a result of populations residing in northern and southern refugia for extended periods of time during the Pleistocene (Armstrong 1972; Arbogast et al. 2001; Wilson et al. in litt.).

Materials and Methods

Animals were collected from 20 mountain ranges in the central Rocky Mountain region (Fig. 1). Voucher specimens (skin, skull, or skeleton) are housed in the Sternberg Museum of Natural History (MHP), Fort Hays State University. Tissues (heart, liver, kidney, spleen, or extracted DNA) are housed in the Oklahoma State University Collection of Vertebrates (OSU). DNA was extracted following the protocol of Longmire et al. (1997) and amplified via the polymerase chain reaction (PCR) using primers for a 2.4-kb segment of the mitochondrial genome (mtDNA) containing the ND5 and ND6 subunits of the NADH dehydrogenase complex (Georgiadis 1996). Amplification reactions were performed in 50μl volumes. Reaction mixes consisted of 50-200 ng DNA, 5 μl of 10X buffer, 1 mM of each dNTP, 0.5 mM of each primer, 2.0 mM $MgCl_2$, and 1.25 units of *Taq* DNA polymerase (Promega, Madison, Wisconsin). PCR cycles were as follows: 94°C for 3 min, followed by 35 cycles of 94°C for 45 sec, 55°C for 30 sec, 72°C for 2.5 min. A final elongation period at 72°C for 30 min concluded the reactions. Verification of

amplification was performed by electrophoresing 2.5 μl of PCR product through a 1% agarose gel stained with ethidium bromide.

We used 10 restriction endonucleases (*Alu* I, *Bst*O I, *Hae* III, *Hha* I, *Hinf* I, *Hsp*92 II, *Mbo* I, *Msp* I, *Rsa* I, and *Taq* I) to digest 5 to 10 μl of PCR product following reaction conditions recommended by the supplier (Gibco, Grand Island, New York; Promega, Madison, Wisconsin). Eight of the 10 restriction endonucleases (*Alu* I, *Hae* III, *Hinf* I, *Hsp*92 II, *Mbo* I, *Msp* I, *Rsa* I, and *Taq* I) have 4-base recognition sequences, whereas *Bst*O I and *Hha* I have 5-base recognition sequences. Digestions were performed using 2.5 to 4 U of enzyme in 96-well microtiter plates covered with clear polyethylene cling wrap.

RFLPs were analyzed by electrophoresis in horizontal 1.5% metaphor agarose gels (FMC BioProducts, Rockland, Maine). Gels were stained with ethidium bromide and fragments were visualized under ultraviolet light. Individuals were grouped into haplotypes based on fragment patterns using a letter code system to represent each individual's composite mtDNA haplotype (Dowling et al. 1996). One individual of each species that possessed a composite haplotype distributed in the greatest number of mountain ranges throughout the study area was selected for DNA sequence analysis of the entire 2.4-kb fragment

(Bermingham et al. 1996). For these animals, the 2.4-kb fragment was first sequenced using primers OSU 5258 and OSU 5257 (Georgiadis 1996) via cycle sequencing according to manufacturer's instructions (BigDye™; Perkin Elmer, Foster City, California). Internal sequencing primers were subsequently developed (Fig. 2) to obtain the complete sequence of the 2.4-kb fragment. Cycling conditions were: 25 cycles at 96°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min. Sequence product was electrophoresed on an Applied Biosystems Inc. (ABI; Foster City, California) 377 automated DNA sequencer. Overlapping fragments of the same sequence were pieced together by use of the AssemblyLIGN computer program (Oxford Molecular 1996). MacVector 6.5 (Oxford Molecular 1998) was used to identify location and order of restriction sites. Double digests were performed to identify the order of restriction sites for RFLP haplotypes for which DNA sequence data were not obtained (Dowling et al. 1996). Log-transformation of fragments was used to estimate fragment sizes by comparison to known size standards (100-bp ladder or 1-kb plus ladder; Gibco). Double digests combined with sequence data allowed construction and verification of a restriction map of all informative sites for each species.

Data were compiled into a presence/absense matrix for all restriction sites. Haplotype (*h*, Nei 1987; Nei and

Tajima 1981) and nucleotide (π, Nei 1987; Tajima 1983) diversity were calculated using version 2.0 of the computer program Arlequin (Schneider et al. 1997). Genetic divergence at different hierarchical levels (e.g., among individuals within populations, between populations among mountain ranges, and among mountain ranges) was analyzed using analysis of molecular variance (AMOVA, Excoffier et al. 1992) of the computer program Arlequin.

Nested clade analysis was used to elucidate relationships among haplotypes (Crandall et al. 1994; Templeton 1998; Templeton et al. 1992; Templeton et al. 1995; Templeton and Sing 1993). This analysis uses the principles of parsimony to assess phylogenetic relationships of haplotypes by illustrating the number of restriction site differences among haplotypes in a network. The ParsProb computer program, which uses algorithms of Templeton et al. (1992), was used to determine the minimal number of mutational events to remain within the 95% confidence interval for construction of the haplotype network. An unrooted cladogram was hand-generated from character-state data by linking haplotypes that differed by a single restriction site until all haplotypes were included (Templeton et al. 1992). The nested design revealed by the unrooted cladogram was used to group haplotypes into 1-step clades, 1-step clades grouped into 2-step clades, and so on,

following the procedure outlined by Templeton et al. (1987). Problems with loops of ambiguity and symmetrically stranded clades, when present, were resolved following Crandall and Templeton (1993) and Templeton and Sing (1993), respectively. The computer program GeoDis (Posada et al. 1999) and a key provided by the authors (http://bioag.byu.edu/zoology/crandall_lab/geodis.htm) was used to distinguish between historic and contemporary processes. Interpretation of the significance of clade distance, $D_c(X)$ [average distance of clade X from the geographical center of that clade], nested clade distance, $D_n(X)$ [average distance of clade X individuals from the geographical center of the higher level clade in which clade X is nested], and average distance between tip (clades that are not interior nodes in the haplotype tree) and interior clades within the nested group, $(I\text{-}T)_c$, and tip to interior distance for the nested clade, $(I\text{-}T)_n$, were used to infer processes that gave rise to observed geographic patterns of mtDNA haplotypes by use of the procedures of Templeton et al. (1995). Older haplotypes are expected to have a broader geographic distribution and be located in the interior portions of the network compared to more recently derived haplotypes which are usually located at the tips of the network. Consequently, significantly large interior distances relative to tip distances are evidence of

restricted gene flow via isolation by distance. If tip distances are significantly larger than interior distances, a historic factor, such as fragmentation, may be inferred as the process responsible for the distribution of haplotypes.

Results

Marmota flaviventris.— A data matrix comprising 53 characters (restriction site positions), 36 of which were shared among all haplotypes, was constructed for 71 individuals (Appendix 1). DNA sequence data generated from *M. flaviventris* and the other 3 taxa have been deposited in GenBank (accession numbers XXXXX-XXXXXX). One (*Hha* I) of the 10 enzymes did not cut the 2.4-kb fragment, and a 2nd (*Msp* I) was monomorphic. Of the 16 unique mtDNA haplotypes, only 5 (A, B, E, F, and G) occurred in more than 1 mountain range (Table 1). Most mountain ranges possessed 2 haplotypes, with a range of 1 to 5. The 2 most common haplotypes (B and G) were the only ones found on both sides of the Green River Canyon and Wyoming Basin. Haplotype A occurred only in the Black Hills and Bighorn Mountains.

An average of 181.3 base pairs (bp) was represented in the recognition sequence of enzymes scored for each haplotype. Mean haplotype diversity (h) was 0.639 (range, 0.167 to 1.000), and average nucleotide diversity (π) was 0.036 (range, 0.000 to 0.091; Table 1). AMOVA revealed an

overall F_{st} of 0.380, indicating significant population substructure ($P < 0.0001$). Most of the diversity (61.96%) occurred within populations, 46.19% was attributed to differences among populations in different mountain ranges, and -8.15% to differences among populations within mountain ranges (Table 2). The negative value can be attributed to either a lack of genetic structure or an artifact of inadequate sampling of populations within mountain ranges. F_{st} values revealed that the Black Hills population is significantly divergent from all other mountain ranges except the Bighorn and Laramie mountains (Table 3).

Nested clade analysis produced 2 major clades (3-1 and 3-2; Appendix 2A). Haplotypes in clade 2-1 occurred throughout the study region. All haplotypes in the Medicine Bow Mountains (B, C, G, V, and W) were confined to clade 2-1. Haplotypes in clade 2-4 (H, M, and N) were restricted to the Wind River Mountains. Haplotypes in clade 2-2 (A, I, and J) occurred in the Black Hills, Bighorn, and Beartooth mountains, whereas haplotypes in clade 2-3 (E, F, L, and O) occurred throughout mountain ranges in Colorado and eastern Wyoming.

The null hypothesis of no association between haplotype tree and geography was rejected for haplotypes nested in clade 1-3, 1-step clades nested in clade 2-1, 2-step clades nested in 3-1 and 3-2, and the total cladogram (Fig. 3).

Because of significantly large clade and nested clade values, restricted gene flow via isolation by distance was inferred for haplotypes nested in clade 1-3 and 2-step clades nested in clade 3-1. These haplotypes occurred in western Wyoming, except for haplotype B, which was found over most of the study region, and 4 haplotypes (C, G, V, and W) restricted to the Medicine Bow Mountains. The geographic sampling scheme was inadequate to discriminate between isolation by distance or fragmentation for 1-step clades nested in clade 2-1. This included haplotypes from eastern and western Wyoming. Because of significantly small clade and nested values, allopatric fragmentation was inferred for 2-step clades nested in 3-2 (haplotypes in the Bighorn Mountains and Black Hills and other haplotypes occurring mainly in eastern Wyoming and northern Colorado) and for the total cladogram (haplotypes distributed on both sides of the Green River Canyon and Wyoming Basin).

Clethrionomys gapperi.—Sixty-one individuals were examined from 12 mountain ranges throughout the central Rocky Mountain region. Fifty-one restriction sites were mapped from fragment patterns, 30 of which were shared among each of the 20 unique haplotypes (Appendix 1). All 10 enzymes cut the 2.4-kb fragment at least once, although 3 enzymes (*Hha* I, *Rsa* I, and *Taq* I) were monomorphic. Most mountain ranges possessed 2 to 4 different haplotypes. Most

haplotypes had limited distributions, with only 6 (F, G, I, K, S, and T) occurring in more than 1 mountain range (Table 4). Most mountain ranges that shared mtDNA haplotypes were close to each other. The most common haplotype (T) occurred only in mountain ranges on the west side of the Green River Canyon and Wyoming Basin.

An average of 170.5 bp was represented in the recognition sequences of enzymes scored for each haplotype. Mean haplotype diversity was 0.555 (range, 0.000 to 1.000), whereas average nucleotide diversity was 0.029 (range, 0.000 to 0.082; Table 4). AMOVA revealed that 60.71% of the genetic diversity was attributable to differences among populations in different mountain ranges, 33.10% to variation within populations, and 6.18% to differences among populations within mountain ranges (Table 2). An overall F_{st} of 0.669 indicated significant population substructure ($P < 0.0001$). Pair-wise population F_{st} values (Table 5) revealed that the Black Hills, Bighorn, Sierra Madre, and Centennial mountains were significantly different from most other mountain ranges.

Nested clade analysis revealed 3 divergent clades (clades 3-1, 3-2, and 3-3; Appendix 2B). The 2 most common haplotypes (T and I) were closely associated with less frequent haplotypes from western Wyoming, eastern Utah, and eastern Idaho. Haplotypes occurring in the Black Hills (A

and B) were grouped in clade 2-3 along with a single haplotype (Z) from the Gore Mountains in northern Colorado. Haplotypes restricted to the Bighorn Mountains (Q, R, and S) were found only in clade 3-2, whereas haplotypes restricted to the Sierra Madre and Medicine Bow mountains (G and H) in eastern Wyoming were found only in clade 3-3.

The null hypothesis of no association between haplotype tree and geography was rejected for haplotypes nested in clade 1-1 and 1-4, 1-step clades nested in clades 2-1 and 2-4, haplotypes nested in clades 1-8, 1-9, and 3-3, 2-step clades nested in clade 3-1, and the total cladogram (Fig. 5). Except for 2-step clades nested in clade 3-1 and the total cladogram, geographic associations were attributable to restricted gene flow via isolation by distance. In most cases, restricted gene flow involved haplotypes from western Wyoming, eastern Utah, and eastern Idaho. Because of significantly small D_c values at higher level clades, allopatric fragmentation was inferred for 2-step clades nested in clade 3-1 and the total cladogram. In most cases, allopatric fragmentation involved haplotypes from eastern Wyoming (Black Hills and mountain ranges to the west) and on either side of the Green River Canyon and Wyoming Basin.

Microtus montanus.—A data matrix comprising 58 characters, 28 of which were shared among all haplotypes, was constructed for 82 individuals (Appendix 1). All 10

enzymes were polymorphic. Only 2 (A and B) of the 21 unique mtDNA haplotypes were found in more than 1 population (Table 6). The most common haplotype (A) was broadly distributed across the study area (both sides of the Green River and Wyoming Basin) and was detected in all 13 mountain ranges from which *M. montanus* was collected except for the Bighorn and Uinta mountains. The Bighorn Mountains possessed 2 private haplotypes (C and D), as did the Uinta Mountains (L and M). Most mountain ranges possessed 3 haplotypes.

An average of 175.9 bp was represented in the recognition sequence of enzymes scored for each haplotype. Mean haplotype diversity was 0.674 (range, 0.133 to 1.000). Average nucleotide diversity was 0.046 (range 0.000 to 0.119; Table 5). AMOVA revealed that most of the genetic variation (52.86%) was partitioned among mountain ranges, whereas 52.45% was partitioned among individuals within populations, and -5.31% was among populations within mountain ranges (Table 2). An overall F_{st} of 0.475 revealed significant population substructure ($P < 0.0001$). Pair-wise population F_{st} values uncovered that the Bighorn Mountains were significantly different from the majority of the other mountain ranges (Table 7).

Nested clade analysis revealed 3 disjunct networks (Appendix 2C). The most common haplotype (A) was found in clade 4-1 and was closely affiliated with haplotypes

distributed in western Wyoming, eastern Utah, and eastern Idaho. Haplotypes in clade 4-3 occurred in eastern Wyoming and the Flat-Tops, a portion of the White River Plateau in northwest Colorado. Clade 4-2 was composed exclusively of haplotypes (C and D) occurring in the Bighorn Mountains in north central Wyoming.

The null hypothesis of no association between haplotype tree and geography was rejected for haplotypes nested in clade 1-2, 1-step clades nested in clade 2-2, 2-step clades nested in clade 3-2, and total cladogram (Fig. 5). Restricted gene flow via isolation by distance was inferred for haplotypes nested in clade 1-2 and 2-step clades nested in clade 3-2. The sampling design was inadequate to discriminate between isolation by distance (short distance movements) versus long distance dispersal for 1-step clades nested in clade 2-2. In most cases, restricted gene flow and short or long distance dispersal involved haplotypes distributed in western Wyoming, eastern Utah, and eastern Idaho. For the total cladogram, the geographic sampling scheme was inadequate to discriminate between fragmentation and isolation by distance and involved haplotypes in the Bighorn Mountains, haplotypes mainly distributed in southeastern Wyoming, and haplotypes in western Wyoming, eastern Utah, and eastern Idaho.

Tamiasciurus hudsonicus.—One hundred-fifty individuals were examined. A data matrix comprising 54 characters, 25 of which were shared among all haplotypes, was constructed (Appendix 1). One (*Hha* I) of the 10 restriction endonucleases did not cut the 2.4-kb fragment, whereas a 2nd enzyme (*Bst*O I) was monomorphic. Presence/absence of the variable sites generated 34 unique haplotypes, 8 of which were distributed in more than 1 mountain range (Table 8). Mountain ranges typically possessed 3 to 6 mtDNA haplotypes. Only 3 haplotypes (A, D, and J) were shared between localities on either side of the Green River Canyon and Wyoming Basin (Table 8). The frequency of haplotype A in the Black Hills and Bighorn mountains was much greater than in other populations. All mountain ranges shared at least 1 haplotype with another mountain range, although several mountain ranges exhibited unique mtDNA haplotypes. The most common haplotype (I) west of the Green River Canyon and Wyoming Basin was not distributed in mountains in other regions of the study area. Nine haplotypes (W, X, Y, Z, AA, AB, AC, AD, and AE) were distributed only in southern Colorado (Wet, Culebra, and Sangre de Cristo mountains).

An average of 159.9 bp was represented in the recognition sequences of enzymes scored for each haplotype. Mean haplotype diversity was 0.617 (range, 0.000 to 1.000), whereas average nucleotide diversity was 0.024 (range, 0.000

to 0.075; Table 7). AMOVA revealed that 72.82% of the genetic diversity was attributable to differences among populations from different mountain ranges, 22.84% to variation within populations, and 4.34% to differences among populations within mountain ranges (Table 2). An overall F_{st} of 0.771 revealed significant population substructure ($P < 0.0001$). Pair-wise F_{st} values showed that populations differed among most mountain ranges, especially the Black Hills, Bighorn, and Laramie mountains in eastern Wyoming (Table 9). In addition, populations from the Wet, Culebra, and Sangre de Cristo mountains represent a homogeneous subset that was significantly different from those on most of the other mountain ranges.

Nested clade analysis produced 3 disjunct networks (clades 3-1, 3-2, and 3-3; Appendix 2D). A clear pattern was evident when examining the distribution of haplotypes in relation to geography. Haplotypes within clade 4-1 occurred throughout central and northern Colorado, Wyoming, eastern Idaho, and eastern Utah. With the exception of haplotype AG, which occurred in the Rampart Range in central Colorado, clade 4-2 contains haplotypes restricted to the Culebra, Wet, and Sangre de Cristo mountains in southern Colorado.

The null hypothesis of no association between haplotype tree and geography was rejected for haplotypes nested in clades 1-1 and 1-3, 1-step clades nested in clades 2-1 and

2-10, 2-step clades nested in 3-1, and the total cladogram (Fig. 6). Restricted gene flow via isolation by distance was inferred for all significant geographic associations except for 1-step clades nested in clades 2-1, 2-step clades nested in 3-1, and the total cladogram, all of which were consistent with expectations from allopatric fragmentation. Except for eastern Wyoming, most inferences of isolation by distance involved haplotypes from contiguous habitat in western Wyoming and from contiguous habitat in southern Colorado. Allopatric fragmentation involved haplotypes on both sides of the Green River Canyon and Wyoming Basin and those in southern Colorado compared to those in the rest of the mountain ranges in the study region.

Discussion

Mitochondrial DNA phylogeography indicates that recent ecological circumstances and climate oscillations during the Pleistocene both have affected the genetic composition of populations of *T. hudsonicus*, *M. flaviventris*, *C. gapperi*, and *M. montanus* in the central Rocky Mountain region. Species with greater tolerance of lowland, more xeric conditions (i.e., *M. montanus* and *M. flaviventris*) exhibit less genetic differentiation than that observed in species whose distributions are more restricted to boreal habitats (i.e., *T. hudsonicus*). Nested clade analysis revealed

concordance in phylogeographic structure of mtDNA lineages for each species on both sides of the Green River Canyon and Wyoming Basin. However, phylogeographic structure, when present, was not congruent in eastern Wyoming.

The contemporary factor of restricted gene flow via isolation by distance seems to explain the distribution of haplotypes for all 4 species in contiguous boreal habitat in northwestern Wyoming, eastern Utah, and eastern Idaho and in more patchy habitat in eastern Wyoming.

AMOVA revealed significant population structure among mountain ranges for all 4 species. Even low levels of gene flow (i.e., 1 effective migrant per generation in the island model of gene flow; 2 to 4 effective migrants in the stepping-stone model) can overcome the effects of genetic drift on divergence between populations (Crow and Aoki 1982; Wright 1965). However, female-mediated gene flow between several mountain ranges in eastern Wyoming did not appear to be sufficient to overcome effects of genetic drift. Based on F_{st} values, population substructure was most pronounced in eastern Wyoming for *C. gapperi*, followed by *T. hudsonicus* and *M. flaviventris*. It is possible that gene flow is lower than indicated by our results, possibly as a result of small sample sizes. Xeric environments dominated by short-grass prairie and scrub habitat appear to serve as ecological

obstacles for gene flow for these species in eastern Wyoming.

Turner (1974) suggested that *T. hudsonicus*, *M. flaviventris*, and *C. gapperi* in the Black Hills represent biogeographic relicts that became isolated at the end of the Pleistocene. This hypothesis is consistent only with our results for *C. gapperi* in the Black Hills and neighboring mountain ranges in eastern Wyoming; *M. flaviventris* exhibited no divergence between populations in the Black Hills and the Bighorn Mountains, but did reveal a deep phylogeographic break between the Black Hills/Bighorn group and other mountain ranges in eastern and western Wyoming. *Tamiasciurus hudsonicus* exhibited only slight divergence among populations in eastern Wyoming mainly due to the contemporary factor of restricted gene flow via isolation by distance. Lack of geographic structure in a rapidly evolving molecular marker such as the NADH-dehydrogenase complex suggests high levels of gene flow, either at present or in the recent past. We suspect, however, that the lack of genetic structure between the Black Hills and neighboring mountain ranges for *T. hudsonicus*, and possibly *M. flaviventris*, may be an artifact of the type of analysis employed. DNA sequence data for the control region of the mitochondrial genome for *T. hudsonicus* (Wilson et al. in litt.) revealed unique lineages between the Black Hills and

neighboring mountain ranges and resulted in an inference of allopatric fragmentation. This probably reflects the greater number of variable characters available in the analysis of DNA sequence data compared with those available in the PCR-RFLP analysis (Dowling et al. 1996). For both *T. hudsonicus* and *M. flaviventris*, the latter analysis did indicate historical allopatric fragmentation between populations from eastern Wyoming and those from western Wyoming, eastern Idaho, and eastern Utah. This finding is congruent with DNA sequence data for *T. hudsonicus* reported by Wilson et al. (in litt).

The current species composition in the Black Hills is represented by a mixture of mammals from a number of regions and biotic affinities in North America, including the Sonoran, eastern deciduous forest, Rocky Mountain, and northern boreal forest (Turner 1974). Similar patterns have been reported for insects (Huntsman et al. 1999; McCafferty 1990) and plants (Harrison et al. 1997; Sakin and Hancock 1997). As a result, the Black Hills probably were associated with a number areas of coniferous forest throughout the Rocky Mountain region. It has been suggested that the connection of coniferous forest of the Black Hills and Bighorn Mountains was indirect, being connected by coniferous forests of the Laramie Mountains along small mountain ranges and escarpments in eastern Wyoming (Elliott-

Fisk et al. 1983; Hoffman and Jones 1970; Mears 1981). Although the Black Hills, Bighorn, and Laramie mountains probably were connected until at least 10,500 to 9,650 years ago, it was not until 8,450 to 4,680 years ago that corridors of boreal habitat between the Black Hills and neighboring mountain ranges are thought to have been replaced by shortgrass prairie (Turner 1974). Turner (1974) noted greater affinities between boreomontane species (i.e., *C. gapperi* and *T. hudsonicus*) in the Black Hills and Bighorn Mountains than between the same species in the Laramie Mountains and either Black Hills or Bighorn Mountains. Based on affinities of cordilleran species (i.e., *M. flaviventris*), the Laramie Mountains should be more closely affiliated with the Bighorn Mountains because they are thought to have been connected for longer periods of time (Turner 1974). Based on the 95% cladograms for *M. flaviventris* and *T. hudsonicus* (Appendix 2A and 2D, respectively), haplotypes in the Black Hills and Bighorn Mountains are more closely related to each other than to haplotypes in the Laramie Mountains, supporting the notion that suitable habitat may have persisted to allow for gene flow between the Bighorn Mountains and Black Hills via wooded river banks in southeastern Montana and northwestern South Dakota (Turner 1974). However, the 95% cladogram for *C. gapperi* (Appendix 2B) revealed that haplotypes in the

Black Hills are more closely related to haplotypes from the Laramie Mountains than to those in the Bighorn Mountains, even though populations in the Laramie and Bighorn mountains are considered the same subspecies (Hall 1981). In addition, several private haplotypes of *C. gapperi* are restricted to mountain ranges in eastern Wyoming. Although these haplotypes may have arisen *in situ*, it is difficult to infer the meaning of the distribution because of small sample sizes. Our data support the hypothesis that *C. gapperi* in the Black Hills may represent a relict population. Gene genealogies of ND5 and ND6 haplotypes for *T. hudsonicus* and *M. flaviventris* in the Black Hills and neighboring mountain ranges each reflect disconcordant patterns when compared to each other and with *C. gapperi*.

Although *M. montanus* does not occur in the Black Hills, pair-wise F_{st} values revealed a significant amount of substructure between the Bighorn Mountains and neighboring mountain ranges. Nested clade analysis revealed that the sampling scheme was inadequate to discriminate between historic (i.e., fragmentation) and contemporary (i.e., isolation by distance) factors. Because the Bighorn population possessed private mtDNA haplotypes and is recognized as a distinct subspecies (*M. m. zygomaticus*--- Anderson 1954), we suspect that past fragmentation may be the most plausible explanation. Haplotypes in the Bighorn

Mountains are more closely related to haplotypes in northwest Wyoming than to haplotypes in eastern Wyoming and northern Colorado (Appendix 2C; Fig. 5). Anderson (1954) speculated that populations of *M. montanus* (*M. m. codiensis*) in the Absaroka Mountains may exhibit interspecific hybridization with other sympatric species of *Microtus*. The mtDNA results show no evidence of such hybridization.

Findley and Anderson (1956) noted that habitat discontinuity caused by the formation of the Green River Canyon and Wyoming Basin during the late Pleistocene may have served as an ecological obstacle for several boreal-adapted species of mammals. They noted that subspecific partitioning on either side of the Green River Canyon and Wyoming Basin appeared to be related to vagility and natural history attributes of each species. For example, 2 of the species included in this study (*M. flaviventris* and *T. hudsonicus*) are represented by different subspecies on either side of the Green River Canyon and Wyoming Basin (Findley and Anderson 1956; Hall 1981; Long 1965). The other 2 species (*C. gapperi* and *M. montanus*) inhabit both sides of the Green River Canyon and Wyoming Basin, but as the same subspecies (Anderson 1954; Hall 1981). Our findings agree with conclusions based on morphologic and phenotypic data (Findley and Anderson 1956; Kirkland 1981). As predicted, mtDNA divergence on either side of the Green

River Canyon and Wyoming Basin was greater for species with increasing affinities to boreal habitat. Based on pair-wise F_{st} values, *T. hudsonicus* exhibited the most pronounced genetic discontinuity; of the other 3 species, *M. flaviventris* and *C. gapperi* showed a moderate amount of genetic structure, whereas *M. montanus* exhibited less genetic discontinuity between populations on the 2 sides of the Green River Canyon and Wyoming Basin.

Allopatric fragmentation also was inferred for haplotypes of *T. hudsonicus* in central and southern Colorado, and is congruent with previous genetic studies (Arbogast et al. 2001; Wilson et al. in litt.). The separation of mtDNA lineages in southern Colorado from localities in central and northern Colorado, which occurs around the headwaters of the Colorado and Arkansas river drainages, reflects longer-term historical disjunctions. These populations probably diverged in separate glacial refugia during the Pleistocene as a result of montane glaciers that formed in the Front, Sawatch, and San Juan mountains in central and southern Colorado. Additional studies have found similar results for several species of mammals in the region. For example, Hafner and Sullivan (1995) found a north-south phylogeographic break for pikas (*Ochotona princeps*) based on protein allozymes. Conroy and Cook (2000) reported deep phylogenetic divergence between

populations of long-tailed vole (*M. longicaudus*) on either side of the Green River Canyon and Wyoming Basin. Lamb et al. (1997) found a deep phylogeographic break between populations of tassel-eared squirrels (*Sciurus aberti*) in the southwestern United States. Additional animal and plant species need to be examined to test hypotheses pertaining to habitat fragmentation and modification of distribution patterns of taxa that occurred during and following the Pleistocene in the Rocky Mountains.

Our analysis of the phylogenetic structure of boreal-adapted rodents has been restricted to the maternally inherited mtDNA genome. Consequently, our results may not reflect the true phylogeographic structure of these 4 species as each may have more male-mediated genetic exchange across the fragmented landscape of the central Rocky Mountains. For example, *M. flaviventris* usually lives in a colony comprising an adult male, several adult females, and their offspring (Van Vuren and Armitage 1994; Armitage 1998). However, individuals also reside outside of colonies and some are transients (Svendsen 1974). Salsbury and Armitage (1994) reported that adult, male *M. flaviventris* made several short (<1,000 m) and long distance excursions (>4,270 m) from each individuals home range. Before a clear comprehensive understanding can be achieved, it is important that nuclear markers, such as microsatellites or protein

allozymes, be investigated to address the affects of male-biased gene flow (Degnan 1993; Wilson et al. 2000) for species in the central Rocky Mountains.

In conclusion, contemporary gene flow among populations of *M. flaviventris*, *T. hudsonicus*, *C. gapperi*, and *M. montanus* is not equal across the heterogeneous landscape of the central Rocky Mountain region. This may be a result of shortgrass prairie, intermontane basins, and arid river valleys. Contemporary restriction of gene flow via isolation by distance appears to be the best explanation for the distribution of ND5 and ND6 haplotypes in areas of contiguous boreal habitat. Species with more specialized diets and habitat requirements revealed more genetic substructure across the heterogeneous landscape of the Rocky Mountains than species with less specialized requirements. For example, *T. hudsonicus*, which coevolved with boreal habitat (Benkman 1999; Smith 1970), revealed the most genetic structure, followed by *C. gapperi*, *M. montanus*, and *M. flaviventris*. *Microtus montanus* and *M. flaviventris* exhibited the least amount of population substructure, probably because they can tolerate a broader range of environmental parameters than the other 2 species. Concordance between the distribution and genealogies of mtDNA lineages of these 4 species of rodents on both sides of the Green River Canyon and Wyoming Basin suggest these

species were influenced by climatic oscillations that occurred during and following Pleistocene. However, populations of these species do not show concordance in eastern Wyoming. This lack of concordance may be attributed to differences in gene flow, responses to geographic filters or barriers, and natural history attributes of individual species.

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APPENDIXES

APPENDIX 1

Presence/absence matrices for *Clethrionomys gapperi*, *Marmota flaviventris*, *Microtus montanus*, and *Tamiasciurus hudsonicus*. Each haplotype for each species is designated with a unique letter. Restriction-site characters are designated as present (1) or absent (0).

Clethrionomys gapperi

Haplotype	Restriction enzyme									
	Alu I	*Bst*O I	*Hae* III	*Hha* I	*Hinf* I	*Rsa* I	*Hsp*92 II	*Msp* I	*Mbo* I	*Taq* I
A	101001001	01	111111	1	111111101	111111111	11111000	11	11	111

Appendix 1.---Continued

B	001001001	01	111111	1	111111101	111111111	11111000	11	11	111
C	101001001	01	111111	1	111111101	111111111	11011010	11	11	111
E	111001001	01	111111	1	111111101	111111111	11011010	11	11	111
F	101001001	00	111110	1	111111101	111111111	11011010	11	11	111
G	101001001	00	111110	1	101111111	110111111	10011010	11	11	111
H	101001001	11	111110	1	101111111	110111111	10011010	11	11	111
I	101001001	11	111111	1	111111101	111111111	11011010	11	11	111
J	101011001	11	111111	1	111111101	111111111	11011110	01	11	111
K	101001011	11	111111	1	111111101	111111111	11011010	11	11	111
M	101001001	11	111111	1	111111101	111111111	10011010	11	11	111
O	101001001	11	111111	1	111111001	111111111	11011110	11	11	111
Q	111001001	00	011111	1	111111111	111111111	11011010	11	11	111
R	101001001	00	011111	1	111111101	111111111	11011010	11	11	111
S	101001001	01	011111	1	111111101	111111111	11011010	11	11	111
T	101001001	11	111111	1	111111101	111111111	11011110	11	11	111
U	101001001	11	011111	1	111111101	111111111	11011111	11	11	111

Appendix 1.---Continued

V	101001001	11	011111	1	111111101	111111111	11011110	11	11	111
X	101001101	11	111111	1	111111101	111111111	11011110	11	11	111
Z	111001001	01	111111	1	111111101	111111111	11111000	11	11	111

Marmota flaviventris

Haplotype	Restriction Enzyme									
	Alu I	*Bst*O I	*Hae* III	*Hha* I	*Hinf* I	*Hsp*92 II	*Mbo* I	*Msp* I	*Rsa* I	*Taq* I
A	1110111001101	0	11001	0	0111111	1110111	11110111	1	1011111	11111
B	1110111101100	0	11101	0	0111111	1110111	11110111	1	0111111	11111
C	1110111101100	0	11101	0	0111111	1110011	11110111	1	0111111	11111
E	1110111111100	1	11001	0	1111111	1111111	11110111	1	0011111	11111

Appendix 1.---Continued

F	1110111111100	1	11001	0	0111111	1111111	11110111	1	0011111	11111
G	1110111101100	0	11101	0	1111111	1110111	11110111	1	0111111	11111
H	1111111101100	0	11001	0	0111111	1110111	11110111	1	0010111	11111
I	1110111001101	0	11001	0	0111111	1110111	11110111	1	1011111	11010
J	1110111001101	0	11001	0	0111111	1110111	11111111	1	1011111	11010
K	1110111101100	0	11101	0	1111111	0110111	11110111	1	0111111	11111
L	1110111101100	1	11001	0	0111111	0110111	11110111	1	0011111	11111
M	1111111101100	0	11101	0	0111111	1110111	11110111	1	0010111	11111
N	1111111101100	0	11011	0	0111111	1110111	11110111	1	0010111	11111
O	1110111101100	1	11001	0	0111111	1111111	11110111	1	0011111	11111
V	1110111101110	0	11101	0	0111111	1110011	11110111	1	0111111	11111
W	1110111101110	0	11101	0	1111111	1110011	11110111	1	0111111	11111

Appendix 1.---Continued

Microtus montanus

Haplotype	Restriction Enzyme								
	Alu I	*Bst*O I	*Hae* III	*Hha* I	*Hinf* I	*Hsp*92 II	*Rsa* I	*Mbo* I	*Msp* I	*Taq* I
A	11111110111011	11	1110	1111	01111110	1111101	1000	01	0010110	110011
B	01110110111011	11	1100	1111	01111110	1111111	1011	01	1010110	111011
C	11110111111010	01	1110	1111	01111110	1111101	1000	01	0010110	110011
D	11110111111010	01	1110	1111	01111110	1111101	1101	01	0010110	110011
E	01110110111011	11	1100	1111	01111110	1111111	1011	01	1111100	111011
F	01110110111011	11	1100	1111	01111110	1111111	1011	01	1010110	110011
G	01110110111011	10	1100	1111	01011111	1111111	1011	01	1010110	110011
H	11111110111111	11	1110	1111	01111110	1111101	1000	01	0010110	110011
I	11111110111011	11	1111	1101	01111110	1111100	1000	01	0010110	110011
J	11111110111011	11	1110	1111	01111111	1111101	1000	01	0010110	110011

Appendix 1.---Continued

K	11111110111011	11	1110	1101	01111110	1111101	1000	11	0010110	110011
L	11111110111011	11	1110	1111	01111110	1111101	1000	01	0010111	110011
M	11111110111011	11	1110	1111	01111110	1111101	1000	01	0000110	110011
N	11111110111011	01	1110	1111	01111111	1111101	1001	11	0010111	110011
O	11111010111011	11	1110	1111	01111110	1111101	1000	01	0010110	110011
P	11111110111011	01	1110	1111	11111111	1111101	1001	11	0010111	110011
R	11111110101011	11	1110	1111	01111110	1111101	1000	01	0010110	110011
S	11111110111011	11	1110	1111	01111111	1111101	1001	11	0010111	110011
T	11111110100011	11	1110	1111	01111110	1111101	1000	01	0010110	110011
U	11111110111011	11	1110	1101	01111110	1111101	1000	01	0010110	110011
V	11111110111011	11	0110	1111	01111110	1111101	1000	01	0010110	110011

Appendix 1.---Continued

Tamiasciurus hudsonicus

Haplotype	Restriction Enzyme									
	Alu I	*Bst*O I	*Hae* III	*Hha* I	*Hinf* I	*Hsp*92 II	*Mbo* I	*Msp* I	*Rsa* I	*Taq* I
A	1100101110	1	1101011	0	011111111	011100011	011100	011	111111	111
B	1100101110	1	1101011	0	011111111	011100011	011110	011	111111	111
C	1100101110	1	1101110	0	011111111	011100011	011110	011	111111	111
D	1100101000	1	1101011	0	011111111	011100011	011100	011	111111	111
E	1100101110	1	1101011	0	011111111	011100011	011110	011	111111	101
F	1100101110	1	1101011	0	011111111	011100011	011100	001	111111	111
G	1100101110	1	1101011	0	011111111	011100011	011100	011	111110	111
H	1100101110	1	1101011	0	011111111	011100011	011100	011	111101	111
I	1100101110	1	1111011	0	011111111	011100011	011101	011	111111	111
J	1100101110	1	1101011	0	011111111	011100011	011101	011	111111	111

Appendix 1.---Continued

K	1100101110	1	1111011	0	011111111	011100011	011100	011	111111	111
L	1100101110	1	1101011	0	011111111	011100011	011100	011	011111	111
M	1111101110	1	1101001	0	011111111	111100011	011100	011	111111	111
N	1111101110	1	1101001	0	011111111	111100011	011101	011	111111	111
O	1100101110	1	1101001	0	011111111	111100011	011100	011	111111	111
P	1111101010	1	1101001	0	011111111	011100011	011100	011	111110	111
Q	1100101110	1	1111011	0	011111111	011100011	011111	011	111111	111
R	1111101010	1	1101001	0	011111111	111100011	011111	011	111111	111
S	1111101010	1	1101011	0	011111111	111100011	011111	011	111111	111
T	1111101010	1	1101011	0	011111111	111100011	011101	011	111111	111
U	1110101111	1	1101011	0	011111111	011100011	011100	011	111111	111
V	1111101110	1	1101001	0	011111111	111100011	011100	111	111111	111
W	1110101111	1	1101001	0	001111111	011100001	010100	011	011101	101
X	1110101111	1	1101001	0	101111111	011100001	010100	011	011101	101
Y	1100101110	1	1101001	0	001111111	011100001	010100	011	011101	101
Z	1110111111	1	1101001	0	001111111	011100001	010100	011	011101	101

Appendix 1.---Continued

AA	1110111111	1	0101011	0	001111111	011100001	010100	011	011101	101
AB	1110101111	1	1101001	0	001111111	011100101	010100	011	011101	101
AC	1110101111	1	1101001	0	000111111	011100001	010100	011	011101	101
AD	1110101111	1	1101001	0	000111111	011100001	010100	011	011101	111
AE	1110101111	1	1101001	0	101111111	011100001	010100	011	011101	111
AF	1100101110	1	1101011	0	011111111	011100011	110100	011	111111	111
AG	1110101111	1	1101001	0	001111111	011100001	010100	011	011101	111
AH	1100101110	1	1111011	0	011111111	011111011	011101	011	111111	111

APPENDIX 2

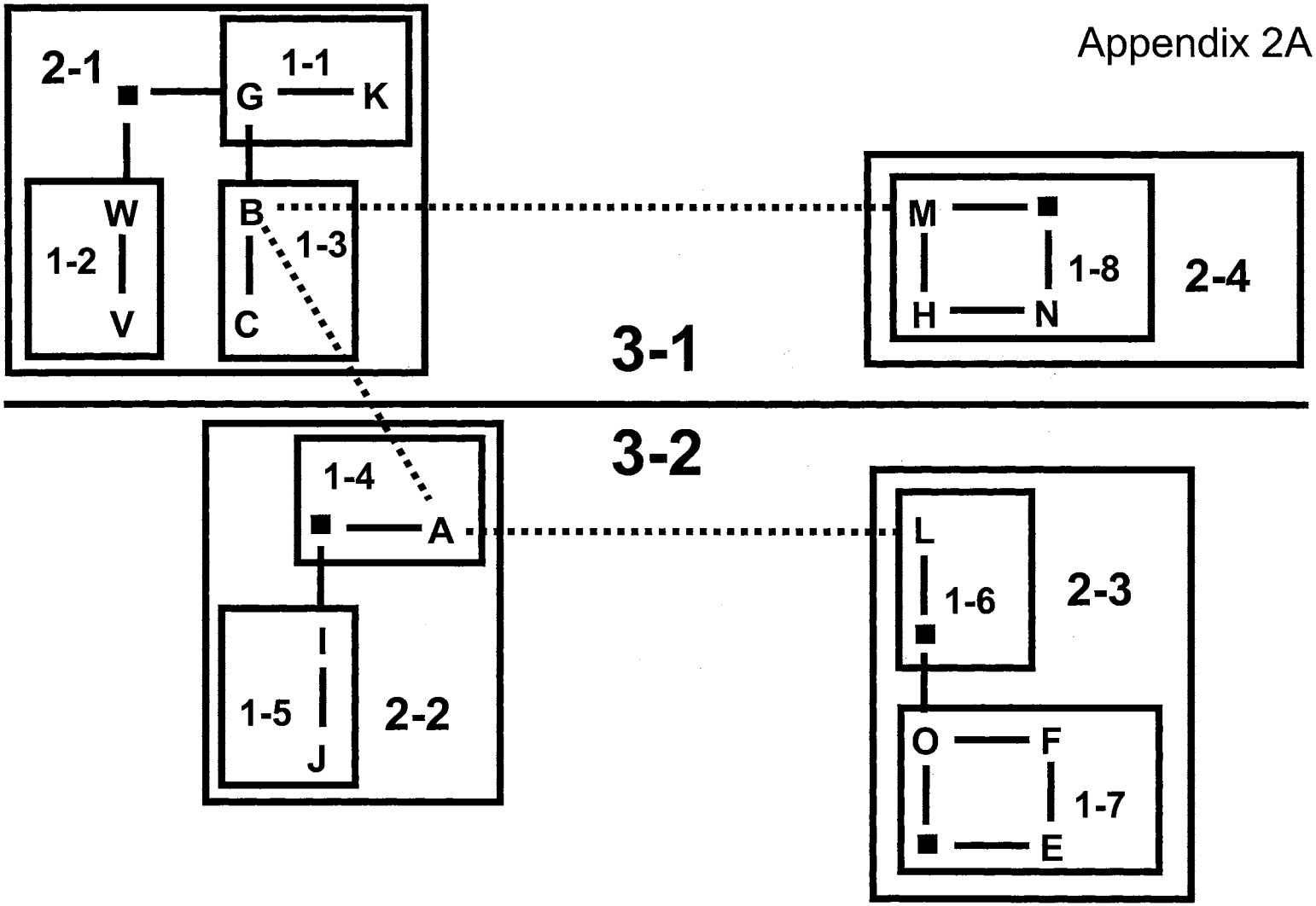
Appendix 2A.---Estimated cladogram with 95% plausible set of haplotype connections with clade nesting for *Marmota flaviventris*. Solid lines indicate single mutational events (restriction-site change). Dashed lines represent a connection that was not supported with 95% confidence. Haplotype B is minimally 3 mutational events from haplotype M, haplotype A is minimally 4 mutational events from haplotype L, and haplotype B is minimally 5 mutational events from haplotype A. Squares represent haplotypes not represented in the sample.

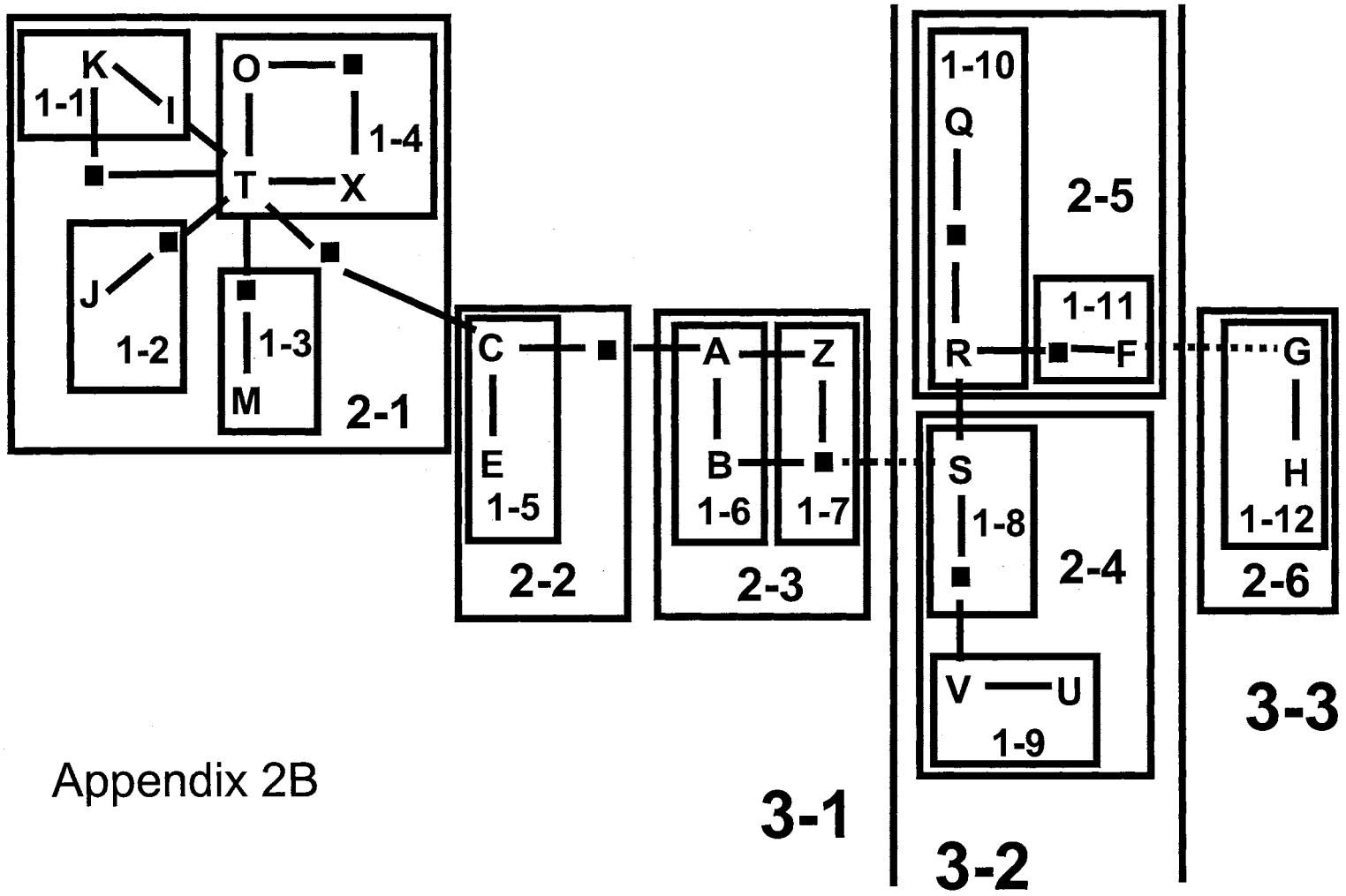
Appendix 2B.---Estimated cladogram with 95% plausible set of haplotype connections with clade nesting for *Clethrionomys gapperi*. Solid lines indicate single mutational events (restriction-site change). Dashed lines represent a connection that was not supported with 95% confidence. Haplotype S is minimally 3 mutational events from the missing haplotype in clade 1-7 and haplotype F is minimally 4 mutational events from G. Squares represent haplotypes not represented in the sample.

Appendix 2C.---Estimated cladogram with 95% plausible set of haplotype connections with clade nesting for *Microtus montanus*. Solid lines indicate single mutational events (restriction-site change). Dashed lines represent a connection that was not supported with 95% confidence. Haplotype L is minimally 3 mutational events from S, haplotype H is minimally 5 mutational events from C, and haplotype A is minimally 7 mutational events from F. Squares represent haplotypes not represented in the sample.

Appendix 2D.---Estimated cladogram with 95% plausible set of haplotype connections with clade nesting for *Tamiasciurus hudsonicus*. Solid lines indicate single mutational events (restriction-site change). Dashed lines represent a connection that was not supported with 95% confidence. All dashed lines represent haplotypes separated by a minimum of 2 mutational events, with the exception of the connection of haplotypes M and P and W and Y, which are separated by a minimum of 3 mutational events, and haplotype Q and S which are separated by a minimum of 5 mutational events. Thick lines represent a large number (≥ 8) of mutational events. Squares represent haplotypes not represented in the sample.

Appendix 2A




K
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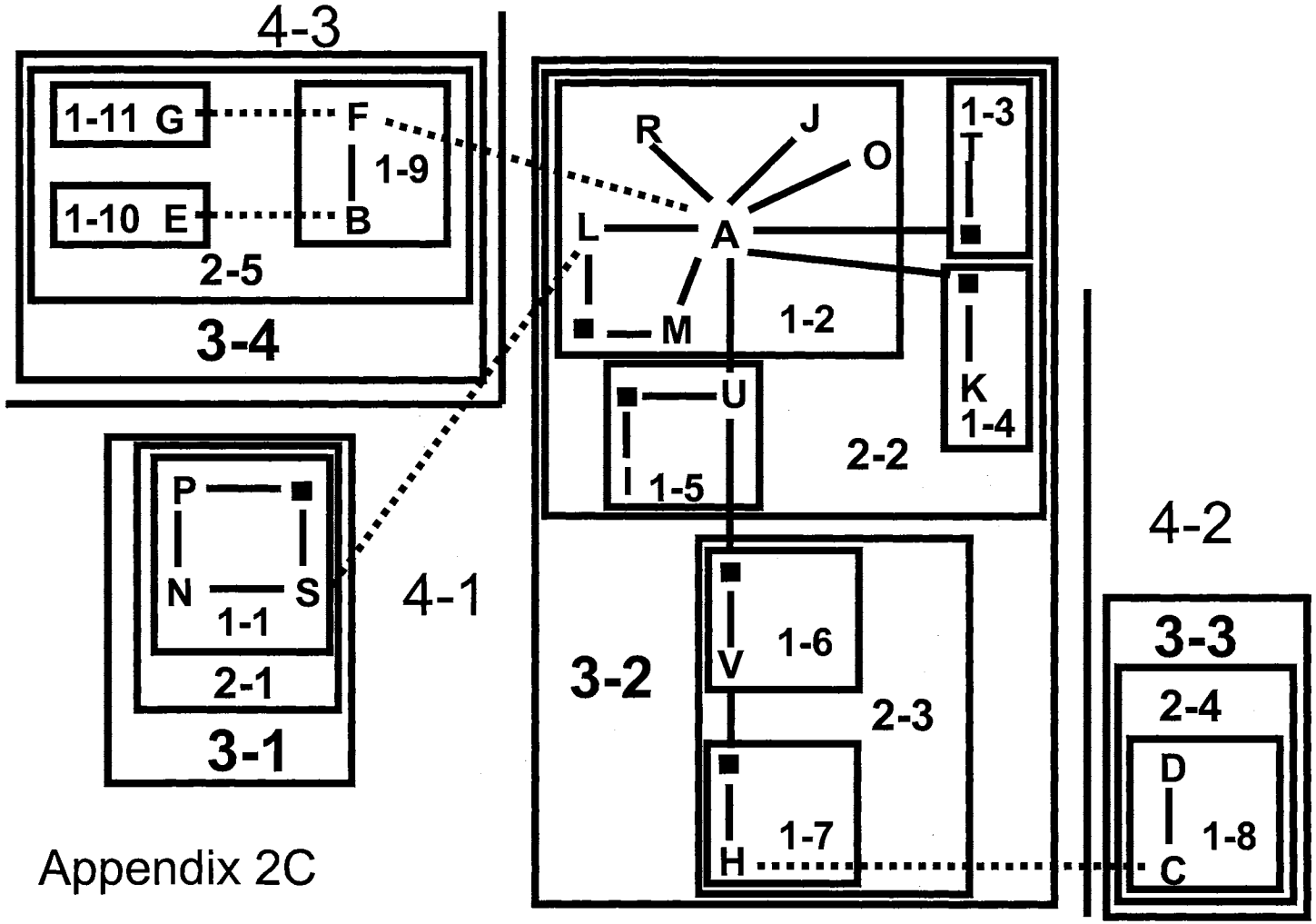
I
O
1-4
T
X
J
1-2
1-3
M
2-1
C
E
1-5
2-2
A
Z
B
1-6
1-7
2-3
1-10
Q
2-5
1-11
R
F
G
H
1-12
2-6
S
1-8
2-4
V
U
1-9
3-3
3-1
3-2

Appendix 2B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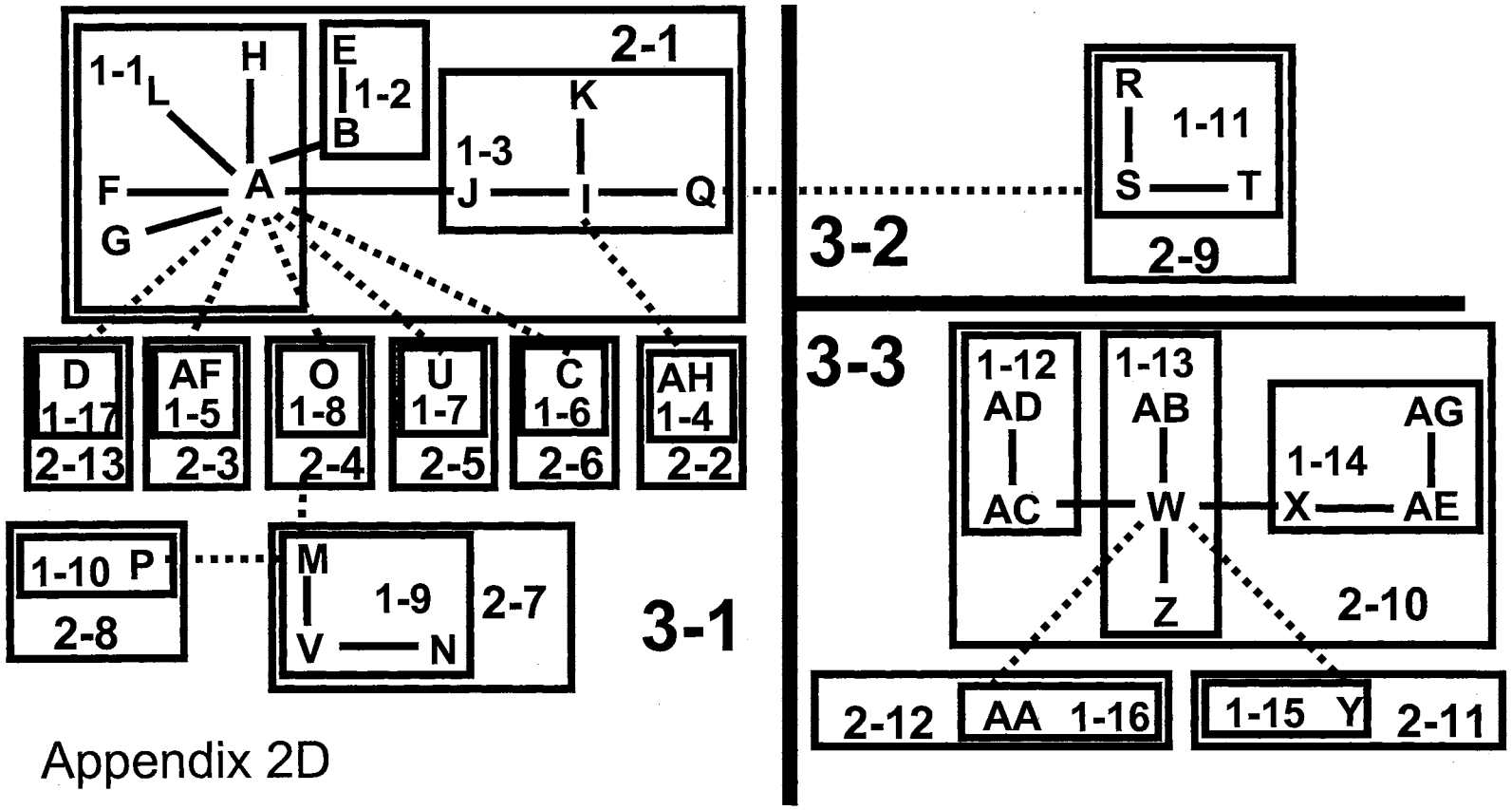
1-11 G
F
1-9
1-10 E
B
2-5
3-4
R
J
O
1-3
T
L
A
M
1-2
K
1-4
U
1-5
2-2
4-2
P
N
S
1-1
2-1
3-1
4-1
3-2
V
1-6
2-3
3-3
2-4
D
1-8
H
1-7
C

Appendix 2C


1-1
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E
1-2
B
2-1
K
1-3
F
A
J
I
Q
G
D
1-17
2-13
AF
1-5
2-3
O
1-8
2-4
U
1-7
2-5
C
1-6
2-6
AH
1-4
2-2
1-10 P
2-8
M
1-9
V
N
2-7
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3-2
R
1-11
S
T
2-9
3-3
1-12
AD
AC
1-13
AB
W
Z
AG
1-14
X
AE
2-10
2-12
AA 1-16
1-15 Y
2-11

Appendix 2D

Table 1.---Distribution and frequency of 16 mtDNA haplotypes and haplotype (h) and nucleotide (π) diversity, plus *SE* of each respective index, for *Marmota flaviventris* from 12 mountain ranges throughout the central Rocky Mountain region.

	Haplotypes											
Mountain Range (n)	A	I	B	F	C	G	V	W	E	L	O	H
Black Hills (9)	6	3										
Bighorn (6)	5		1									
Laramie (2)			1	1								
Medicine Bow (9)			2		2	2	2	1				
Sierra Madre (7)			4	1					1	1		
Gore (4)			3								1	
Flat-Tops (7)			3	2					2			
Teton (1)			1									
Wind River (6)						3						1
Gros Ventre (4)			1			3						
Uinta (4)			3									
Beartooth (12)						11						
Total	11	3	19	4	2	19	2	1	3	1	1	1

Table 1.---Extended.

Haplotypes							
M	N	K	J	*h*	*SE*	π	*SE*
				0.500	0.128	0.018	0.015
				0.333	0.215	0.030	0.024
				1.000	0.500	0.091	0.099
				0.889	0.071	0.028	0.021
				0.714	0.181	0.059	0.039
				0.500	0.265	0.036	0.030
				0.762	0.115	0.061	0.040
				1.000	0.000	0.000	0.000
1	1			0.800	0.172	0.059	0.041
				0.500	0.265	0.009	0.011
		1		0.500	0.265	0.018	0.018
			1	0.167	0.134	0.027	0.019
1	1	1	1	0.639	0.193	0.036	0.030

Table 2.---Partitioning of mtDNA diversity in 4 species of boreal-adapted mammals in the central Rocky Mountain region. Numbers represent percent of total diversity.

	Species			
	M. flaviventris	*C. gapperi*	*M. montanus*	*T. hudsonicus*
Within populations	62.0	33.1	52.5	22.8
Among populations within mountain ranges	-8.2	6.2	-5.3	4.3
Among populations in different mountain ranges	46.2	60.7	52.9	72.8

Table 3.---Pair-wise F_{st} values for *Marmota flaviventris* collected from several mountain ranges in the central Rocky Mountain region. F_{st} values marked with an asterisk are significant (Tablewide $\alpha = 0.05$) based on the sequential Bonferroni test (Rice 1989).

	Location											
	1	2	3	4	5	6	7	8	9	10	11	12
1. Black Hills	0.000											
2. Bighorn	0.129	0.000										
3. Laramie	0.736	0.510	0.000									
4. Medicine Bow	0.814*	0.703*	0.380	0.000								
5. Sierra Madre	0.672*	0.488	-0.387	0.284	0.000							
6. Wind River	0.703*	0.533	0.094	0.317	0.141	0.000						
7. Gros Ventre	0.867*	0.754*	0.425*	0.223*	0.217*	0.179	0.000					
8. Beartooth	0.796*	0.691*	0.448	0.305	0.323	0.260	-0.113	0.000				
9. Gore	0.769*	0.584	-0.239	0.222*	-0.135	0.123	0.286	0.331	0.000			
10. Flat-Tops	0.687*	0.528	-0.411	0.432	-0.066	0.250	0.366	0.435*	0.081	0.000		
11. Uinta	0.838*	0.700*	0.238	0.144	0.090	0.174	0.143	0.192	0.000	0.318	0.000	
12. Teton	0.823*	0.600	-1.000	-0.273	-0.511	-0.307	0.333	0.053	-1.000	-0.061	-1.000	0.000

Table 4.---Distribution and frequency of 20 mtDNA haplotypes and haplotype (h) and nucleotide (π) diversity, plus *SE* of each respective index, for *Clethrionomys gapperi* from 12 mountain ranges throughout the central Rocky Mountain region.

	Haplotypes														
Mountain Range (n)	A	B	R	Q	S	C	E	G	F	H	Z	I	J	K	T
Black Hills (6)	5	1													
Bighorn (6)			3	1	2										
Laramie (2)						2									
Medicine Bow (5)							2	2	1						
Sierra Madre (5)								4		1					
Gore (2)									1		1				
Absaroka (4)												1	1	2	
Beartooth (8)					1							1			2
Gros Ventre (6)												1		4	1
Bear River (5)												5			
Caribou (4)															3
Centennial (8)															5
Total	5	1	3	1	3	2	2	6	2	1	1	8	1	6	11

Table 4.---Extended.

Haplotypes								
M	O	X	U	V	*h*	*SE*	π	*SE*
					0.333	0.215	0.007	0.009
					0.733	0.155	0.024	0.020
					0.000	0.000	0.000	0.000
					0.800	0.164	0.082	0.057
					0.400	0.237	0.016	0.016
					1.000	0.500	0.098	0.107
					0.833	0.222	0.042	0.035
1	3				0.857	0.108	0.036	0.026
					0.600	0.215	0.017	0.016
					0.000	0.000	0.000	0.000
		1			0.500	0.265	0.010	0.012
			2	1	0.607	0.164	0.019	0.016
1	3	1	2	1	0.555	0.187	0.029	0.026

Table 5.---Pair-wise F_{st} values for *Clethrionomys gapperi* collected from 12 mountain ranges in the central Rocky Mountain region. F_{st} values marked with an asterisk are significant (Tablewide $\alpha = 0.05$) based on the sequential Bonferroni test (Rice 1989).

	Location											
	1	2	3	4	5	6	7	8	9	10	11	12
1. Black Hills	0.000											
2. Laramie	0.874*	0.000										
3. Medicine Bow	0.607*	0.092	0.000									
4. Sierra Madre	0.934*	0.895	0.349	0.000								
5. Absaroka	0.761	0.337	0.399	0.822*	0.000							
6. Gros Ventre	0.850*	0.630	0.522*	0.887*	-0.099	0.000						
7. Gore	0.449*	0.000	-0.089	0.735	0.359	0.586	0.000					
8. Bear River	0.942	1.000	0.500	0.939*	0.197	0.444	0.655	0.000				
9. Caribou	0.910	0.837	0.534	0.915	0.333	0.590	0.624	0.825	0.000			
10. Beartooth	0.726*	0.342	0.462	0.810*	0.151	0.308	0.459	0.252	0.080	0.000		
11. Bighorn	0.816*	0.538*	0.370*	0.845*	0.626*	0.730*	0.416	0.781*	0.782*	0.601*	0.000	
12. Centennial	0.855*	0.697*	0.612*	0.890*	0.402*	0.566*	0.671	0.643*	0.118	0.159	0.725*	0.000

Table 6.---Distribution and frequency of 21 mtDNA haplotypes and haplotype (h) and nucleotide (π) diversity, plus *SE* of each respective index, for *Microtus montanus* from 13 mountain ranges throughout the central Rocky Mountain region.

	Haplotypes															
Mountain Range	A	F	B	E	G	K	C	D	U	V	H	I	J	P	N	O
Laramie (2)	1	1														
Medicine Bow (11)	5		5	1												
Sierra Madre (4)	3				1											
Flat-Tops (3)	1		1			1										
Gore (1)	1															
Bighorn (15)							14	1								
Beartooth (10)	6								1	3						
Absaroka (6)	3										1	2				
Gros Ventre (13)	11												1	1		
Bear River (6)	2														3	1
Wind River (3)	1															
Caribou (5)	1															
Uinta (3)																
Total	35	1	6	1	1	1	14	1	1	3	1	2	1	1	3	1

Table 6.---Extended.

Haplotypes								
R	S	T	L	M	*h*	*SE*	π	*SE*
					1.000	0.500	0.119	0.127
					0.636	0.089	0.083	0.049
					0.500	0.265	0.068	0.051
					1.000	0.272	0.113	0.091
					1.000	0.000	0.000	0.000
					0.133	0.112	0.002	0.003
					0.600	0.130	0.011	0.011
					0.733	0.155	0.037	0.027
					0.295	0.156	0.018	0.014
					0.733	0.155	0.056	0.039
2					0.667	0.314	0.011	0.014
	2	2			0.800	0.164	0.061	0.043
			1	2	0.667	0.314	0.023	0.023
2	2	2	1	2	0.674	0.202	0.046	0.038

Table 7.---Pair-wise F_{st} values for *Microtus montanus* collected from 13 mountain ranges in the central Rocky Mountain region. F_{st} values marked with an asterisk are significant (Tablewide $\alpha = 0.05$) based on the sequential Bonferroni test (Rice 1989).

	Location												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Laramie	0.000												
2. Medicine Bow	-0.298	0.000											
3. Sierra Madre	-0.197	0.136	0.000										
4. Absaroka	0.308	0.382*	0.144	0.000									
5. Gore	-1.000	-0.059	-1.000	-0.467	0.000								
6. Flat-Tops	-0.512	-0.105	-0.160	0.148	-1.000	0.000							
7. Uinta	0.230	0.322	0.064	0.250	-0.333	0.077	0.000						
8. Bear River	0.235	0.370	0.129	0.336	-0.250	0.132	0.227	0.000					
9. Gros Ventre	0.461	0.433*	0.094	0.252	-0.952	0.269	0.234	0.277*	0.000				
10. Wind River	0.272	0.318	0.059	0.250	0.000	0.083	0.400	0.296	0.227	0.000			
11. Caribou	0.159	0.333	0.053	0.267	-0.500	0.062	0.147	0.001	0.218	0.061	0.000		
12. Beartooth	0.529	0.434*	0.184	0.272	-0.667	0.303	0.381	0.418	0.078	0.375	0.344*	0.000	
13. Bighorn	0.886*	0.708*	0.843*	0.869*	0.967	0.818*	0.941	0.810*	0.874*	0.957*	0.843*	0.962*	0.000

Table 8.---Distribution and frequency of 34 mtDNA haplotypes and haplotype (h) and nucleotide (π) diversity, plus *SE* of each respective index, for *Tamiasciurus hudsonicus* from 17 mountain ranges throughout the central Rocky Mountain region.

	Haplotypes															
Mountain Range (n)	A	D	B	C	E	F	G	H	L	J	U	AF	AG	I	K	M
Black Hills (9)	9															
Bighorn (13)	12	1														
Laramie (15)			9	2	1	3										
Medicine Bow (4)	4															
Sierra Madre (6)	4						1	1								
Gore (8)	3	1							4							
Rampart (12)	3		3							2	2	1	1			
Beartooth (8)	3									1				4		
Wind River (13)		1								2				6	3	1
Gros Ventre (8)														3		1
Caribou (5)										1				1	1	1
Wasatch (1)																1
Bear River (6)														1		
Centennial (1)															1	
Wet (11)																
Culebra (9)																
Sangre de Cristo (21)																
Total	38	3	12	2	1	3	1	1	4	6	2	1	1	15	5	4

Table 8.---Extended.

Haplotypes																				
				A								A			A	A	A	A		
N	O	P	V	H	Q	R	S	T	W	Y	B	Z	X	A	C	D	E	*h*	*SE*
																		0.000	0.000
																		0.154	0.126
																		0.619	0.120
																		0.000	0.000
																		0.600	0.215
																		0.679	0.122
																		0.878	0.059
																		0.679	0.122
																		0.756	0.097
1	1	1	1															0.893	0.111
				1														1.000	0.126
																		1.000	0.000
					2	1	1	1										0.933	0.121
																		1.000	0.000
									8	1	2							0.473	0.161
									8			1						0.222	0.166
									13				3	2	1	1	1	0.609	0.114
1	1	1	1	1	2	1	1	1	29	1	2	1	3	2	1	1	1	0.617	0.098

Table 8.---Extended.

π	SE
0.000	0.000
0.006	0.007
0.024	0.018
0.000	0.000
0.012	0.012
0.019	0.016
0.051	0.033
0.020	0.017
0.034	0.024
0.075	0.047
0.065	0.046
0.000	0.000
0.070	0.047
0.000	0.000
0.012	0.011
0.004	0.006
0.022	0.016
0.024	0.018

Table 9.---Pair-wise F_{st} values for *Tamiasciurus hudsonicus* collected from 17 mountain ranges in the central Rocky Mountain region. F_{st} values marked with an asterisk are significant (Tablewide $\alpha = 0.05$) based on the sequential Bonferroni test (Rice 1989).

	Location						
	1	2	3	4	5	6	7
1. Black Hills	0.000						
2. Bighorn	-0.031	0.000					
3. Laramie	0.436*	0.441*	0.000				
4. Medicine Bow	0.000	-0.130	0.336	0.000			
5. Sierra Madre	0.072*	0.040	0.359*	-0.081	0.000		
6. Wind River	0.408*	0.419*	0.486*	0.298	0.332*	0.000	
7. Gros Ventre	0.414*	0.450*	0.481*	0.265	0.310	0.209	0.000
8. Caribou	0.392*	0.419*	0.441*	0.195	0.249	-0.074	0.000
9. Centennial	1.000	0.733	0.437	1.000	0.500	-0.370	-0.133
10. Bear River	0.628*	0.648*	0.519*	0.479	0.514*	0.299*	0.122
11. Wasatch	1.000	0.926	0.753	1.000	0.846	0.632*	-0.314
12. Beartooth	0.529*	0.495*	0.499*	0.390	0.378	-0.059	0.255
13. Gore	0.308*	0.249	0.417*	0.158	0.185	0.380*	0.367*
14. Rampart	0.071	0.103*	0.187*	-0.040	0.019	0.238*	0.230
15. Wet	0.958*	0.947*	0.897*	0.941*	0.924*	0.872*	0.790*
16. Culebra	0.988*	0.970*	0.912*	0.982*	0.957*	0.885*	0.799*
17. Sangre de Cristo	0.908*	0.907*	0.879*	0.889*	0.881*	0.861*	0.801*

Table 9.---Extended.

Location									
8	9	10	11	12	13	14	15	16	17
0.000									
-0.636	0.000								
0.123	0.108	0.000							
0.250	1.000	0.227	0.000						
-0.039	0.016	0.362*	0.784	0.000					
0.315*	0.388	0.533*	0.774	0.419*	0.000				
0.137	-0.097	0.346*	0.424	0.185	0.107	0.000			
0.850*	0.931	0.850*	0.925	0.914*	0.903*	0.788*	0.000		
0.869*	0.978*	0.862*	0.976	0.938*	0.930*	0.801*	0.026	0.000	
0.843*	0.880	0.850*	0.870	0.885*	0.869*	0.789*	0.053	-0.002	0.000

FIG. 1.---Distribution of boreal habitats in the central Rocky Mountain region as modified from Findley and Anderson (1956). Numbers represent mountain ranges from which *Clethrionomys gapperi*, *Tamiasciurus hudsonicus*, *Microtus montanus*, and *Marmota flaviventris* were collected, including 1) Black Hills, 2) Bighorn, 3) Laramie, 4) Medicine Bow, 5) Sierra Madre, 6) Gore, 7) Rampart, 8) Flat-Tops, 9) Uinta, 10) Wasatch, 11) Bear River, 12) Gros Ventre, 13) Wind River, 14) Caribou, 15) Centennial, 16) Absaroka, 17) Beartooth, 18) Wet, 19) Sangre de Cristo, and 20) Culebra.

FIG. 2.---Position and sequences (identified by OSU numbers) of PCR amplifiation and sequencing primers used to amplify and sequence coding regions of the mitochondrial ND5 and ND6 subunits of the NADH dehydrogenase complex for *Clethrionomys gapperi*, *Microtus montanus*, *Tamiasciurus hudsonicus*, and *Marmota flaviventris*. All primers are listed 5′ to 3′. OSU 5258 and OSU 5257 represent flanking primers listed in Georgiadis (1996). * = primer and primer location for *C. gapperi* only; ** = primer and primer location for *T. hudsonicus* only.

FIG. 3.---Results of nested clade analysis of the geographic distance of ND5 and ND6 haplotypes of *Marmota flaviventris*. Haplotypes are listed at the top of the figure and grouped

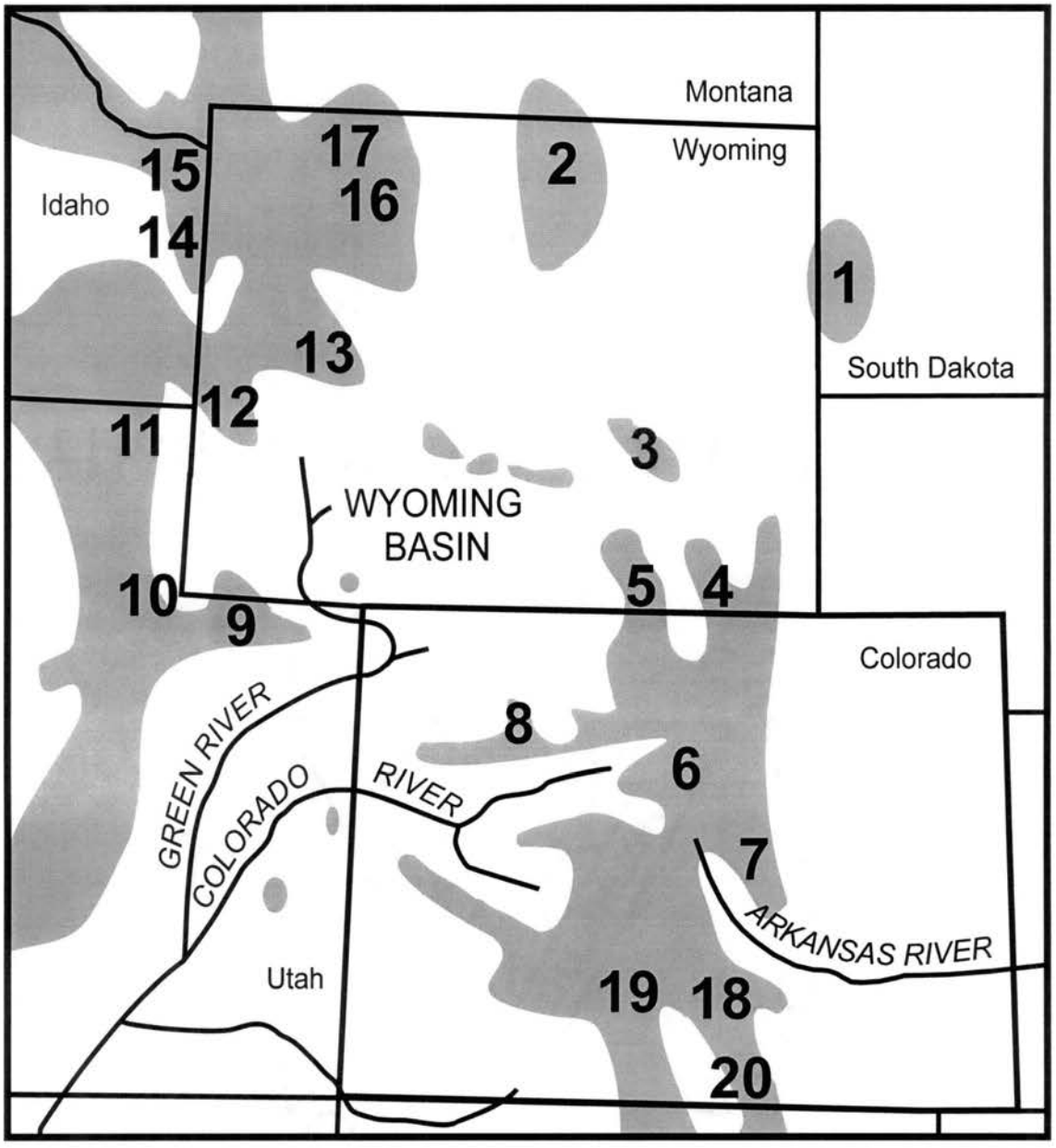
according to the clade nesting depicted in Appendix 2A. A superscript 'S' means that the distance measure was significantly small at the 5% level, and a superscript 'L' means that the distance measure was significantly large. See Materials and Methods for definitions of D_c, D_n, $(I\text{-}T)_c$, and $(I\text{-}T)_n$. Final inference for each clade was obtained from the key available at (http://bioag.byu.edu/zoology/crandall_lab/geodis.htm). IBD = restricted gene flow via isolation by distance; AF = allopatric fragmentation; IBD/Frag. = geographic sampling scheme inadequate to discriminate between isolation by distance or fragmentation.

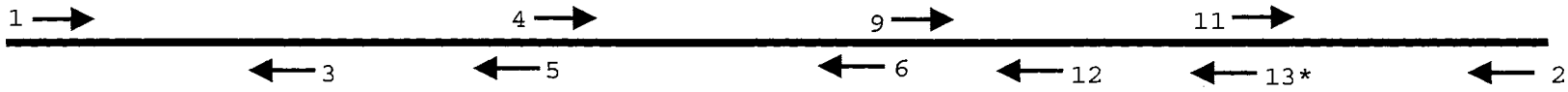
FIG. 4.---Results of nested clade analysis of the geographic distance of ND5 and ND6 haplotypes of *Clethrionomys gapperi*. Haplotypes are listed at the top of the figure and grouped according to the clade nesting depicted in Appendix 2B. A superscript 'S' means that the distance measure was significantly small at the 5% level, and a superscript 'L' means that the distance measure was significantly large. See Materials and Methods for definitions of D_c, D_n, $(I\text{-}T)_c$, and $(I\text{-}T)_n$. IBD = restricted gene flow via isolation by distance; AF = allopatric fragmentation.

FIG. 5.---Results of nested clade analysis of the geographic distance of ND5 and ND6 haplotypes of *Microtus montanus*. Haplotypes are listed at the top of the figure and grouped according to the clade nesting depicted in Appendix 2C. A superscript 'S' means that the distance measure was significantly small at the 5% level, and a superscript 'L' means that the distance measure was significantly large. See Materials and Methods for definitions of D_c, D_n, $(I\text{-}T)_c$, and $(I\text{-}T)_n$. IBD = restricted gene flow via isolation by distance; IBD/LDD = sampling design inadequate to discriminate between isolation by distance (short distance movements) versus long distance dispersal; IBD/Frag. = geographic sampling scheme inadequate to discriminate between isolation by distance or fragmentation.

FIG. 6.---Results of nested clade analysis of the geographic distance of ND5 and ND6 haplotypes of *Tamiasciurus hudsonicus*. Haplotypes are listed at the top of the figure and grouped according to the clade nesting depicted in Appendix 2D. A superscript 'S' means that the distance measure was significantly small at the 5% level, and a superscript 'L' means that the distance measure was significantly large. See Materials and Methods for definitions of D_c, D_n, $(I\text{-}T)_c$, and $(I\text{-}T)_n$. IBD = restricted

gene flow via isolation by distance; AF = allopatric fragmentation.

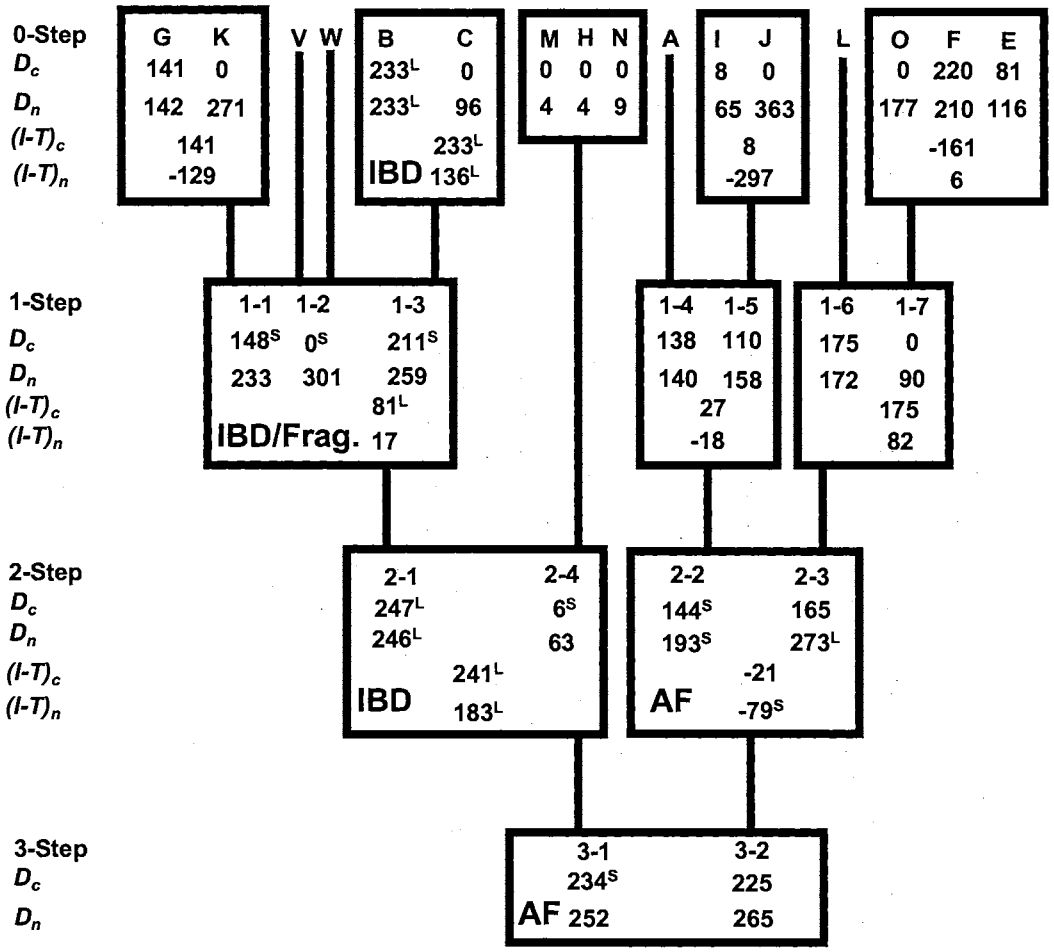

Montana
Wyoming
Idaho
South Dakota
Colorado
Utah
WYOMING
BASIN
GREEN RIVER
COLORADO
RIVER
ARKANSAS RIVER
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20



A) *Clethionomys gapperi* and *Microtus montanus*

B) *Tamiasciurus hudsonicus* and *Marmota flaviventris*

1.	OSU 5258	TTACAACGATGGGTTTTTCATRTCA	8.	OSU 8103	CCAACGCCTGAGCCCTAAT
2.	OSU 5257	AATAGTTTATCCRTTGGTCTTAGG	9.	OSU 8104	CCATRCCARRCACATC
3.	OSU 8067	GAYATRATDCCTACDCCTTC	10.	OSU 8138	TCWAACCAAAAAGGC
4.	OSU 8040	AACTCVTGAGAHCTHCAACA	11.	OSU 8140	ACTTCCTCTCATTCCTAA
5.	OSU 8041	GKRAKGGTAGYCAKGGGTG	12.	OSU 8179	GGTATTTAGTGTTATTG
6.	OSU 8054	AGGGCTCAGGCGTTGGT	13.	OSU 8180	GGTTTATGATGGTGATG
7.	OSU 8039	CCAWGGATGRAGRCCAAAT	14.	OSU 8182	GCCAGGGTGGTGGAGATTA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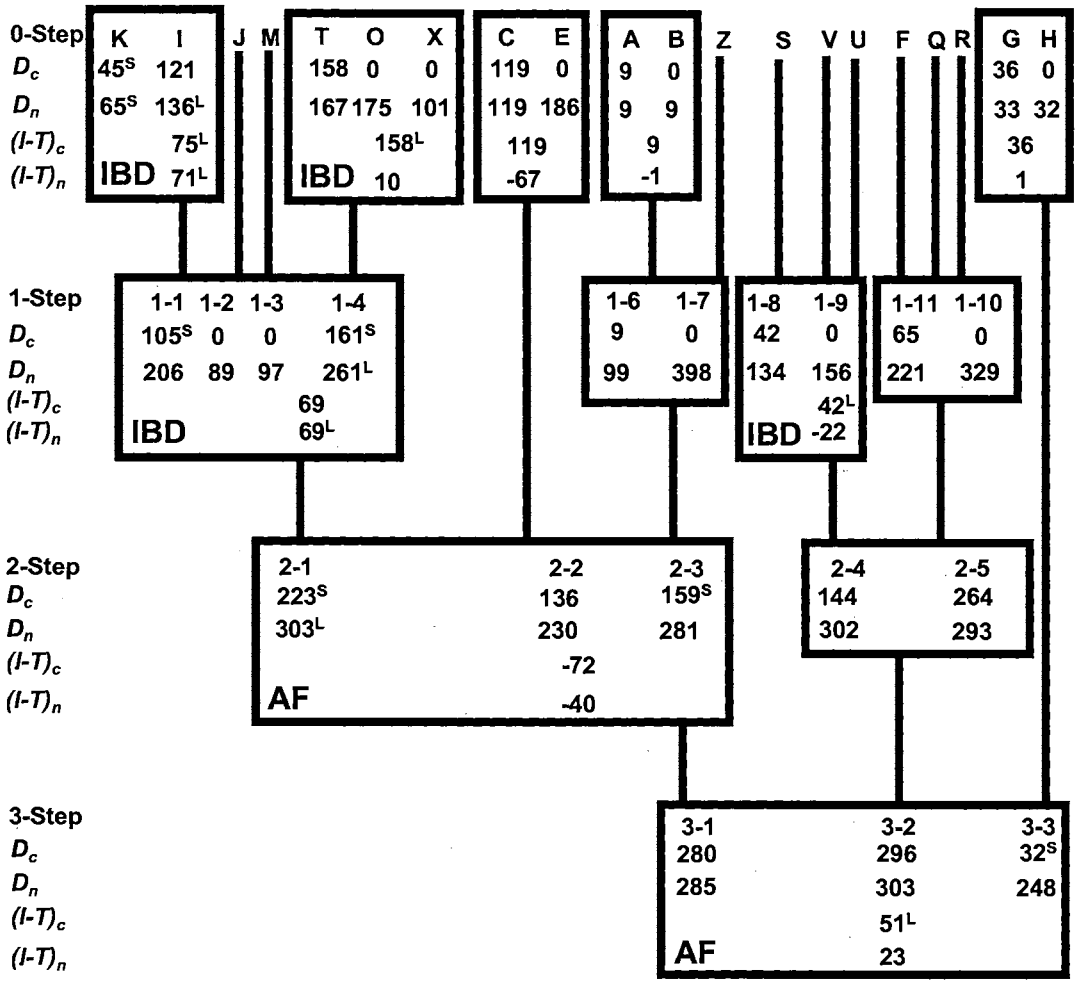
D_c
D_n
$(l\text{-}T)_c$
$(l\text{-}T)_n$
G K
141 0
142 271
141
-129
V W
B C
233^L 0
233^L 96
233^L
IBD 136^L
M H N
0 0 0
4 4 9
A
I J
8 0
65 363
8
-297
L
O F E
0 220 81
177 210 116
-161
6
1-Step
D_c
D_n
$(l\text{-}T)_c$
$(l\text{-}T)_n$
1-1 1-2 1-3
148^S 0^S 211^S
233 301 259
81^L
IBD/Frag. 17
1-4 1-5
138 110
140 158
27
-18
1-6 1-7
175 0
172 90
175
82
2-Step
D_c
D_n
$(l\text{-}T)_c$
$(l\text{-}T)_n$
2-1 2-4
247^L 6^S
246^L 63
241^L
IBD 183^L
2-2 2-3
144^S 165
193^S 273^L
-21
AF -79^S
3-Step
D_c
D_n
3-1 3-2
234^S 225
AF 252 265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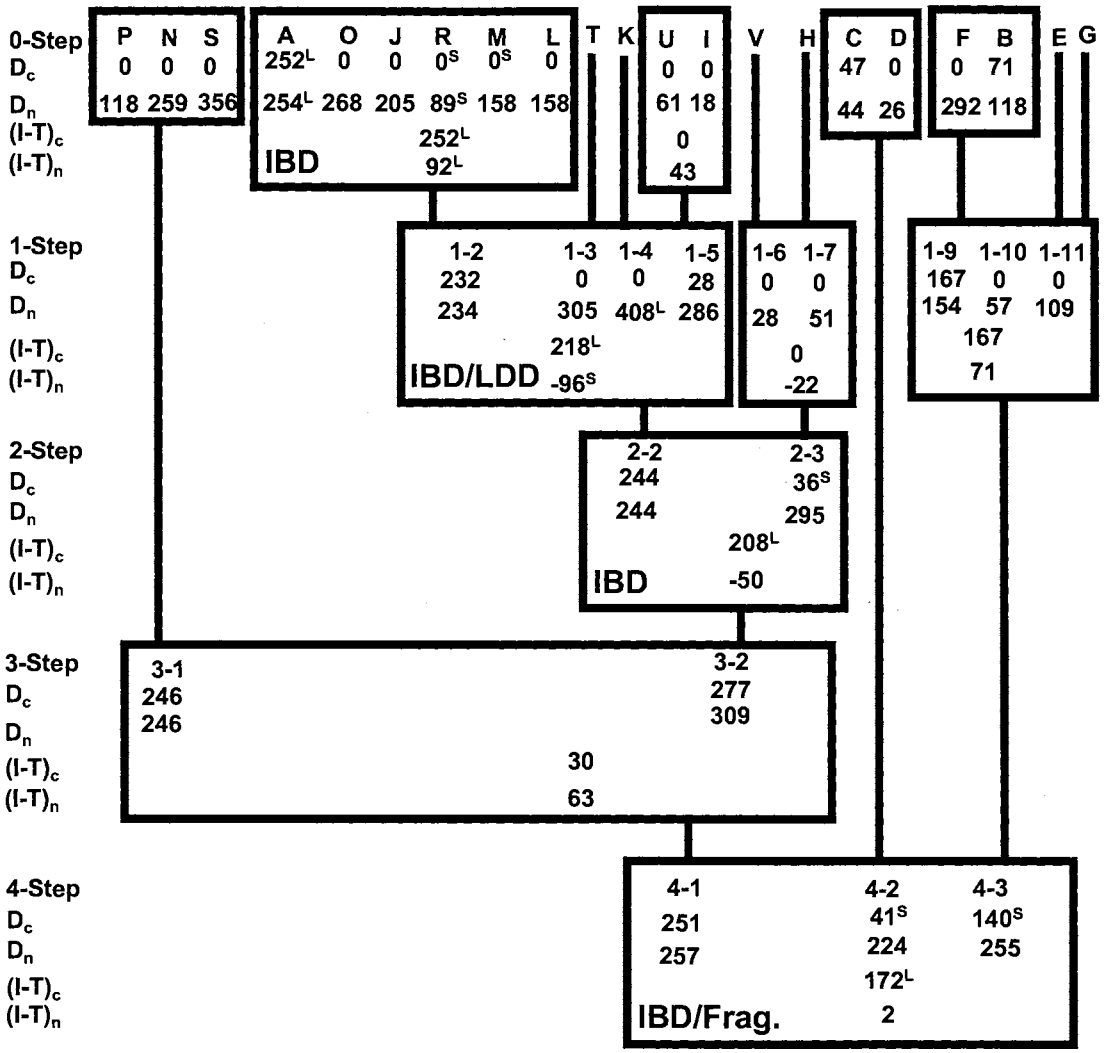
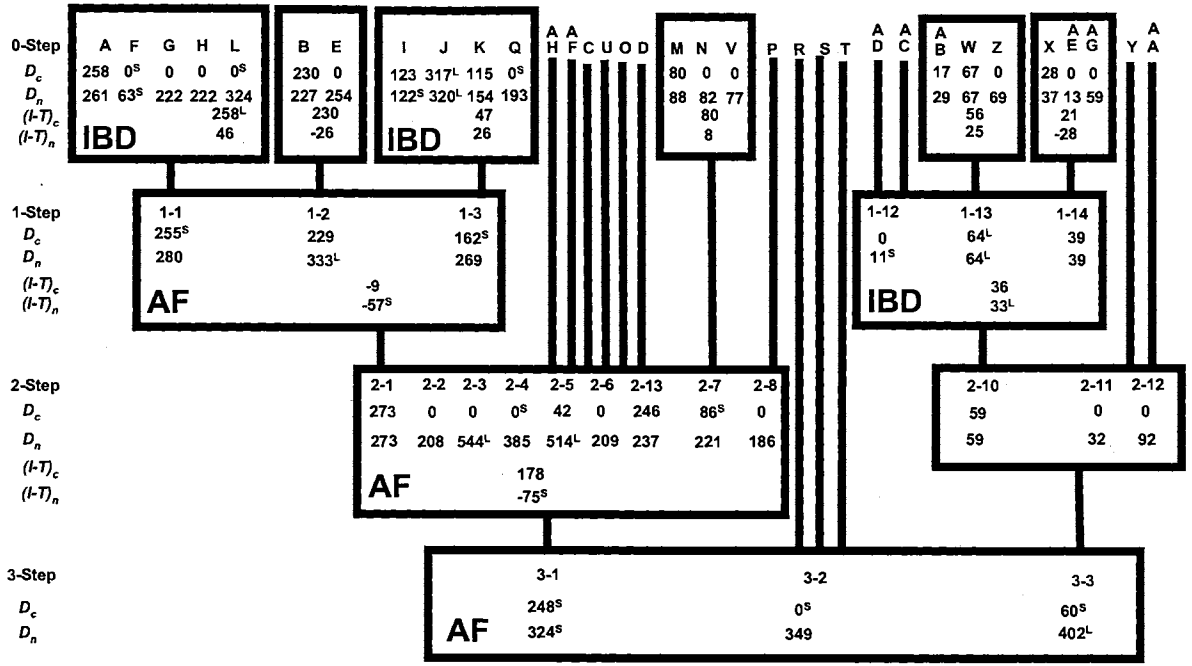
D_c
D_n
$(l\text{-}T)_c$
$(l\text{-}T)_n$
K I
45S 121
65S 136L
75L
IBD 71L
J M
T O X
158 0 0
167 175 101
158L
IBD 10
C E
119 0
119 186
119
-67
A B
9 0
9 9
9
-1
Z
S V U
F Q R
G H
36 0
33 32
36
1
1-Step
D_c
D_n
$(l\text{-}T)_c$
$(l\text{-}T)_n$
1-1 1-2 1-3 1-4
105S 0 0 161S
206 89 97 261L
69
IBD 69L
1-6 1-7
9 0
99 398
1-8 1-9
42 0
134 156
42L
IBD -22
1-11 1-10
65 0
221 329
2-Step
D_c
D_n
$(l\text{-}T)_c$
$(l\text{-}T)_n$
2-1
223S
303L
2-2
136
230
-72
AF -40
2-3
159S
281
2-4
144
302
2-5
264
293
3-Step
D_c
D_n
$(l\text{-}T)_c$
$(l\text{-}T)_n$
3-1
280
285
3-2
296
303
51L
AF 23
3-3
32S
248


0-Step
D_c
D_n
$(I-T)_c$
$(I-T)_n$
P N S
0 0 0
118 259 356
A O J R M L
252^L 0 0 0^S 0^S 0
254^L 268 205 89^S 158 158
252^L
92^L
IBD
T K
U I
0 0
61 18
0
43
V H
C D
47 0
44 26
F B
0 71
292 118
E G
1-Step
D_c
D_n
$(I-T)_c$
$(I-T)_n$
1-2 1-3 1-4 1-5
232 0 0 28
234 305 408^L 286
218^L
IBD/LDD -96^S
1-6 1-7
0 0
28 51
0
-22
1-9 1-10 1-11
167 0 0
154 57 109
167
71
2-Step
D_c
D_n
$(I-T)_c$
$(I-T)_n$
2-2 2-3
244 36^S
244 295
208^L
IBD -50
3-Step
D_c
D_n
$(I-T)_c$
$(I-T)_n$
3-1 3-2
246 277
246 309
30
63
4-Step
D_c
D_n
$(I-T)_c$
$(I-T)_n$
4-1 4-2 4-3
251 41^S 140^S
257 224 255
172^L
IBD/Frag. 2


0-Step
D_c
D_n
$(l\text{-}T)_c$
$(l\text{-}T)_n$
A F G H L
258 0S 0 0 0S
261 63S 222 222 324
258L
IBD
46
B E
230 0
227 254
230
-26
I J K Q
123 317L 115 0S
122S 320L 154 193
47
IBD
26
A H A F C U O D
M N V
80 0 0
88 82 77
80
8
P R S T
A D A C
A B W Z
17 67 0
29 67 69
56
25
A X A E A G
28 0 0
37 13 59
21
-28
Y A A
1-Step
D_c
D_n
$(l\text{-}T)_c$
$(l\text{-}T)_n$
1-1 1-2 1-3
255S 229 162S
280 333L 269
-9
AF
-57S
1-12 1-13 1-14
0 64L 39
11S 64L 39
IBD
36
33L
2-Step
D_c
D_n
$(l\text{-}T)_c$
$(l\text{-}T)_n$
2-1 2-2 2-3 2-4 2-5 2-6 2-13 2-7 2-8
273 0 0 0S 42 0 246 86S 0
273 208 544L 385 514L 209 237 221 186
178
AF
-75S
2-10 2-11 2-12
59 0 0
59 32 92
3-Step
D_c
D_n
3-1 3-2 3-3
248S 0S 60S
AF
324S 349 402L

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