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Molecular Phylogeny of the Marmots (Rodentia: Sciuridae): Tests of Evolutionary and Biogeographic Hypotheses

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Abstract.—There are 14 species of marmots distributed across the Holarctic, and despite extensive systematic study, their phylogenetic relationships remain largely unresolved. In particular, comprehensive studies have been lacking. A well-supported phylogeny is needed to place the numerous ecological and behavioral studies on marmots in an evolutionary context. To address this situation, we obtained complete cytochrome (cyt) *b* sequences for 13 of the species and a partial sequence for the 14th. We applied a statistical approach to both phylogeny estimation and hypothesis testing, using parsimony and maximum likelihood–based methods. We conducted statistical tests on a suite of previously proposed hypotheses of phylogenetic relationships and biogeographic histories. The cyt *b* data strongly support the monophyly of *Marmota* and a western montane clade in the Nearctic. Although some other scenarios cannot be rejected, the results are consistent with an initial diversification in North America, followed by an invasion and subsequent rapid diversification in the Palearctic. These analyses reject the two major competing hypotheses of *M. broweri*'s phylogenetic relationships—namely, that it is the sister species to *M. camtschatica* of eastern Siberia, and that it is related closely to *M. caligata* of the Nearctic. The Alaskan distribution of *M. broweri* is best explained as a reinvasion from the Palearctic, but a Nearctic origin can not be rejected. Several other conventionally recognized species groups can also be rejected. Social evolution has been homoplastic, with large colonial systems evolving in two groups convergently. The cyt *b* data do not provide unambiguous resolution of several basal nodes in the Palearctic radiation, leaving some aspects of pelage and karyotypic evolution equivocal. {Beringia; cytochrome *b*; Holarctic; hypothesis testing; *Marmota;* phylogenetics.}

There is a growing movement in systematics from simply making estimates of phylogeny to hypothesis testing and reliability estimation (Huelsenbeck and Rannala, 1997). This statistical perspective allows more precise delineation of which relationships are well understood and which need additional investigation. It also promotes the generation of explicit evolutionary and biogeographic models while providing the tools to reject hypotheses. These developments promise to accelerate our understanding of evolution by improving our hypotheses, defining well-resolved nodes of the phylogeny, and identifying unresolved relationships, thereby focusing our efforts more efficiently.

Here, we employ a statistical approach, using both parsimony and likelihood analyses of molecular sequence data to test a suite of previously proposed hypotheses of phylogenetic relationships in marmots (*Marmota*). A sequential phylogenetic estimation procedure was used that culminated in a maximum likelihood analysis utilizing a model of sequence evolution with parameters estimated from the data.

Marmots are large terrestrial rodents found today throughout much of northern Eurasia and North America, including the Bering Strait region of western Alaska and eastern Siberia (Fig. 1). Their ecology and ethology has been studied extensively (e.g.,

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Armitage, 1975, 1987; Barash, 1989; Arnold, 1990; Blumstein et al., 1997) and thus, along with their close relatives the ground squirrels and prairie dogs, marmots have figured prominently in general theories of mammalian social evolution. Social systems vary widely in marmots, from the solitary *monax* to the large, almost prairie dog-like colonies of *bobak, sibirica,* and *himalayana.* Moreover, the phylogenetic relationships, derived from morphological and karyological data, between marmots and related ground squirrels are poorly known and controversial (Black, 1963; Gromov et al., 1965; Hafner, 1984), as are relationships within the genus (Hoffman and Nadler, 1968; Vorontsov et al., 1969; Bibikov, 1996). There are no well-supported and corroborated phylogenetic hypotheses within the genus. Explicit models of marmot social evolution have been proposed (Barash, 1989) but they cannot be tested without a robust phylogeny.

Marmots also provide biogeographic insights to an important but still insufficiently well understood faunal interchange across the Bering land bridge (Beringia). During the Plio–Pleistocene period of the last several million years, this region was an important connection between the two hemispheres during periods of glacial maxima, when the Bering land bridge was in existence. During interglacial times, however, this region has been a water barrier to the movement of terrestrial mammals, the rising sea level flooding the land bridge to form the Bering Strait (Hopkins et al., 1982; Hoffmann, 1984). Fossil evidence suggests that marmots first arose in North America (Black, 1963). Their spread into Eurasia would likely have occurred across Beringia, but the timing of this event is only loosely constrained by currently available fossil evidence (Gromov et al., 1965; Savage and Russell, 1983). The Alaskan species *M. broweri* has been proposed to be a reinvasion of the Nearctic from the Palearctic (Hoffmann et al., 1979), owing to its hypothesized Palearctic affinities, and thus may provide additional information re-

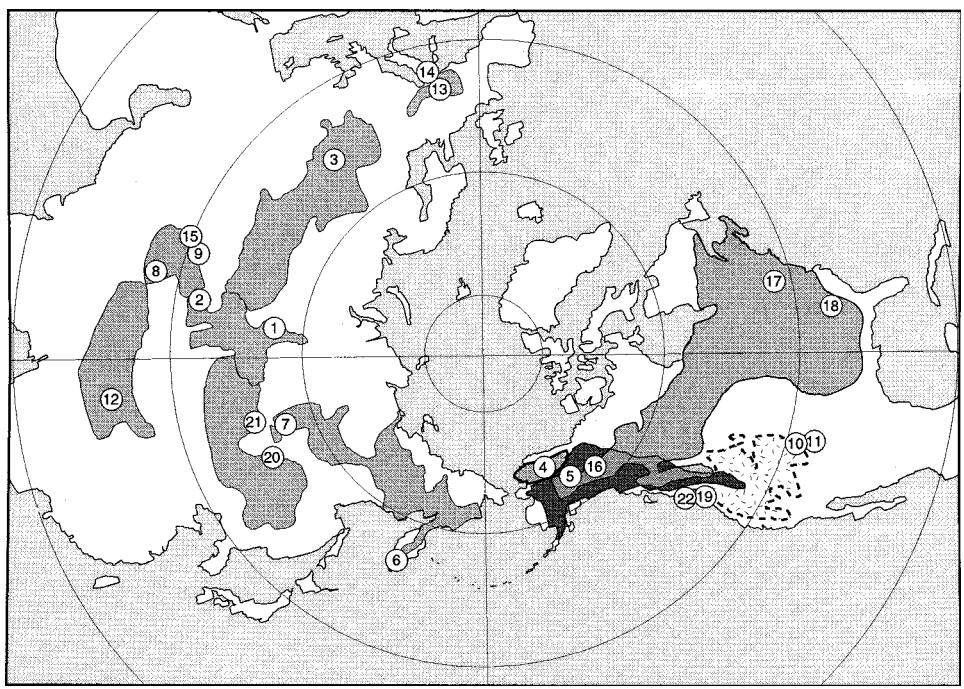

FIGURE 1. Geographic distribution of *Marmota* species. Numbers indicate collecting localities: 1 = *baibacina* 1; 2 = *baibacina* 2; 3 = *bobak* 1 and 2; 4 = *broweri* 1 and 2; 5 = *caligata;* 6 = *camtschatica* 1; 7 = *camtschatica* 2; 8 = *caudata* 1; 9 = *caudata* 2 and 3; 10 = *flaviventris* 1; 11 = *flaviventris* 2; 12 = *himalayana;* 13 = *marmota* 1; 14 = *marmota* 2; 15 = *menzbieri;* 16 = *monax* 1; 17 = *monax* 2; 18 = *monax* 3; 19 = *olympus;* 20 = *sibirica* 1; 21 = *sibirica* 2; 22 = *vancouverensis.*

garding the timing and directionality of the faunal interchanges.

Taxonomic Background

The taxonomy of the genus *Marmota*, particularly its Old World members, has until recently been unstable. Ellerman and Morrison-Scott (1951) recognized only three widely ranging species of this genus in the Palearctic, *M. bobak* (incl. *himalayana* and *sibirica*), *M. caudata,* and *M. marmota* (including *camtschatica, baibacina,* and *menzbieri*). Rausch (1953) extended their polytypic species concept of *M. marmota* to also include *M. caligata* (incl. *M. broweri, olympus,* and *vancouverensis*). In contrast, Hall and Kelson (1959) placed *caligata* (incl. *M. broweri*), *olympus,* and *vancouverensis* at the species level, as well as *flaviventris* and *monax,* which had been universally regarded as distinct species. Thus, in the 1950s, at the height of popularity of the "biological species concept" (Huxley, 1940), only five or six species of Holarctic marmots were accepted by many systematists.

The following decades, which saw the widespread application of karyological data to systematic questions, resulted in a turnabout. In the Palearctic, eight taxa were considered full species, and in the Nearctic, six, for an Holarctic total of 14 (Gromov et al., 1965; Rausch and Rausch, 1965; Hoffmann and Nadler, 1968; Vorontsov et al., 1969; Vorontsov and Lyapunova, 1970; Rausch and Rausch, 1971). Their status remains the same at present (Hoffmann et al., 1993).

The first attempt to assess relationships among species of marmots was that of Howell (1915), who defined three species groups of Nearctic marmots, the *caligata* group (also including *olympus* and *vancouverensis*), the *flaviventris* group, and the *monax* group; Howell also pointed out similarities between the latter and the Palearctic *M. marmota.* Ognev (1947) suggested affiliation of *bobak, himalayana, baibacina,* and *sibirica,* and possibly, *camtschatica* and *caligata* in one group; he believed *marmota, caudata,* and *menzbieri* each to be separate groups. Gromov et al. (1965) modified Ognev's groupings; their *bobak* group included *baibacina, himalayana, sibirica,* and *camtschatica,* these latter linking the *bobak* group with *marmota,* which they affiliated with *monax.* They also considered the Alaskan *broweri* as a representative of the Asiatic marmots in the Nearctic, while recognizing the *caligata* group (including *olympus* and *vancouverensis*) as close to the *marmota* group. Finally, Lyapunova et al. (1992) considered *camtschatica* a member of the American, rather than the Asian, group of marmots.

The first comprehensive hypothesis of the origin and evolution of the genus *Marmota* in the Holarctic was published by Hoffmann and Nadler (1968). On the basis of both morphological and chromosomal characters as well as the fossil record, marmots were at that time supposed to have evolved from ground squirrels in North America in the early Pliocene. Radiating from there, a species that probably resembled *monax* and *marmota* migrated into Eurasia across the Bering land bridge in the late Pliocene, and reached western Eurasia by the Pleistocene. A radiation in North America gave rise to the *monax, flaviventris,* and *caligata* groups, while a Eurasian radiation produced *marmota* and the Asiatic marmots (*bobak* group, *menzbieri,* and *caudata*), plus *broweri,* which migrated back across the Bering land bridge in the late Pleistocene to northern Alaska. *M. broweri* thus represented the Asiatic *camtschatica* (of the *bobak* group) in the Nearctic (Hoffmann et al., 1979).

All Palearctic species excluding *marmota* possess black-tipped guard hairs, which are softer and finer than the guard hairs in the rest of the genus and has led to those Palearctic species being heavily exploited for their fur. The greater insulation provided by fine, dense hair has been suggested to be advantageous to marmots spreading across the Palearctic during the Pleistocene, when the climate was rapidly cooling in Siberia. According to Zimina and Gerasimov (1973:335), ". . . modern habitats of *M. baibacina* and *M. camtschatica* may be similar to the conditions under which marmots lived in the periglacial zone . . . {being} better adapted to periglacial conditions . . ." If this scenario is correct, black-tipped guard hairs would represent one of the few explicit morphological synapomorphies and biogeographically significant adaptations.

A Priori Hypotheses

Many biogeographic and evolutionary models make predictions about phylogenetic relationships; they can therefore be translated into phylogenetic trees, which can then be evaluated using statistical tests (Fig. 2). We tested a series of hypotheses that either represented strongly argued hypotheses from the systematic literature or were particularly important to the biogeography or social and chromosomal evolution of marmots. The phylogenetically explicit hypotheses tested herein are: 1) *Marmota* is not monophyletic; 2) *broweri* and *camtschatica* are sister species (Hoffmann et al., 1979); 3) *broweri* and *caligata* are conspecific or sister species (Rausch and Rausch, 1971; Hall, 1981). Hypotheses 2 and 3 imply dichotomous biogeographic scenarios, involving either a dispersal or vicariant event across the Bering land bridge (hypothesis 2) or simple local differentiation (hypothesis 3). The following biogeographic and evolutionary scenarios were also evaluated (with phylogenetic hypotheses): 4) There was a single crossing of Beringia (Palearctic species monophyletic; the most-parsimonious biogeographic scenario); 5) original diversification was in North America (North American species basal and paraphyletic; paleontological hypothesis); 6) black-tipped hairs evolved once without reversal (monophyly of all Asian species excluding *marmota;* proposed morphological synapomorphy); 7) increased sociality to large colonies evolved once without reversal (monophyly of *bobak, sibirica, himalayana;* most-parsimonious ethological scenario); 8) there has been minimum karyotypic evolution among Nearctic species, consistent with the transformation series $2n = 38$ (most marmots) $\rightarrow$ 40 (*olympus*) $\rightarrow$ 42 (*caligata, flaviventris, vancouverensis*).

METHODS

Specimens Examined

Twenty-five specimens representing all 14 currently recognized species in the genus

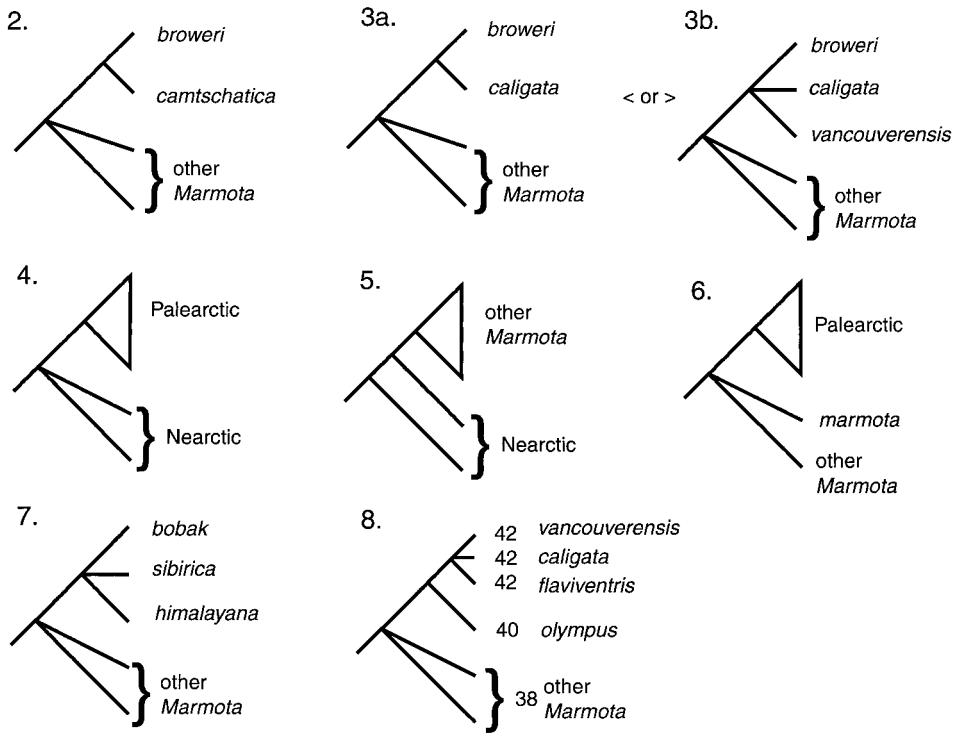

FIGURE 2. Model cladograms representing a priori hypotheses presented in the text. Hypothesis 1, *Marmota* monophyly, is not shown. Numbers on hypothesis 8 represent chromosome numbers.

Marmota (Hoffmann et al., 1993) were sequenced for this study. Five species are represented by one specimen, and the remaining nine by two or more specimens, usually from different localities and assignable to named subspecies (see Appendix). The endangered species *vancouverensis* was imported under USFWS permit no. PRT-802845. Additional taxa included in the analyses as outgroups were five published ground and tree squirrel sequences—*Sciurus carolinensis, Spermophilus (Spermophilus) columbianus, S. (S.) richardsoni, S. (Callospermophilus) lateralis,* and *S. (Ictidomys) tridecemlineatus* (Thomas and Martin, 1993)—and four unpublished ground squirrel sequences supplied by R. Harrison and P. Sherman: *S. (Otospermophilus) beecheyi, S. (S.) fulvus, S. saturatus,* and *S. (S.) undulatus.* Sequences have been submitted to Genbank and assigned accession numbers AF14314–39.

DNA Extraction, Amplification, and Sequencing

Total genomic DNA was extracted from frozen liver or blood of 24 individuals (all but *olympus*) by following standard phenol/chloroform extraction techniques (Sambrook et al., 1989). The entire cytochrome (cyt) *b* gene was amplified from the mitochondrial genome via polymerase chain reaction (PCR) using primers L14725 (P484; TGAAAAAYCATCGTTGT) and H15915 (P485; TYTYCWTYTTNGGTTTACAARAC) modified from the universal mammalian primers of Irwin et al. (1991). L and H refer to the light and heavy strands, respectively, and the numbers following them refer to the position of the 3' base of the primers in relation to human mtDNA (Anderson et al., 1981). P numbers refer to specific primer batches in use at the Laboratory of Molecular Systematics, Smithsonian Institution. Amplifications were performed in 50–100 μl total reaction volumes containing 1.5–3.0 mM $MgCl_2$, 1.0 μM of each primer, 5.0 U of *Taq* DNA polymerase or Amplitaq Gold (Perkin-Elmer–Cetus or Promega, respectively) in a buffer supplied by the respective enzyme manufacturer. Typical cycling conditions were initial denaturation at 94–95°C for 3 min 45 s (17 min for Amplitaq Gold), followed by 25 to 30 cycles of denaturation (1 min at 94–95°C), primer annealing (1 min at 52–55°C), and DNA extension (2 min at 72°C). A final extension for 7 min at 72°C was included to minimize the number of partial strands. Five microliters of the double-stranded product was electrophoresed on a 1% agarose gel, stained with ethidium bromide, and visualized under UV to assess reaction success. The products were then cleaned by filtration through Ultrafree-MC 100,000 NMWL filters (Millipore Corp., Bedford, MA) or by precipitation with polyethylene glycol (PEG) in saline (NaCl) and resuspended in 25 μl of purified H_2O. One to two microliters of the cleaned PCR product was electrophoresed again to estimate concentration visually by comparison with size standards.

For initial sequencing of the double-stranded templates, we used T7 DNA polymerase in the Sequenase 2.0 kit (United States Biochemical, Cleveland, OH), following the manufacturer's protocols. The products of the sequencing reaction were resolved in 6% polyacrylamide gels, which were autoradiographed for 5 to 10 days at room temperature and read manually. The initial sequences were confirmed and extended by automated sequencing of the full-length amplicons with use of 26 to 40 ng of double-stranded template and ABI Prism dye terminator sequencing chemistry on ABI 373A (Perkin-Elmer) automated sequencers. All 24 individuals were sequenced completely in both directions for the entire cyt *b* gene by using the amplication primers and the following sequencing primers: P499 (L14994: 5'-TGACTTATCCG CTATATACA-3'), P1185 (L15279: 5'-AA AGCYACYYTAACACGATT-3'), L15615 (5'-ATTCTTAACAAACTAGGAGG-3'), P1188 (L15615: 5'-ATCCCCAACAAATTAGGAG G-3'), P1189 (H15756: 5'-CRGGYTGTCCT CCRATTC-3'), P500 (H15481: 5'-CTCCTA GAAGGTCAGGTGAA-3'), P386 (H15149: 5'-TTTCTGCAGCCCCTCAGAATGATATT TGTCCTCA-3'), P1190 (H14993: 5'-ATTATT GATGCACCGTTRGCATG-3'). Additional primers were used for manual sequencing: P442 (L14841: 5'-AAGCTTCCATCCAAC A-3'), P586 (L15171: 5'-CAAATKTCATTYT GAGGNGCAAC-3'), P509 (L15350: 5'-GK TTKTTIGAICCIGTYTC-3'), P443 (H15498: 5'-CTGCAGGGAATAAAGT-3').

Because no frozen tissue was available for *olympus,* dried tissue adhering to a cleaned museum skull was collected from a specimen prepared in 1927. DNA was extracted from ~2 mg of dry tissue by grinding in liquid nitrogen, adding 100 µl of Chelex to the ground tissue, and incubating at 98°C for 10 min. Three pieces of tissue were processed and extraction-negative controls were prepared for each. All extractions were conducted in a laboratory that has never been exposed to PCR products.

For *olympus,* standard PCR procedures were used except that experiments were run on the three extraction-positive and three extraction-negative samples in addition to the PCR-positive and PCR-negative controls used throughout. Nine primer pair combinations with amplicon sizes ranging from 113 to 308 bp were used. For those primer pairs that resulted in products, all three extractions showed product in repeated experiments, whereas negatives usually showed none. In the two cases where a faint band was visible in a negative control (both involving the same primer pairs), the product was purified and sequenced for comparison. A BLAST search in GenBank on the potential contaminants returned human cyt *b* as the closest matches for both, which were otherwise >20% divergent from the probable *olympus* products produced during the same experiments. These *olympus* products were <6.5% divergent in sequence—but not identical to—the other Nearctic species to which *olympus* was expected to be most closely related. Sequencing of *olympus* PCR products was as specified above except that a total of 577 bp nucleotides were determined (on both strands).

Phylogeny Estimation

Sequences were aligned by using Sequencher 3.0 (Gene Codes Corp.). Alignments were unambiguous because insertions and deletions were absent and protein structure was sufficiently conserved. Pairwise genetic distances (e.g., absolute and maximum likelihood) were calculated and all phylogenetic analyses were conducted by using a beta test version of PAUP* 4.0d61a (D. Swofford, pers. comm.) Trees were rooted by using *Sciurus.* Examination of preliminary results indicated that the region between 700 and 900 bp downstream from the start codon of a published *flaviventris* sequence (Thomas and Martin, 1993) was highly divergent from all other taxa, including 16.7% Kimura two-parameter (K2P distance; Kimura, 1980) from our *flaviventris* over this region. Analyzing this 200-bp region separately from the rest of the gene produced cladograms in which the published *flaviventris* was a divergent branch outside the *Marmota* clade. All well-supported relationships among other taxa from the analyses on all the data were otherwise recovered from this fragment. We also sequenced two other *flaviventris* that were 2.5% divergent over this region. Given the uncertainty regarding that published sequence, we did not include it in the analyses.

Phylogenetic analyses were conducted by using distance (neighbor joining; NJ), maximum parsimony (equally weighted, MP; weighted parsimony, WP), and maximum likelihood (ML) criteria. A sequential optimization approach (Fratti et al., 1997) was used to estimate the phylogeny. Initial trees were generated by NJ with use of multiple distance measures and by MP with all characters weighted equally. The distance metric used in NJ had no effect on topology. Transversion/transition ratios (tv/ts) and relative variability for first, second, and third codon positions were estimated by using ML with a K2P model on the MP tree. WP analyses were then conducted with character weights for transversions and codon position based on the inverse of the estimated evolutionary rates (tv/ts = 7:1, 7:1, 12:1 for the three codon positions respectively, and positions were weighted 1st:2nd:3rd = 5:12:1). All MP and WP analyses used heuristic searches with tree bisection–reconnection (TBR) branch-swapping and 30 random addition replicates. ML parameter values were estimated under a nested array of substitution models for each of these three trees: NJ, MP, and WP (Fratti et al., 1997). These models were Jukes–Cantor (JC: Jukes and Cantor, 1969), K2P (Kimura, 1980), HKY85 (Hasegawa et al., 1985), and general time reversible (GTR). Each of these models was adjusted for among-site rate variation in four ways:

All sites were assumed to have equal rates, a portion of the sites was assumed to be invariable (I), rates among all sites were assumed to vary following a gamma distribution (Γ: Yang, 1994), and a combination of invariable sites and gamma-distributed rates was used. The combination of four substitution and four rate-distribution models resulted in each tree being evaluated for 16 models. Estimated parameter values differed by <3% among the three starting topologies within each model.

Because each of the models can be considered a special case of the most general GTR + I + Γ, a likelihood-ratio test can be used to test for significant differences in the fits of the models (Yang et al., 1995), with the degrees of freedom being equal to the difference in the number of parameters. The GTR + I + Γ model was a significantly better fit for each of the trees. A ML search was then conducted by using the GTR + I + Γ model with parameters fixed to the values estimated on all three initial trees. Heuristic searches were conducted with 20 random addition replicates and TBR branch-swapping. All three searches yielded the same single topology. A final ML analysis utilizing the same search conditions was conducted by using the parameters estimated from the topology produced by the first three ML searches.

Robustness and Hypothesis Testing

Robustness of the results for each set of analyses (initial NJ, MP, WP, and final ML) was estimated for 100 bootstrap replicates (Felsenstein, 1985). Decay (or Bremer support) indices (Bremer, 1994) were calculated for selected nodes by searching for the shortest trees containing the hypothesized constraint under equal-weighting.

A priori hypotheses were tested by using parsimony- and likelihood-based approaches. Equally weighted MP searches were conducted with constraints enforced to match predicted topologies for each hypothesis. Differences in tree lengths between constrained searches and the most-parsimonious tree were tested by using the Kishino–Hasegawa (Kishino and Hasegawa, 1989) and Templeton (Templeton, 1987) tests. Because the topology of the reference (optimal) tree was not defined a priori, we applied one-tailed tests at the 2.5% significance level as a conservative test. The same approach was employed with ML by using the optimal ML tree as the reference.

TABLE 1. Nucleotide composition averaged over the sciurid species examined in this study.

Codon position	A	C	G	T
First	0.292	0.245	0.211	0.253
Second	0.199	0.238	0.140	0.423
Third	0.375	0.296	0.027	0.303
Total	0.289	0.260	0.126	0.326

Results

Each *Marmota* sequence has an open reading frame of 1140 bp. There were 507 variable sites, 399 of which were parsimony-informative. Only 25% of the variable sites involve nonsynonymous substitutions. Nucleotide composition is very close to mean mammalian values (Irwin et al., 1991) for first and second positions (Table 1). The sciurids examined in the study (including outgroups) show a higher frequency of Ts at the third position (0.303 vs. 0.103–0.221), with correspondingly lower

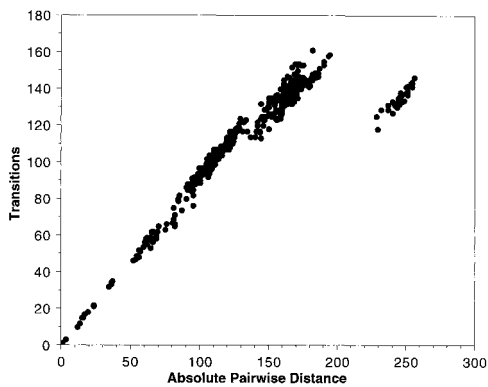

FIGURE 3. Saturation curve for transitions. The pairwise comparisons of absolute number of differences fall into three groups: within genera (<134 total distance), between *Marmota* and *Spermophilus* (134–200), and between *Sciurus* and all others (>200). The comparison between *Sciurus* and *M. olympus* is deleted for clarity because the combination of fewer nucleotides in *M. olympus* and saturation effects makes it appear incorrectly to be an outlier.

TABLE 2. Distance matrix for all *Marmota* sequenced. Absolute number of differences above diagonal; maximum likelihood distances using model and parameters (GTR + I + Γ) estimated from ML tree below the diagonal.

	1	2	3	4	5	6	7	8	9	10	11
(1) olympus	0	37	34	36	36	68	66	64	56	56	63
(2) flaviventris 1	0.083	0	17	66	65	116	117	114	126	123	132
(3) flaviventris 2	0.073	0.016	0	60	61	113	114	113	118	115	127
(4) vancouverensis	0.080	0.072	0.063	0	13	106	107	106	119	116	121
(5) caligata	0.079	0.070	0.064	0.012	0	107	108	107	116	113	123
(6) monax 2	0.205	0.151	0.146	0.138	0.138	0	15	16	109	110	109
(7) monax 3	0.191	0.152	0.146	0.138	0.138	0.014	0	19	112	113	114
(8) monax 1	0.186	0.146	0.145	0.137	0.137	0.015	0.018	0	111	112	107
(9) marmota 1	0.149	0.166	0.151	0.156	0.150	0.140	0.143	0.141	0	3	115
(10) marmota 2	0.149	0.161	0.146	0.151	0.144	0.142	0.145	0.144	0.003	0	114
(11) broweri 1	0.166	0.177	0.168	0.161	0.163	0.140	0.148	0.136	0.146	0.144	0
(12) broweri 2	0.171	0.179	0.170	0.164	0.166	0.142	0.150	0.138	0.148	0.146	8.8e-4
(13) menzbieri	0.127	0.155	0.140	0.159	0.158	0.143	0.145	0.140	0.136	0.137	0.139
(14) caudata 3	0.146	0.142	0.136	0.141	0.147	0.110	0.123	0.119	0.115	0.117	0.115
(15) caudata 2	0.146	0.142	0.136	0.141	0.147	0.110	0.123	0.119	0.115	0.117	0.115
(16) caudata 1	0.145	0.141	0.139	0.147	0.145	0.120	0.131	0.126	0.117	0.119	0.114
(17) baibacina 2	0.151	0.142	0.131	0.131	0.131	0.124	0.114	0.123	0.138	0.137	0.132
(18) baibacina 1	0.167	0.154	0.138	0.144	0.144	0.136	0.127	0.139	0.144	0.146	0.143
(19) bobak 1	0.142	0.136	0.127	0.136	0.136	0.116	0.120	0.122	0.127	0.129	0.134
(20) bobak 2	0.163	0.142	0.133	0.144	0.144	0.121	0.125	0.127	0.135	0.137	0.139
(21) himalayana	0.189	0.156	0.141	0.163	0.167	0.127	0.121	0.117	0.133	0.135	0.150
(22) sibirica 2	0.168	0.151	0.140	0.163	0.167	0.134	0.127	0.130	0.131	0.133	0.131
(23) sibirica 1	0.169	0.153	0.141	0.163	0.167	0.134	0.127	0.130	0.132	0.134	0.131
(24) camtschatica 2	0.136	0.171	0.158	0.171	0.168	0.155	0.146	0.148	0.135	0.137	0.160
(25) camtschatica 1	0.134	0.170	0.154	0.167	0.164	0.156	0.146	0.148	0.134	0.136	0.153

frequencies of A (0.375 vs. 0.363–0.474) and C (0.296 vs. 0.329–0.479). There was no significant variation in nucleotide composition among the sciurids. Transitions show no evidence of saturation within *Marmota* but do appear to be saturated in comparisons between *Sciurus* and the marmotines (Fig. 3), a pattern commonly observed for mammalian groups with these cyt *b* divergences. Uncorrected and ML estimates (GTR + I + Γ; in parentheses) in divergence are as follows: within species, 0–3.2%, $\bar{x}$ = 1.27%, n = 13 (0–3.5%; $\bar{x}$ = 1.36%); among *Marmota* species, 1.1–11.9%; $\bar{x}$ = 8.5%, n = 300 (1.2– 20.5%; $\bar{x}$ = 13.3%); and between *Marmota* and *Spermophilus,* 11.8–17.0%, $\bar{x}$ = 14.7%, n = 125 (19.6–37.2%; $\bar{x}$ = 27.6%) (Table 2). Only one interspecific distance overlaps the range of values observed for intraspecific comparisons: *caligata* and *vancouverensis* were 1.2% distant (Fig. 4). All other interspecific ML distances exceeded 6%. Estimated ts/tv ratios (K2P) for each codon position were 7.7, 6.5, and 11 for first, second, and third positions, respectively. The aligned sequences are available from the *Systematic Biology* Website (www.utexas.edu/ftp/depts/systbiol).

There is agreement among the different phylogenetic analyses for all well-supported nodes. The equally weighted (MP) analysis produced a single tree, 1586 steps long. The weighted-parsimony analysis (WP) produced a single tree 7231 steps long. Monophyly of *Marmota* is supported by 100% bootstraps in all analyses and a decay index of 19. Two major clades constitute *Marmota.* A western North American (Nearctic) clade is formed by *caligata, flaviventris, olympus,* and *vancouverensis* (node A; Fig. 5). This clade is well supported, with 100% ML bootstrap, and a decay index of

TABLE 2. Extended

12	13	14	15	16	17	18	19	20	21	22	23	24	25
64	52	57	57	57	59	62	56	61	66	62	62	54	53
133	121	113	113	113	112	118	110	112	121	118	119	129	128
128	112	110	110	112	106	109	105	107	112	111	112	122	119
122	121	111	110	114	105	111	109	112	123	123	123	128	125
124	121	115	115	114	106	112	110	113	126	126	126	127	124
110	111	92	92	98	100	107	96	98	103	107	107	120	119
115	113	101	101	105	95	102	99	101	100	104	104	115	114
108	110	98	98	102	100	109	100	102	97	105	105	116	115
116	109	96	96	97	110	113	104	108	107	107	107	109	108
115	109	97	97	98	109	114	105	109	108	108	108	110	109
1	110	96	96	95	105	111	107	109	116	105	105	123	118
0	111	97	97	96	106	112	108	110	117	106	106	124	119
0.142	0	62	62	63	94	107	100	105	99	107	106	122	116
0.117	0.066	0	0	23	92	99	94	96	94	91	91	107	105
0.117	0.066	0	0	23	92	99	94	96	94	91	91	107	105
0.116	0.068	0.022	0.022	0	94	102	101	103	94	93	93	110	110
0.134	0.112	0.109	0.109	0.112	0	36	68	68	99	96	96	108	101
0.145	0.134	0.120	0.120	0.125	0.035	0	67	69	99	98	98	107	98
0.136	0.119	0.111	0.111	0.122	0.075	0.074	0	12	96	91	91	99	90
0.141	0.129	0.116	0.116	0.126	0.076	0.078	0.011	0	101	94	94	100	91
0.152	0.120	0.111	0.111	0.112	0.120	0.121	0.114	0.122	0	70	70	84	81
0.133	0.133	0.107	0.107	0.110	0.114	0.117	0.106	0.112	0.076	0	0	85	84
0.133	0.131	0.107	0.107	0.110	0.114	0.117	0.106	0.112	0.077	0	0	85	84
0.162	0.156	0.131	0.131	0.136	0.134	0.133	0.117	0.120	0.097	0.097	0.097	0	23
0.155	0.147	0.129	0.129	0.137	0.124	0.120	0.104	0.107	0.093	0.097	0.097	0.022	0

10, which increases to 17 if *olympus* is excluded because of the smaller number of bases sequenced for *olympus* (577). The second major clade (node B) includes the remaining 10 species, with a high bootstrap of 95% but a decay index of only 1. This high bootstrap but low decay index illustrates that these two indices measure different aspects of nodal support as well as the potential impact of refined evolutionary models. Bootstrap values for this node in the other analyses are 44% (MP), 63% (NJ), and 89% (WP). The clade is unambiguously supported by two unique transversions at first and third positions (at positions 997 and 870; plus 12 homoplastic transitions) that receive weights of 1 in decay index calculations but have more influence on the more parameter-rich analyses.

Within this major clade (B), all analyses show very short basal branches and weak resolution. The MP and ML trees both place *monax* as sister to the remaining species, which are all Palearctic except for *broweri*. Support for this node (Palearctic species plus *broweri*) is weak, with only 36% ML bootstrap. The NJ tree places *monax* as sister to the *sibirica–baibacina* clade, whereas WP places it sister to *marmota* plus *broweri*. The European *marmota* appears to be a basal member of clade B, either as sister to all remaining clade B species (ML, NJ, MP) or as the sister species to *broweri* (WP), but again, none of the deeper nodes in clade B is well supported. The best supported clades in this group are the southwest Asian species pair *caudata–menzbieri* (100% bootstrap, decay index of 12), the central/western pair *bobak–baibacina* (95% bootstrap, decay index of 6), and an eastern *camtschatica* group (95% bootstrap, decay index of 6), which includes the probable species pair *sibirica*—

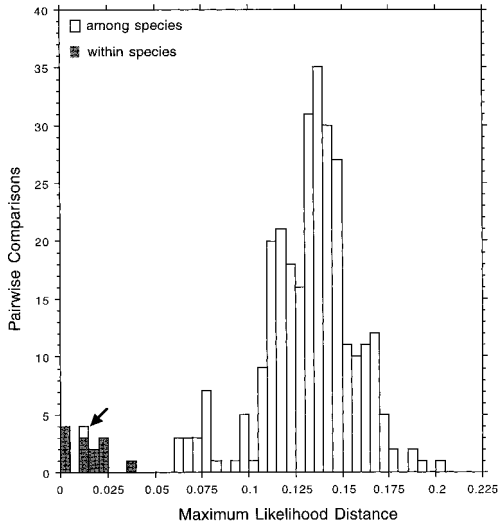

FIGURE 4. Histogram of maximum likelihood genetic distances, calculated using the same model (GTR + I + Γ) and parameters as in the ML phylogenetic analysis. The single interspecific distance <5% (*caligata/vancouverensis*) is indicated by the arrow.

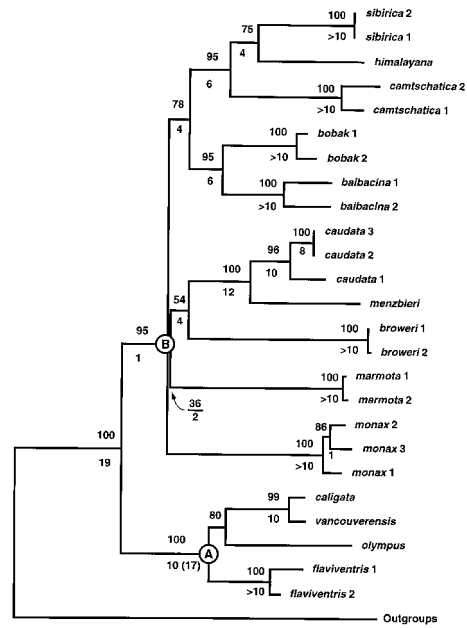

FIGURE 5. Maximum likelihood phylogram of *Marmota* obtained by using best-fitting model (GTR + I + Γ) and parameters. ML bootstrap percentages are above the branches; decay indices calculated for the MP tree are below the branches. Outgroups are pruned for presentation.

himalayana (75% bootstrap, decay index of 4). The *bobak* and *camtschatica* groups appear to be sister taxa with moderate support (78% bootstrap, decay index of 4), and all the analyses except WP place the Alaskan *broweri* as sister to the *caudata* group (54% ML bootstrap, decay index of 4).

Among the four western Nearctic species, the species pair of *caligata* and *vancouverensis* is well supported at 99% ML bootstrap and decay index of 10. Other relationships are uncertain. ML and MP place *olympus* as the sister species to *caligata* plus *vancouverensis,* whereas NJ and WP place it one node lower. In all analyses, the branch leading to *caligata/vancouverensis/olympus* or *caligata/vancouverensis/flaviventris* is short but receives moderate bootstrap support (63–80%), given that there are effectively only three possible alternate placements.

Hypothesis Testing

Using the optimal trees from the MP and ML analyses, we performed Kishino–Hasegawa and Templeton tests on the a priori hypotheses presented in the Introduction and in Figure 2. These tests were evaluated in terms of the estimates of support for each node from the cyt *b* data. Results are summarized in Table 3.

1. Marmota *is not monophyletic.*—Rejected. All analyses strongly support the monophyly of *Marmota.* Trees conforming to the alternative hypothesis are significantly longer (1605 steps, $P = 0.008$–0.02) and less likely (8108 log-likelihood units, $P < 0.0001$). With 100% bootstrap support and a decay index of 19, *Marmota* is the best supported supraspecific clade in the analysis.
2. M. broweri *and* M. camtschatica *are sister species.*—Rejected. The most thoroughly expounded presentation of this hypothesis was by Hoffmann et al. (1979). Most, but not all, equally most-parsimonious trees under this constraint were significantly worse, depending on the particular character distributions associated with the different topologies. Although some topologies consistent with this hypothesis cannot be rejected by all tests

TABLE 3. Results of Kishino–Hasegawa (KH) and Templeton (T) tests for alternative hypotheses. Hypothesis numbers refer to those used in the text and Figure 2. MP and ML scores are for those optimal trees conforming to the a priori constraints. *P*-values are for the constrained trees, determined by using KH and T tests.

Hypothesis	MP	*P* (KH–MP)	*P* (T–MP)	ML	*P* (KH–ML)
1	1605	0.0077–0.009	0.021–0.022	8108	<0.0001
2	1601	0.011–0.051	0.027–0.078	8069	0.029
3a	1641	<0.0001	<0.0001	8133	<0.0001
3b	1621	0.0002	0.0006	8115	<0.0001
4	1596	0.13	0.17	8061	0.11
5	1586	na[a]	na	8046	na
6	1588	n.s.	n.s.	8054	n.s.
7	1602	0.0002	0.0009	8070	0.023
8	1587	n.s.	n.s.	8055	n.s.

[a]na = not applicable, because test tree identical to optimal tree.

(i.e., $0.05 < P < 0.08$ for one of the tests), all constrained topologies are rejected by at least one test.

3. M. broweri *and* M. caligata *are conspecific or sister species.*—Rejected. Because the original proposals did not exclude the possibility that *vancouverensis* was as closely related to *caligata* as was *broweri,* we divided this hypothesis into two subhypotheses; the strict interpretation 3a that *broweri* and *caligata* are most closely related, and the less restrictive interpretation 3b, which allows *vancouverensis* to be most closely related to one of the former species. Both alternatives are strongly rejected, and even the less restrictive alternative requires 35 extra steps ($P = 0.0001–0.0002$). We can be confident in excluding *broweri* from the *caligata* group sensu stricto, thus making the scenario of a return invasion of North America by *broweri* more plausible (see hypothesis 4).
4. *Single crossing of Beringia.*—Not rejected. This biogeographic scenario would suggest that a monophyletic group exists that encompasses all the Palearctic species and no others. Nearctic species could be either monophyletic or paraphyletic with respect to the Palearctic species. Yet, optimal trees in each analysis nested the Alaskan *broweri* within an otherwise Palearctic group, suggesting that it may be derived from a secondary dispersal across Beringia. However, the alternative hypothesis presented in Figure 2 is not significantly longer or less likely than the optimal trees ($P = 0.11–0.17$). Thus, the possibility that *broweri* has retained an ancestral Beringian distribution and separated from the Palearctic clade by a single vicariance event cannot be rejected, and either single or multiple crossings of Beringia are consistent with current data.
5. *Original diversification in North America.*—Not rejected. If the fossil evidence is correct and *Marmota* originated in North America, one might expect that the root of the tree would lie within North America, leaving the Nearctic species basal and paraphyletic with respect to the Palearctic species. That is precisely the pattern seen in the optimal trees, with *monax* more closely related to the Palearctic species (including *broweri,* node B, Fig. 5) than to the western Nearctic clade (node A). This indicates that North American diversification is the most likely scenario, given the data. However, two additional hypotheses can be evaluated; reciprocal monophyly of these two groups, which would be equivocal regarding the biogeographic scenario, and the Palearctic group being basal and paraphyletic, which would suggest an origination in the Palearctic. Trees conforming to these alternatives are five and three steps longer, respectively. These are not significant differences, and thus the statistical tests do not provide a basis for choosing among these scenarios.
6. *Black-tipped hairs evolved once.*—Not rejected. Assuming that black-tipped hairs

evolved once with no reversals requires monophyly of all Palearctic species (plus *broweri*) to the exclusion of *marmota.* This involves moving *marmota* one node basally so that it is sister to the remaining Palearctic clade. Such trees are only two steps longer and are not significantly different. Therefore, the mitochondrial data are uninformative regarding this aspect of pelage evolution.

7. *Increased sociality to large colonies evolved once.*—Rejected. Assuming no reversal, this hypothesis implies that *bobak, sibirica,* and *himalayana* form a monophyletic group. All the optimal trees place *himalayana* and *sibirica* together, but *bobak* is always the sister species to *baibacina.* The shortest trees consistent with hypothesis 7 are 16 steps longer ($P = 0.0002–0.0009$) and differ by 24 log-likelihood units ($P = 0.023$). Thus the possibility that this component of social evolution has been homoplastic has strong support. This homoplasy thus allows the examination of associations between the gain or loss of coloniality and other factors to which it might be an adaptation. If coloniality has been a unique case, no association could be assessed.
8. *Minimum karyotypic evolution among Nearctic species, consistent with the transformation series* 2n = *38* → *40* → *42.*—Not rejected. Assuming no homoplasy, this transformation series predicts that *olympus* is the sister species to a clade formed by *caligata, flaviventris,* and *vancouverensis.* The ML and MP analyses nest *olympus* within this clade, whereas NJ and WP are consistent with the karyotypic hypothesis. MP and ML trees matching the prediction are only one step longer and slightly less likely, and thus hypothesis 8 can not be rejected. The mitochondrial data remain equivocal regarding the precise relationship of *olympus,* although it clearly belongs to a western Nearctic clade.

Thus, hypotheses 1 (*Marmota* not monophyletic), 3a (*broweri* sister to *caligata*), 3b (*broweri* sister to *caligata/vancouverensis*), and 7 (coloniality evolved once) can be rejected. Significance levels are marginal for hypothesis 2 (*broweri* sister to *camtschatica*), but hypotheses 4 (single crossing of Beringia), 5 (original diversification in North America), 6 (black-tipped hairs evolved once), and 8 (simple karyotypic transformation) cannot be rejected.

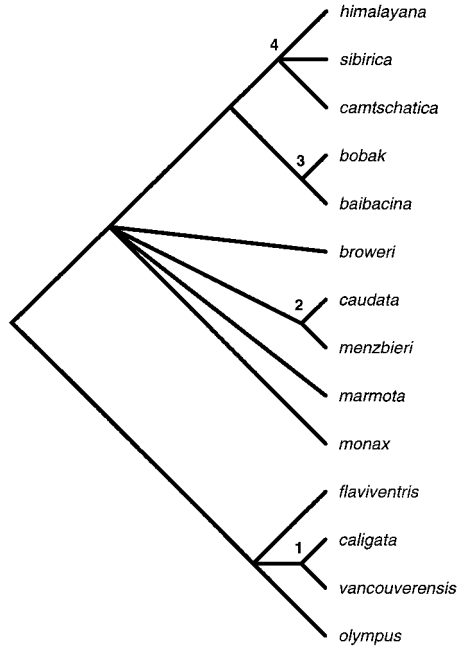

FIGURE 6. Summary topology incorporating clades with robust support that provides the basis of the classification presented in the text. 1 = *caligata* group; 2 = *caudata* group; 3 = *bobak* group; 4 = *camtschatica* group.

DISCUSSION

Systematics

Figure 6 summarizes those phylogenetic hypotheses about which we are confident. In accordance with those results, we propose the classification scheme in Table 4.

Petromarmota, subgen. nov., is defined as the most recent common ancestor of *M. olympus, M. flaviventris,* and *M. caligata* and all its descendants. This western North American clade is characterized by dorsal pelage with light-tipped guard hairs and a white nose and chin. A light patch is also present between or just in front of the eyes, separated from the white nose by a dark brown to black transverse stripe (Hoffmann et al., 1979). Basal members of the nominal subgenus, *M. (Marmota) marmota* and *M.*

TABLE 4. Proposed classification of the genus *Marmota.*

Genus Marmota Blumenbach, 1779
Synonyms: *Marmota* Frisch, 1775; *Glis* Erxleben, 1777; *Arctomys* Schreber, 1780; *Lagomys* Storr, 1780; *Lipura* Illiger, 1811; *Marmotops* Pocock, 1922
Subgenus *Petromarmota* Subgen. Nov.
flaviventris
caligata group
caligata
vancouverensis
olympus
Subgenus *Marmota*
monax
marmota
broweri
caudata group
caudata
menzbieri
bobak group
bobak
baibacina
camtschatica group
camtschatica
himalayana
sibirica

(M.) monax, also possess light-tipped dorsal guard hairs and a light-colored nose, but lack the contrasting light-and-dark pattern on the face seen in *Petromarmota,* most prominent in *M. (P.) flaviventris* and *M. (P.) caligata.* The facial pattern is present but subdued in *M. (P). olympus* (more noticeable in subadults and juveniles), but is obliterated in the melanistic allospecies *M. (P.) vancouverensis* (Hoffmann et al., 1979). The etymology refers to the rocky habitat preference of the new subgenus's constituent species; type species, M. caligata.

The terminal clades of the subgenus *Marmota,* namely, the *bobak* and *camtschatica* groups, have a solid black cap from the tip of the nose backward, merging into the black-tipped dorsal guard hairs; their winter pelage is long and soft, in contrast to *Petromarmota* and the less derived species of subgenus *Marmota.* All species in this subgenus except *M. broweri* have yellowish to bright ochraceous fur covering the venter.

Evolutionary History

The most recent survey of the genus was that of Bibikov (1996). In his view, *Marmota* first appeared in the Central Asian mountains in the Pliocene, adapted to montane meadow and steppe habitats. However, he considers the oldest of the living species to be the North American marmots, plus *marmota* of Western Europe. North America and Asia were independent loci of speciation; in the former, *monax* became secondarily adapted to lowland forest and meadow, and in the latter, various species adapted to both xeric steppe and periglacial steppe–tundra and permafrost. Both *caudata* and *menzbieri* represent early Asian branches, whereas a *baibacina*-like ancestor gave rise to the modern *bobak* group, with its related taxa, *himalayana, sibirica,* and *camtschatica.* Thus, he believes that *bobak* and *himalayana/sibirica* evolved into xeric steppe populations in parallel. Bibikov also suggests that *broweri* derived from the lineage represented by *camtschatica* and then colonized Alaska.

In contrast, the molecular data are consistent with the traditional view that the genus arose in North America. The oldest fossil of *Marmota* is now known to be from the middle Miocene of Nebraska (Clarendonian/Hemphilian) (Black, 1963; Savage and Russell, 1983), whereas the oldest Old World fossils are Pliocene to early Pleistocene (Bibikov, 1996; Savage and Russell, 1983). The oldest fossil of a modern species is assignable to *monax* from the mid-Pleistocene (Irvingtonian) in several sites in the southeastern USA, within the present range of the species (Kurten and Anderson, 1980). Giboulet et al. (1997) date the divergence of marmots from some ground squirrels at 6 million years ago (MYA), based on DNA hybridization evidence. However, given the evidence of *Spermophilus* paraphyly (Thomas and Martin, 1993), a different selection of *Spermophilus* more closely related to *Marmota* could have had their data support an even earlier divergence for *Marmota.* This again illustrates the importance of careful taxon sampling in applications of the molecular clock. Still, the estimated date is at odds with a revised North American paleontological time scale, which places the earliest species of *Marmota, vetus,* at 9.5 MYA (J. Alroy, pers. comm.).

Other modern species are not known until later in the Pleistocene. These records support Bibikov's argument that the New World species are, among the surviving Ho-

larctic species, the older radiation, and this conclusion is supported by paraphyly of Nearctic species in our phylogenetic tree (Fig. 6). The tree does not support a close relationship between *monax* and *marmota,* as advocated by Hoffmann and Nadler (1968) and Bibikov (1996). Instead, the similarity between these taxa may be due to retention of plesiomorphic character states. The remaining species of *M. (Marmota)* form two large clades, one consisting of *broweri, menzbieri,* and *caudata,* and the other two species groups (*bobak* and *camtschatica*) that were placed in the *bobak* group by Bibikov and many earlier authors.

The major surprise in our analysis is the position of *broweri* within the tree. After its specific distinctness was recognized (Rausch and Rausch, 1965), most authors believed it to be closely allied with *camtschatica,* representing a late Pleistocene migration to Alaska from Siberia and subsequent speciation (Hoffmann and Nadler, 1968; Hoffmann et al., 1979; Bibikov, 1996). This hypothesis receives little support from our analyses. However, Rausch and Rausch (1971:96) stated: "From the evidence now available, we consider *broweri* to be probably a relict North American species which became established in the Brooks Range during pre-Wurm time, rather than a late Pleistocene invader of middle Asian derivation." If they meant that *broweri* was a member of a paraphyletic North American grade, possibly sister to a Palearctic group, then their hypothesis cannot be rejected. If however, they meant that *broweri* is a member of an exclusively North American clade (e.g., *Petromarmota*), then their hypothesis is rejected strongly by the cyt *b* data (results not shown).

The long-tailed, or red marmot (*caudata*) and Menzbier's marmot (*menzbieri*) are the most divergent morphologically of all the members of the genus; the former is the largest, with the longest tail, and the latter is the smallest, with the shortest tail. Nevertheless, they form a monophyletic group with strong support from our data. Bibikov (1996) remarks that they share certain primitive features with the American marmots (rougher pelage and bright, contrasting color with light spots on sides of head and body), and earlier immunological and nucleotide sequence comparisons indicated that the two species were closer to each other than to species in the *bobak* or *camtschatica* groups (Baranov and Vorontsov, 1973; Zholnerovskaya et al., 1992; Lyapunova et al., 1995). Genetic distances between these two species (6.5–6.8%) are similar to other closely related taxa: *flaviventris* and *caligata/vancouverensis* (6.3–7.2%), *baibacina* and *bobak* (7.4–7.8%), and *himalayana* and *sibirica* (7.6%). Their montane Middle Asian distributions are contiguous and are the smallest ranges of any of the Asian marmots.

The other large clade comprises the *bobak* and *camtschatica* groups. The first consists of *baibacina* and *bobak;* the other includes the species *himalayana, sibirica,* and *camtschatica.* These species are allo- to parapatric in distribution across an enormous expanse of territory, from the Ukraine to Kamchatka in far northeastern Siberia, and share similar karyotypes ($2n = 38$; FN = 64–66) except for *camtschatica* ($2n = 40$; FN = 62) (Lyapunova and Vorontsov, 1969; Vorontsov et al., 1969). These species exhibit different patterns of habitat adaptations. The most generalized in this respect is *baibacina,* which occupies montane steppe and meadow in the Altai (*M. b. baibacina*) and Tien Shan (*M. b. centralis*) mountains but is also found in foothill woodland in southern Siberia and in rolling hills of central Kazakhstan, where it is in contact with *M. bobak shaganensis.* Hybrids between the two species have been reported in this contact zone (Kapitonov, 1966; Sludskii, 1969), but specimens from Kizil-Rai and Karkaralinsk retain their morphological distinction, and there is no evidence of intergradation between the two species (Nikol'skii et al., 1983). *M. bobak,* the sister species to *baibacina* in our tree, is more specialized, is able to occupy xeric steppes, and forms large colonies; Bibikov (1996) regarded it as having diverged from the more generalized *baibacina.*

The *camtschatica* group consists of the species pair *himalayana* and *sibirica,* and *camtschatica,* which is more divergent in morphology, ecology, and karyology (Baranov and Vorontsov, 1973; Vorontsov and Lyapunova, 1984). All three of these species are adapted to life in the permafrost zone, as is the American *broweri.* The tarbagan

(*sibirica*) is similar morphologically to *baibacina,* and there were early reports of intergradation between *M. b. baibacina* and *M. s. caliginosus* in the montane steppes of the western Mongolian Altai (Bannikov, 1954). However, Ziminia (1978) provided evidence of specific separation, and Smirin et al. (1985), analyzing the contact zone, found that the two taxa occupied distinct colonies and differed in vocalizations; Sokolov and Orlov (1980) also indicated sympatry in the contact zone, although limited hybridization is possible.

Both species occupy montane steppe habitats but differ in preferred biotopes. "In the areas of joint settlement the gray marmots {*M. b. baibacina*} occupy wet meadow slopes and interfluvial areas . . . while *M. s. sibirica* usually settle in the dry steppe valleys" (Bibikov, 1996:36). Like *bobak, sibirica* is adapted to more open, rolling landscapes and avoids steep, rocky terrain; because "the type of settlement is a steppe . . . , the animals are distributed rather evenly . . ." and larger colonies are formed than is the case with montane species (Bibikov, 1996:36).

The sister species to *sibirica* is *himalayana,* distributed widely on the Tibetan Plateau. Because it is quite similar morphologically to members of the *bobak* group, and its distribution is entirely allopatric relative to other species in the group, it has sometimes been considered a race of *bobak.* However, the genetic distances (Table 2) and tree topology show it is well separated from that species. Its habitat varies geographically; around the edges of the plateau, it is found in rocky montane biotopes similar to those frequented by *baibacina* and American montane marmots, but in areas of less rugged relief across the expanse of the plateau, it occupies the flat to rolling open surface of the alpine steppe and semidesert. There it occurs in large colonies (Jameson, 1847) similar to those formed by *bobak* and *sibirica.*

This pattern seems to indicate that the adaptation of marmot lineages to xeric open steppe habitats has occurred independently in several species, and that these adaptations relate to a number of facets of their biology, including seasonality, food habits, and social behavior. A similar parallelism can also be seen in the adaptations of *camtschatica* and *broweri* to the extremes of cold and short growing season in northeast Siberia and northern Alaska, respectively. As noted above, most previous authors have considered *broweri* to be the sister species to *camtschatica*—an Asian marmot that migrated into Alaska in the late Pleistocene—but as Rausch and Rausch (1971) postulated, the cyt *b* tree suggests that its ancestry goes back at least to the beginning of the radiation of the Asian marmots (Zimina and Gerasimov, 1973).

Despite similarities of geography and Pleistocene history between the Olympic Peninsula and Vancouver Island (Hoffmann, 1981), the relationship of the two marmots endemic to these regions, *olympus* and *vancouverensis,* differ. The latter is very closely related to *caligata,* which occurs in the adjacent Coast Ranges of British Columbia as well as more widely in the northern Cascades and Rocky Mountains. The low genetic divergence of *vancouverensis* from *caligata* (1.2%; Table 2, Fig. 5), suggests that it is recently isolated, but fixed differences not only in color and vocalizations but also habitat and behavior (Barash, 1989) indicate that it is an insular allospecies of the superspecies *caligata* (Hoffmann et al., 1979). In contrast, *olympus* may be a basal member of *Petromarmota* (Fig. 5); its genetic distance from *caligata, vancouverensis,* and *flaviventris* is greater—5.3–6.8% (GTR distance, calculated only over the 577 nucleotides), about the same level as the species pairs *bobak–baibacina* (5.8–7.0%) or *himalayana–sibirica* (6.1–6.4%) for the same regions of the gene. The Olympic marmot may be a relict species that differentiated during isolation in an early- to mid-Pleistocene ice-free refugium (Hoffmann, 1981).

After the acceptance of this manuscript, a marmot phylogeny was published by Kruckenhauser et al. (1999). Their study included complete cyt *b* sequences for 10 of the 14 species and yielded the same MP phylogeny as ours (after pruning the four species) with one exception. They found *M. olympus* to be most closely related to *vancouverensis,* in contrast to its basal position in *Petromarmota* in our analyses. The two *olympus* sequences are 9% divergent. When both data sets are analyzed together, their

olympus and *vancouverensis* form a clade that is sister to our *vancouverensis,* whereas the remaining topology matches that reported here. We think it likely that at least one of the *olympus* samples was contaminated. The procedures we used to preclude and test for that possibility are discussed in Methods. Neither *olympus* sequence appears to be related to the pseudogenes discovered by Kruckenhauser et al. (1999), being >14% divergent (absolute differences). The *olympus* and *vancouverensis* sequences from Kruckenhauser et al. (1999) differ at seven positions, four of which result in unique amino acid substitutions (among the 47 sequences) and three of those being between hydophobic and ambivalent amino acids. Despite the greater overall divergence seen between our *olympus* and other *Petromarmota,* only 5 of 34–37 differences are nonsynonymous, and only 2 of those are unique to *olympus.* Given that their *olympus* was a museum skin collected in 1950 and that they preferentially amplified a pseudogene, the pattern of nucleotide substitution is consistent with their sequence being a *vancouverensis* contaminant coupled with 0.6% sequencing error. The substitution pattern in our *olympus* sample is not consistent with the high error rate necessary to account for its basal phylogenetic position if it was contaminated.

We note that *caligata, olympus,* and *vancouverensis* are represented in our data set by single individuals, and unsampled intraspecific variation could influence this conclusion. However, none of the 13 intraspecific comparisons we have (Table 2, Fig. 4) show divergence >3.5% (distance over entire gene), and all divergences >1.1% involve different subspecies. It seems unlikely, therefore, that intraspecific variation in *Petromarmota* will prove to be great enough to affect the topology of relationships estimated from the data at hand.

A similar argument can be made regarding the robustness of the entire tree topology to the effect of lineage sorting, which can cause any particular gene tree to differ from the true species tree (Maddison, 1997). Pairwise intra- and interspecific divergence overlap only marginally (Fig. 4). Thus the available data indicate that many nodes in the phylogeny will be resistant to lineage-sorting effects. However, basal internodes in the subgenus *Marmota* (for which we claim no resolution) are within the bounds of intraspecific variation and possibly are subject to lineage-sorting (Moore, 1995); thus, even with additional mitochondrial sequence data, we should be cautious in assessing our confidence for any resulting resolution.

Conclusions

The cyt *b* data and analyses are consistent with the following scenario. *Marmota* diverged from *Spermophilus* in North America and radiated into several lineages, although the data cannot reject an Asian origin. One lineage led to the subgenus *Petromarmota,* which is today found in the montane west. *Petromarmota* includes two species with highly restricted distributions: *M. vancouverensis* is endemic to Vancouver Island and is only recently derived from *caligata;* on the other hand, *olympus,* which is endemic to the Olympic Peninsula, is a basal member of the subgenus, and if karyotypic evolution has been simple in this group, may be the sister species to the remaining members. The other surviving lineage of Nearctic origin may have crossed Beringia into Asia not long after its divergence from *Petromarmota,* leaving *monax* (the woodchuck) in North America, and then spread across the Palearctic. Why this ancestral marmot crossed the Bering land bridge, whereas the *Petromarmota* lineage did not, may have been influenced by both geographic distribution and habitat differences. Judging from the habitat preferences of modern *monax,* its ancestral form probably occupied a larger array of habits and occurred closer to (perhaps within) Beringia. Ancestral *Petromarmota* may have been a more specialized montane form occupying a more southerly range in the western mountains and thus was less likely to cross Beringia into the Palearctic. Alternatively, a *Petromarmota*-like species did cross Beringia—a northerly sister-species to *Petromarmota* that subsequently evolved the derived *monax*—which was followed by the extinction of its *Petromarmota*-like descendant in the Nearctic. Perhaps *broweri* is that descendant that did not go extinct. A more definitive estimate of the plesiomorphic habitat

preferences in *Marmota* would assist in choosing between these alternatives.

Differentiation into the major Palearctic lineages appears to have been rapid, judging from the short internal branches and poor phylogenetic resolution. Subsequent speciation in the three to five Palearctic lineages appears to have resulted in parapatric sister taxa. The optimal trees support the hypothesis that the Alaskan *broweri* represents a recrossing of Beringia from the east. However, the data cannot reject the possibility that *broweri* may, like *monax,* be a remnant of the earlier Nearctic phase of the *M. (Marmota)* radiation prior to the Palearctic invasion. The data are sufficient to demonstrate that *broweri* is not sister to *camtschatica* nor a member of *Petromarmota,* as had been the preferred hypotheses.

We have used the molecular phylogeny to examine one important aspect of marmot biology, the evolution of increased sociality and large colony formation. A single origination of this trait without reversal can be rejected. A parsimony optimization of increased sociality on the optimal tree indicates that it has been gained (or lost) twice, and possibly three times, because *olympus* forms moderately large colonies. These multiple cases of social evolution will allow statistical tests of explicit ethological hypotheses, such as those proposed by Barash (1989) and Armitage (1999). Another likely example of convergence is between the *camtschatica* group and *broweri* for morphological traits associated with life in the permafrost.

Taking into account the evidence presented above and in Figure 5 regarding individual nodes on the tree, Figure 6 represents the summary topology for which we feel these data provide robust support and is the graphical representation of our taxonomy. Eight of 13 possible nodes are resolved, which represents a significant increase over the perhaps single node, *Marmota,* that could be considered well resolved by the sum of previous systematic studies. Although all nodes and hypotheses should be subject to further testing, resources should be concentrated on resolving those nodes that remain unresolved on this summary. The explicit statistical framework within which this analysis was conducted has as a benefit the ability to identify those hypotheses consistent with the data as well as rejecting inconsistent hypotheses. By doing so, this approach can more sharply focus the direction of subsequent investigations and streamline our efforts, given finite resources and time. A particular focus of future research should be the relationships among the major lineages of the nominate subgenus.

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APPENDIX

List of *Marmota* specimens sequenced; specimen IDs are as in the tables and figure. Abbreviations: AF = University of Alaska, Fairbanks; ASU = Appalachian State University; IDB = Institute of Developmental Biology, Moscow; LMS = Laboratory of Molecular Systematics, Smithsonian Institution; NMMNH = New Mexico Museum of Natural History; NPIB = Northwest Plateau Institute of Biology, Xining, China; USNM = National Museum of Natural History. Tissues collected by J. F. Jacobs (JFJ) and R. S. Hoffmann (RSH) are housed in the collections of the Smithsonian Institution.

baibacina 1. *baibacina kastschenkoi*. Russia, Novosibirsk obl., vic. Novosibirsk; 55°N, 83°E. IDB 23766.

baibacina 2. *baibacina centralis.* Kazakhstan, Chimkentsk obl., Bol'shoi Kokpak valley; 41°45′N, 69°30′E. IDB 23929.

bobak 1. *bobak bobak.* Ukraine, Khar'kovsk obl., Velikii, Byrluksk rai.; 50°N, 37°20′E. IDB 23765.

bobak 2. *bobak bobak.* Ukraine, Khar'kovsk obl., Velikii, Byrluksk rai.; 50°N, 37°20′E. IDB 23803.

broweri 1. *broweri*. USA, Alaska, Brooks Range, vic. Anaktuvuk Pass; 68°10′N, 152°W. USNM 583154, AF 7212.

broweri 2. *broweri*. USA, Alaska, Brooks Range, vic. Anaktuvuk Pass; 68°10′N, 152°W. USNM 583155, JFJ 974.

caligata. caligata caligata. USA, Alaska, vic. Fairbanks; 65°N, 145°W. AF 2384.

camtschatica 1. *camtschatica camtschatica.* Russia, Kamchatsk obl., Nilkovski rai.; 53°N, 157°30′E. IDB 23764.

camtschatica 2. *camtschatica doppelmayri*. Russia, Buryatiya, Severo-Baikalsk rai., upper Chai River; 55°30′N, 109°E. IDB 23901.

caudata 1. *caudata caudata.* Pakistan, Northern Terr., Hunza, vic. Khunjerab Pass; 36°50′N, 75°20′E.

caudata 2. *caudata aurea.* Kazakhstan, Dzhambulsk obl., Nerke; 43°N, 71°30′E. IDB 23767.

caudata 3. *caudata aurea.* Kazakhstan, Dzhambulsk obl., Nerke; 43°N, 71°30′E. IDB 23708.

flaviventris 1. *flaviventris luteola.* USA, Colorado, Gunnison Co., ~7 mi. N of Crested Butte, along East River; 38°53′N, 106°58′W. USNM 575170, JFJ 816.

flaviventris 2. *flaviventris obscura.* USA, New Mexico, Taos Co., Sangre De Cristo Mountains, N of Santa Fe; 36°30′N, 105°30′W. NMMNH 128.

himalayana. himalayana robusta. China, Qinghai Prov., Yushu Aut. Pref., Nangqen Co., Bei-zha Forestry Sta., Ba Qu (river); 31°45′N, 96°30′E. RSH 4478.

marmota 1. *marmota marmota*. Switzerland, Canton Grisons, Davos. 46°47′N, 9°50′E. LMS M00017.

marmota 2. *marmota marmota*. Italy, Modena, Monte Cimone. 44°12′N, 10°42′E. LMS M00018.

menzbieri. menzbieri zachidovi. Uzbekistan, Tashkentsk obl., Chatkalsk zapovednik, vic. Parkent; 41°15′N, 70°E. IDB 23863.

monax 1. *monax ochracea.* Canada, Yukon, Ethel Lake; 63°21′N, 136°W. Univ. RSH 4249

monax 2. *monax rufescens.* USA, New York, Tompkins Co., vic. Ithaca; 42°30′N, 76°30′W. JFJ 947.

monax 3. *monax monax*. USA, North Carolina, no exact locality. 35°30′N, 82°30′W. ASU 16756.

olympus. olympus. USA, Washington, Quinault River; 49°30′N, 125°W. USNM 241947.

sibirica 1. *sibirica sibirica.* Russia, Chitinsk Obl. Ononsk rai., Pobeda; 57°30′N, 116°E. IDB 9324.

sibirica 2. *sibirica caliginosus.* Russia, Buryatiya, Selenginsk rai., Toion, Gusinoe Lake; 51°N, 106°15′E. IDB 23906.

vancouverensis. vancouverensis. Canada, British Columbia, Vancouver Island, no exact locality. 49°30′N, 123°30′W, blood sample collection number 989244.