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

SCOTT J. STEPPAN,1,2,5 MIKHAIL R. AKHVERDYAN,3,4 ELENA A. LYAPUNOVA,3 DARRELYN G. FRASER,1,6 NIKOLAI N. VORONTSOV,3 ROBERT S. HOFFMANN,2 AND MICHAEL J. BRAUN1,2

1Laboratory of Molecular Systematics, Smithsonian Institution, 4210 Silver Hill Road, Suitland, Maryland 20746, USA; E-mail: steppan@bio.fsu.edu (S.J.S.)
2Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA
3Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia
4Institute of Zoology, National Academy of Sciences, Republic of Armenia, 375 014 Yerevan, Armenia

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 reinvansion from the Palearctic, but a Nearctic origin cannot 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.,
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 himalyana. 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 reinvansion 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 = himalyana; 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.](image-url)
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 a 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) $→ 40$ (olympus) $→ 42$ (caligata, flaviventris, vancouverensis).

METHODS

Specimens Examined

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

![Figure 2](image_url)
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; TGA AAA AYCATC GTTGT) and H15915 (P485; TTYCWTYTTNGGT TACAARAC) 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 MgCl₂, 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 H₂O. 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 amplification primers and the following sequencing primers: P499 (L14994: 5′-TGACTTTATCGCTATATACA-3′), P1185 (L15279: 5′-AA AGCYACYTTAACAGATT-3′), L15615 (5′-ATCTTAACAAACTAGGAGG-3′), P1188 (L15615: 5′-ATCCCCAACAATTAGGAGG-3′), P1189 (H15756: 5′-CRGGYTGTCTCT CRATTC-3′), P500 (H15481: 5′-CTCCTA GAAGTCAATGAA-3′), P386 (H15149: 5′-TTTCTCGAGCCCTCAGATGATT TTCTACACA-3′), P1190 (H14993: 5′-ATTATT GATGCAACCGTTTRCATG-3′). Additional primers were used for manual sequencing: P442 (L14841: 5′-AAGCTTCCATCCACAAC-3′), P586 (L15171: 5′-CAAATKTCATTTY GAGNGCAAC-3′), P509 (L15350: 5′-GK TTKTIGAICCGTYC-3′), P443 (H15498: 5′-CTGCAAGGAATAAGT-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 ($\Gamma$; 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 $+ I + \Gamma$, 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 $+ I + \Gamma$ model was a significantly better fit for each of the trees. A ML search was then conducted by using the GTR $+ I + \Gamma$ 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.

### RESULTS

Each *Marmota* sequence has an open reading frame of 1140 bp. There were 507 variable sites, 399 of which were parsimony-informative. The sites in the *Spermophilus* outgroup (H. and *Sciurus* outgroups) show a higher frequency of Ts at the third position (0.303 vs. 0.103–0.221), with correspondingly lower frequencies for *Marmota*.

<table>
<thead>
<tr>
<th>Codon position</th>
<th>A</th>
<th>C</th>
<th>G</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>0.292</td>
<td>0.245</td>
<td>0.211</td>
<td>0.253</td>
</tr>
<tr>
<td>Second</td>
<td>0.199</td>
<td>0.238</td>
<td>0.140</td>
<td>0.423</td>
</tr>
<tr>
<td>Third</td>
<td>0.375</td>
<td>0.296</td>
<td>0.027</td>
<td>0.303</td>
</tr>
<tr>
<td>Total</td>
<td>0.289</td>
<td>0.260</td>
<td>0.126</td>
<td>0.326</td>
</tr>
</tbody>
</table>

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

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.

Pyrograms showing the distribution of the four nucleotide frequencies are shown for each group. **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.
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. 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. 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. 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 + G; 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 (Ne-arctic) 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>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) olympus</td>
<td>0</td>
<td>37</td>
<td>34</td>
<td>36</td>
<td>36</td>
<td>68</td>
<td>66</td>
<td>64</td>
<td>56</td>
<td>56</td>
<td>63</td>
</tr>
<tr>
<td>(2) flaviventris 1</td>
<td>0.083</td>
<td>0</td>
<td>17</td>
<td>66</td>
<td>65</td>
<td>116</td>
<td>117</td>
<td>114</td>
<td>126</td>
<td>123</td>
<td>132</td>
</tr>
<tr>
<td>(3) flaviventris 2</td>
<td>0.073</td>
<td>0.016</td>
<td>0</td>
<td>61</td>
<td>60</td>
<td>113</td>
<td>114</td>
<td>113</td>
<td>118</td>
<td>142</td>
<td>137</td>
</tr>
<tr>
<td>(4) vancouverensis</td>
<td>0.080</td>
<td>0.072</td>
<td>0.063</td>
<td>0</td>
<td>13</td>
<td>106</td>
<td>107</td>
<td>106</td>
<td>119</td>
<td>116</td>
<td>121</td>
</tr>
<tr>
<td>(5) caligata</td>
<td>0.079</td>
<td>0.070</td>
<td>0.064</td>
<td>0.012</td>
<td>0</td>
<td>107</td>
<td>108</td>
<td>107</td>
<td>116</td>
<td>113</td>
<td>123</td>
</tr>
<tr>
<td>(6) monax 2</td>
<td>0.205</td>
<td>0.151</td>
<td>0.146</td>
<td>0.138</td>
<td>0.138</td>
<td>0</td>
<td>15</td>
<td>16</td>
<td>109</td>
<td>110</td>
<td>109</td>
</tr>
<tr>
<td>(7) monax 3</td>
<td>0.191</td>
<td>0.152</td>
<td>0.146</td>
<td>0.138</td>
<td>0.138</td>
<td>0.014</td>
<td>0</td>
<td>19</td>
<td>112</td>
<td>113</td>
<td>114</td>
</tr>
<tr>
<td>(8) monax 1</td>
<td>0.186</td>
<td>0.146</td>
<td>0.145</td>
<td>0.137</td>
<td>0.137</td>
<td>0.015</td>
<td>0.018</td>
<td>0</td>
<td>111</td>
<td>112</td>
<td>107</td>
</tr>
<tr>
<td>(9) marmota 1</td>
<td>0.149</td>
<td>0.166</td>
<td>0.151</td>
<td>0.156</td>
<td>0.150</td>
<td>0.140</td>
<td>0.143</td>
<td>0.141</td>
<td>0</td>
<td>3</td>
<td>115</td>
</tr>
<tr>
<td>(10) marmota 2</td>
<td>0.149</td>
<td>0.161</td>
<td>0.146</td>
<td>0.151</td>
<td>0.144</td>
<td>0.142</td>
<td>0.145</td>
<td>0.145</td>
<td>0.144</td>
<td>0.003</td>
<td>0</td>
</tr>
<tr>
<td>(11) broweri 1</td>
<td>0.166</td>
<td>0.177</td>
<td>0.168</td>
<td>0.161</td>
<td>0.163</td>
<td>0.140</td>
<td>0.148</td>
<td>0.136</td>
<td>0.146</td>
<td>0.144</td>
<td>0</td>
</tr>
<tr>
<td>(12) broweri 2</td>
<td>0.171</td>
<td>0.179</td>
<td>0.170</td>
<td>0.164</td>
<td>0.166</td>
<td>0.142</td>
<td>0.150</td>
<td>0.138</td>
<td>0.148</td>
<td>0.146</td>
<td>8.8e-4</td>
</tr>
<tr>
<td>(13) menzibieri</td>
<td>0.127</td>
<td>0.155</td>
<td>0.140</td>
<td>0.159</td>
<td>0.158</td>
<td>0.143</td>
<td>0.145</td>
<td>0.140</td>
<td>0.136</td>
<td>0.137</td>
<td>0.139</td>
</tr>
<tr>
<td>(14) caudata 3</td>
<td>0.146</td>
<td>0.142</td>
<td>0.136</td>
<td>0.141</td>
<td>0.147</td>
<td>0.110</td>
<td>0.123</td>
<td>0.119</td>
<td>0.115</td>
<td>0.117</td>
<td>0.115</td>
</tr>
<tr>
<td>(15) caudata 2</td>
<td>0.146</td>
<td>0.142</td>
<td>0.136</td>
<td>0.141</td>
<td>0.147</td>
<td>0.110</td>
<td>0.123</td>
<td>0.119</td>
<td>0.115</td>
<td>0.117</td>
<td>0.115</td>
</tr>
<tr>
<td>(16) caudata 1</td>
<td>0.145</td>
<td>0.141</td>
<td>0.139</td>
<td>0.147</td>
<td>0.145</td>
<td>0.120</td>
<td>0.131</td>
<td>0.126</td>
<td>0.117</td>
<td>0.119</td>
<td>0.114</td>
</tr>
<tr>
<td>(17) baibacina 2</td>
<td>0.151</td>
<td>0.142</td>
<td>0.131</td>
<td>0.131</td>
<td>0.131</td>
<td>0.124</td>
<td>0.114</td>
<td>0.123</td>
<td>0.138</td>
<td>0.137</td>
<td>0.132</td>
</tr>
<tr>
<td>(18) baibacina 1</td>
<td>0.167</td>
<td>0.154</td>
<td>0.138</td>
<td>0.144</td>
<td>0.144</td>
<td>0.136</td>
<td>0.127</td>
<td>0.139</td>
<td>0.144</td>
<td>0.146</td>
<td>0.143</td>
</tr>
<tr>
<td>(19) bobak 1</td>
<td>0.142</td>
<td>0.136</td>
<td>0.127</td>
<td>0.136</td>
<td>0.136</td>
<td>0.116</td>
<td>0.120</td>
<td>0.122</td>
<td>0.127</td>
<td>0.129</td>
<td>0.134</td>
</tr>
<tr>
<td>(20) bobak 2</td>
<td>0.163</td>
<td>0.142</td>
<td>0.133</td>
<td>0.144</td>
<td>0.144</td>
<td>0.121</td>
<td>0.125</td>
<td>0.127</td>
<td>0.135</td>
<td>0.137</td>
<td>0.139</td>
</tr>
<tr>
<td>(21) himalayana</td>
<td>0.189</td>
<td>0.156</td>
<td>0.141</td>
<td>0.163</td>
<td>0.167</td>
<td>0.127</td>
<td>0.121</td>
<td>0.117</td>
<td>0.133</td>
<td>0.135</td>
<td>0.150</td>
</tr>
<tr>
<td>(22) sibirica 2</td>
<td>0.168</td>
<td>0.151</td>
<td>0.140</td>
<td>0.163</td>
<td>0.167</td>
<td>0.134</td>
<td>0.127</td>
<td>0.130</td>
<td>0.131</td>
<td>0.133</td>
<td>0.131</td>
</tr>
<tr>
<td>(23) sibirica 1</td>
<td>0.169</td>
<td>0.153</td>
<td>0.141</td>
<td>0.163</td>
<td>0.167</td>
<td>0.134</td>
<td>0.127</td>
<td>0.130</td>
<td>0.132</td>
<td>0.134</td>
<td>0.131</td>
</tr>
<tr>
<td>(24) camtschatica 2</td>
<td>0.136</td>
<td>0.171</td>
<td>0.158</td>
<td>0.171</td>
<td>0.168</td>
<td>0.155</td>
<td>0.146</td>
<td>0.148</td>
<td>0.135</td>
<td>0.137</td>
<td>0.160</td>
</tr>
<tr>
<td>(25) camtschatica 1</td>
<td>0.134</td>
<td>0.170</td>
<td>0.154</td>
<td>0.167</td>
<td>0.164</td>
<td>0.156</td>
<td>0.146</td>
<td>0.148</td>
<td>0.134</td>
<td>0.136</td>
<td>0.153</td>
</tr>
</tbody>
</table>
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–

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—

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 64 | 52 | 57 | 57 | 57 | 59 | 62 | 56 | 61 | 66 | 62 | 62 | 54 | 53 |
| 128 | 112 | 110 | 110 | 112 | 106 | 109 | 105 | 107 | 112 | 111 | 112 | 112 | 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 | 107 | 119 |
| 115 | 113 | 101 | 101 | 105 | 95 | 102 | 99 | 101 | 100 | 104 | 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 | 109 | 109 | 108 |
| 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 | 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 | 0.022 | 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 |
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...
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 Nearctic 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 *himalyana* form a monophyletic group. All the optimal trees place *himalyana* 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 Berinia), 5 (original diversification in North America), 6 (black-tipped hairs evolved once), and 8 (simple karyotypic transformation) cannot be rejected.

**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 Nearctic 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.
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... 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-

### 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

### Table 4. Proposed classification of the genus *Marmota*.

<table>
<thead>
<tr>
<th>Genus Marmota Blumenbach, 1779</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synonyms: <em>Marmota</em> Frisch, 1775; <em>Glis</em> Erxleben, 1777; <em>Arctomys</em> Schreber, 1780; <em>Lagomys</em> Storr, 1780; <em>Lipura</em> Illiger, 1811; <em>Marmotops</em> Pocock, 1922</td>
</tr>
<tr>
<td>Subgenus Petro<em>Marmota</em> Subgen. Nov.</td>
</tr>
<tr>
<td>flaviventris</td>
</tr>
<tr>
<td>caligata group</td>
</tr>
<tr>
<td>caligata</td>
</tr>
<tr>
<td>vancouverensis</td>
</tr>
<tr>
<td>olympus</td>
</tr>
<tr>
<td>Subgenus <em>Marmota</em></td>
</tr>
<tr>
<td>monax</td>
</tr>
<tr>
<td>marmota</td>
</tr>
<tr>
<td>broweri</td>
</tr>
<tr>
<td>caudata group</td>
</tr>
<tr>
<td>caudata</td>
</tr>
<tr>
<td>menzbieri</td>
</tr>
<tr>
<td>bobak group</td>
</tr>
<tr>
<td>bobak</td>
</tr>
<tr>
<td>baibacina</td>
</tr>
<tr>
<td>camtschatica group</td>
</tr>
<tr>
<td>camtschatica</td>
</tr>
<tr>
<td>himalayana</td>
</tr>
<tr>
<td>sibirica</td>
</tr>
</tbody>
</table>

(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 *Petro*marmota, 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 allelespecies *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 *Petro*marmota 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.
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 nu-

cleotide 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
lelism 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
**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 wood-chuck) 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 *Petro-marmota*, 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.

**ACKNOWLEDGMENTS**

We thank R. Harrison, J. Mercer, V. L. Roth, R. Thor-ington, R. Page, M. Wilkinson, and an anonymous re-viewer for their suggestions and constructive critique of earlier drafts of the manuscript. Field research was supported by the Institute of Developmental Biology, Russian Academy of Sciences, Moscow; the Northwest Plateau Institute of Biology, Chinese Academy of Sciences, Xining; and by the Division of Mammals, National Museum of Natural History, Smithsonian Institution, Washington, D.C. We thank Drs. W. Arnold and Thomas Ruff of Phillips-Universität, Marburg; J. Cook of the University of Alaska, Fairbanks; P. Tongiori of the Department of Animal Biology, University of Modena, Italy; and D. Hafner of the New Mexico Museum of Natural History for loans of frozen tissue. We also thank A. Bryant of the Vancouver Island Marmot recovery team for supplying blood samples and Dr. Richard W. Thorington, Jr., Curator in Charge, for permission to obtain dry tissue from a specimen of *M. olympus* in the USNM collection. W. Kelley Thomas of the University of Missouri, Kansas City, kindly provided several primers and electronic copies of their published sequences. Collecting in Eurasia was funded in part from NSF grant DEB-9221504 to Drs. Richard Harrison and Paul Sherman, Cornell University (R. S. Hoffmann and E. Jensen, Co-Investigators). Laboratory work was supported by the Laboratory of Molecular Systematics, Smithsonian Institution and by a Scholarly Studies Grant from the Office of Fellow-ships and Grants, Smithsonian Institution.

**REFERENCES**


Hoffmann, R. S. 1984. An ecological and zoogeographical analysis of animal migration across the Bering land bridge in the Cenozoic. Pages 464–481 in *Beringia in the Cenozoic* (V. L. Kontrimavichus, ed.). Amerind, New Delhi, India.


MOORE, W. S. 1995. Inferring phylogenies from 
mtDNA variation: Mitochondrial-gene trees versus 

NIKOL’SKII, A. A., I. Y. YANINA, M. V. RUTOVSKAYA, AND 
N. A. FORMOZOV. 1983. Izmenchivost’ zvukova sign-
ala stepnovo i serovo surkov (Marmota bobac, M. 
baibacina; Sciuridae, Rodentia) v zone vtorichnovno 
kontakta [Variation in sound signals of steppe and 
gray marmots (Marmota bobac, M. baibacina; Sciuri-
da, Rodentia) in a zone of secondary contact]. Zool. 
Zh. 62:1258–1266.

OGNEV, S. I. 1947. Zveri SSSR i prilezhashchikh stran 
[Mammals of the USSR and adjacent countries], 
Volume 5. Gryzuny [Rodents]. Academy of Sciences of 
the USSR, Moscow.

RAUSCH, R. L. 1953. On the status of some arctic 

evidence for the specific distinction of an Alaskan 
marmot, Marmota broweri Hall and Gilmore (Mam-

RAUSCH, R. L., AND V. R. RAUSCH. 1971. The somatic 
chromosomes of some North American marmots 
(Sciuridae), with remarks on the relationships of 
Marmota broweri Hall and Gilmore. Mammalia 

SAMBROOK, E., F. FRITSCH, AND T. MANIATIS. 1989. Mo-
olecular cloning. Cold Spring Harbor Laboratory 

paleofaunas of the world. Addison-Wesley, Reading, 
Massachusetts.

SLUDSKII, A. A. 1969. Mlekopitayushchikh Kazakh-
stan [Mammals of Kazakhstan], Volume 1. 
Gryzuny (Surki i susliki) [Rodents (Marmots and 
ground squirrels)]. Nauka, Alma-Ata.

SMIRIN, Y. M., N. A. FORMOZOV, D. I. BIBIKOV, AND 
D. MYAGMARZH. 1985. Kharakteristika poselenii 
dvukh vidov surkov (Marmots, Rodentia, Sciuridae) 
v zone ikh Kontakta na Mongol’kom Altaye [Charac-
teristics of settlement in two species of marmots 
(Marmots, Rodentia, Sciuridae) in their contact zone 

SOKOLOV, V. E., AND V. N. ORLOV. 1980. Opredelitel’ 
mlekopitayushchikh Mongol’skoi Narodnoi Re-

Received 13 August 1998; accepted 12 January 1999 
Associate Editor: R. Page
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.


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


*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.


*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.


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

*olympus* 1. *olympus olympus*. USA, Washington, Quinault River; 49°30’N, 125°W. USNM 241947.

