MITOCHONDRIAL DNA PHYLOGEOGRAPHY OF THE NEW WORLD FLYING SQUIRRELS (*GLAUCOMYS*): IMPLICATIONS FOR PLEISTOCENE BIOGEOGRAPHY

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Cytochrome-*b* sequence data of mitochondrial DNA (mtDNA) were used to infer evolutionary and biogeographic histories of the New World flying squirrels, *Glaucomys sabrinus* and *G. volans*. Two distinct mtDNA lineages were recovered within *G. sabrinus*: a western lineage consisting of populations from western California, Oregon, and Washington, and a much larger "eastern" lineage comprised of populations from the remainder of the species' range (North Carolina, West Virginia, Michigan, Utah, Alaska, eastern Washington, British Columbia, and Alberta). In contrast, only one major mtDNA lineage was recovered within *G. volans*. Little sequence variation was observed among populations of *G. volans* (≤0.6%), but sequence variation within *G. sabrinus* was much higher (2.3% and 2.6% within the eastern and western clades, respectively, and 4.3–7.2% between the two clades). The level of sequence divergence observed between the eastern and western mtDNA clades of *G. sabrinus* (4.3–7.2%) was slightly greater than that observed between the two species, *G. sabrinus* and *G. volans* (4.0–6.1%), suggesting the possibility of an unrecognized species within *G. sabrinus*. Minimum levels of sequence divergence among the three mtDNA clades were nearly equal (ca. 4% in all pairwise comparisons), suggesting that *Glaucomys* underwent a relatively rapid diversification in the early-to-middle Pleistocene. The mtDNA discontinuity in the Pacific Northwest within *G. sabrinus* is congruent with similar disjunctions in a variety of vertebrate taxa, suggesting that an ancestral North American boreal ecosystem may have been divided into two distinct communities at this time.

Key words: *Glaucomys*, flying squirrel, mtDNA phylogeography, historical biogeography, cytochrome-*b*, Pleistocene

Molecular methods of detecting genetic variation within species have led to exciting advances in studies of historical biogeography. Mitochondrial DNA (mtDNA), in particular, has facilitated the use of genealogical trees to examine evolutionary relationships of conspecific populations, or "intraspecific phylogeography" (Avise et al., 1987:519). Several features of mtDNA, including a matrilineal mode of inheritance and rapid rate of evolution, promote rapid geographic sorting of haplotype lineages in the absence of gene flow (Avise et al., 1984). As such, analysis of mtDNA variation often is able to recover intraspecific patterns of population subdivision and genetic differentiation beyond the resolving power of non-molecular approaches.

The New World flying squirrels (*Glaucomys*) are nocturnal sciurids that are capable of gliding locomotion. Although >50 species of flying squirrels currently are recognized, most species are restricted to the Old World, primarily Asia (Nowak, 1991). Two species occur in the New World, the northern flying squirrel, *Glaucomys sabrinus*, and the southern flying squirrel, *G. volans*. The evolutionary relationship between *G. sabrinus* and *G. volans* remains enigmatic. Whereas bacular morphology (Burt, 1960) and immunological distance data (Hight et al., 1974) suggest a distant rela-

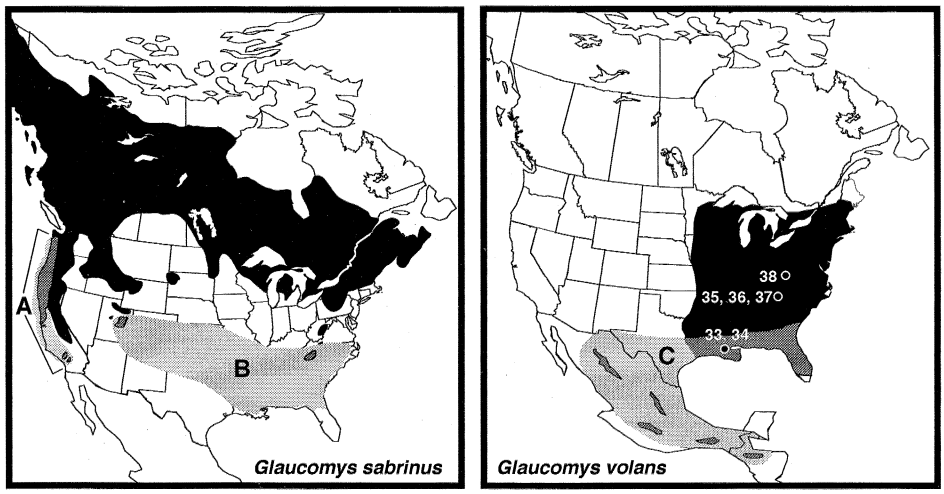

FIG. 1.—Geographic distributions of *Glaucomys sabrinus* (Wells-Gosling and Heaney, 1984) and *G. volans* (Dolan and Carter, 1977) and locations of proposed Pleistocene refugia for the three mtDNA clades recovered in this study. Present distributions of *G. sabrinus* and *G. volans* are shown in black. Generalized locations of proposed Pleistocene refugia (lightly stippled areas) for the western and eastern mtDNA clades of *G. sabrinus* and for *G. volans* are labeled A, B, and C, respectively. Dark stipples designate areas of overlap between present distributions and distributions of proposed refugia. Numbers represent collection localities for *G. volans* in Table 1 and Fig. 2 (collection localities for *G. sabrinus* in Fig. 3).

tionship, chromosomal data (Nadler and Sutton, 1967; Rausch and Rausch, 1982; Schindler et al., 1973) and numerous morphological characters (Essner, 1996; Thorington et al., 1996) suggest that the two species may be closely related.

Glaucomys sabrinus and *G. volans* have largely non-overlapping geographic distributions in North America (Fig. 1). *G. sabrinus* is tied closely to boreal forests and has a large distribution, continuous across northern North America through central Canada, with several disjunct populations occurring in mountainous regions of the United States. In contrast, *G. volans* is associated closely with hardwood forests. Its distribution is continuous across the eastern one-half of the United States as far north as southern Canada; like *G. sabrinus, G. volans* has a few small, disjunct southern populations, in this case, in Mexico and Central America.

Throughout the Pleistocene, vegetational and faunal distributions over much of North America were modified radically and repeatedly in response to changes in climate, sea level, and position of glacial fronts (Ritchie, 1987; Wright and Frey, 1965). From geological and paleobotanical investigations of these events, it appears that forest types of North America were telescoped greatly south of the glacial margins (Ritchie, 1987; Whitehead, 1965). Boreal forests occupied relatively broad areas south of advancing glaciers in the south-central United States and also persisted along the Pacific coast. Similarly, deciduous forests occupied the southern Appalachian valleys and other parts of the southeastern United States and Middle America. Based on Pleistocene fossil remains, it appears that many faunal distributions followed movement of their characteristic vegetational types quite closely (Hibbard et al., 1965).

I used mtDNA data to clarify the evolutionary relationship between *G. sabrinus* and *G. volans* and assess the relative im-

portance of Pleistocene vegetational shifts and other environmental impediments to gene flow in shaping patterns of genetic variation and population subdivision within the New World flying squirrels.

Materials and Methods

Blood or tissue samples were obtained from 32 individuals of *G. sabrinus* and 6 individuals of *G. volans* from representative localities throughout their respective geographic distributions (Table 1). In addition, a portion of the foot pad of a mounted specimen of the giant flying squirrel, *Petaurista petaurista,* was obtained from the Sarawak Museum, Borneo, Malaysia. All unused tissues from this study have been deposited in the Collection of Genetic Resources at either the Museum of Natural Science, Louisiana State University (LSUMZ) or the Burke Museum of Natural History, University of Washington (UWBM).

Tissue samples were stored at −70°C until DNA isolations were performed. Total DNA extractions from either tissues or blood were conducted using either the phenol-chloroform method described by Hillis et al. (1996) or the chelex-solution method (Walsh et al., 1991). Isolated DNA was stored at −20°C.

A 402 base-pair (bp) fragment of the cytochrome-*b* gene of mtDNA was amplified using the polymerase chain reaction (PCR—Mullis and Faloona, 1987; Saiki et al., 1988). The primers L14724 (5′ CGAAGCTTGATATGAAAAA CCATCGTTG−3′) and H15149 (5′ AAACTGCA GCCCCTCAGAATGATATTTGTCCTCA−3′) were used for double-stranded amplifications and sequencing. Primer names indicate the DNA strand (H, heavy or L, light) and the position of the 3′ end of the oligonucleotide sequence of humans (Anderson et al., 1981). Both primers were taken from Irwin et al. (1991). Concentrations and volumes of the reagents used in amplifications were as follows: 1 μl DNA template (10 ng/μl), 2.5 μl of each primer (10 mmol); 5 μl of 10X Promega thermo buffer (Promega, Madison, WI); 0.95 μl of NTPs (10 mmol each); 3 μl of $MgCl_2$ (25 mmol); 34.8 μl of H_2O; and 0.25 μl of Promega *Taq* polymerase (total reaction volume = 50 μl). Thermal-cycling parameters were: 93°C for 3 min, 41°C for 10 s, increasing to 72°C over 3 min, followed by 72°C for 2 min (1 cycle); 93°C for 45 s, 42°C for 10 s, increasing to 72°C over 3 min, followed by 72°C for 2.5 min (3 cycles); 93°C for 45 s, 56°C for 1.5 min, and 72°C for 2.5 min (30 cycles); and 72°C for 10 min (1 cycle).

Both automated and Sanger sequencing (Sanger et al., 1977) were used to sequence 315 bp of the 402 bp fragment. Sanger sequencing was conducted using 5 μl of the double-stranded PCR product with the Sequenase® PCR Sequencing Kit (Version 2.0, T7 DNA Polymerase; United States Biochemical, Cleveland, OH) and sequencing grade ^{35}S labeled dATP. The same primers were used for PCR amplification and sequencing. To minimize sequencing errors, both strands were sequenced for each individual. Samples were run on polyacrylamide gels (6–8%) and visualized by autoradiography. Automated sequencing was performed with an ABI Prism 377 automated sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA) at the Bowman Gray School of Medicine, Wake Forest University (Winston-Salem, NC).

MacClade (Version 3.01—Maddison and Maddison, 1992) was used to identify unique haplotypes, reconstruct amino-acid sequences, and estimate transition bias in the data set. Estimates of sequence divergence for all possible pairwise combinations of taxa were calculated under the two-parameter model of Kimura (1980) in the computer program PHYLIP (Felsenstein, 1995). Both maximum-likelihood and parsimony methods of phylogenetic analysis were used to infer gene genealogies of mtDNA from sequence data. Maximum-likelihood analysis was performed using the DNAML (DNA maximum-likelihood) program in PHYLIP (Felsenstein, 1995), and parsimony analysis was conducted in the computer program PAUP (Phylogenetic Analysis Using Parsimony Version 3.1.1—Swofford, 1993). *Petaurista petaurista* was designated as an outgroup taxon in all phylogenetic analyses; although the monophyly of the flying squirrels has been questioned (Weigl, 1969), molecular data suggests that *P. petaurista* is more closely related to *Glaucomys* than any non-volant sciurid assayed to date (V. L. Roth, pers. comm.). Parsimony analyses were performed with all character state changes weighted equally and also with transversions weighted 3.15:1 over transitions (based on the transition bias estimated across all positions and all taxa, including the outgroup, in MacClade—Maddison and Maddison, 1992).

The data set was subjected to 1,000 bootstrap replicates, employing the heuristic search and strict consensus-tree options (PAUP—Swofford, 1993). Only unique haplotypes were included in bootstrap analyses, and all sites were used as characters. Maximum-likelihood analysis was performed using empirically observed base frequencies and the estimated transition : transversion ratio of 3.15:1. Dates of divergence among major mtDNA clades were based on estimated minimum levels of between-clade sequence divergence observed at third positions of codons and an estimated rate of divergence for these positions of ca. 10–15% per 1.0×10^6 years (modified from Irwin et al., 1991, and Thomas and Martin, 1993).

RESULTS

Twenty unique haplotypes were observed among the 38 individuals of *Glaucomys* sampled. Of the 315 bp of the cytochrome-*b* gene examined, 88 characters were variable and 44 were parsimony informative. Estimated values of pairwise-sequence divergence among taxa range from 0 to 7.2% within *G. sabrinus,* from 0 to 0.6% within *G. volans,* and from 4.0 to 6.1% between the two species. Within *Glaucomys,* observed transition : transversion ratios were approximately eight-fold higher for third positions than for first and second positions combined (21:1 and 2.7:1, respectively). As typically observed in mammalian cytochrome-*b* sequences, there was a marked base-composition bias (i.e., the four bases are not represented in equal proportions) in the form of a lack of guanines (G) in the sense strand (Brown et al., 1982; Irwin et al., 1991). This bias is strongest at third positions, where guanines comprise <3% of the total base composition.

Maximum-likelihood and parsimony analyses of the sequence data yielded similar trees, all of which contained the same three major clades (Fig. 2). In addition to a clade consisting of all individuals of *G. volans* sampled, two distinct mtDNA lineages were recovered within *G. sabrinus*: a western lineage consisting of populations from western Washington, California, and Oregon; and a larger "eastern" lineage comprised of populations from the remainder of the species' range (eastern Washington, British Columbia, Alberta, Alaska, Utah, Michigan, West Virginia, and North Carolina). Both unweighted and weighted parsimony produced the same six most-parsimonious trees. The six most-parsimonious trees produced by unweighted parsimony had 92 steps each, with a consistency index of 0.819 (all characters included). Both the maximum-likelihood tree and each of the six most-parsimonious trees supported a paraphyletic association of the eastern and western mtDNA clades of *G. sabrinus.* However, this node received relatively weak bootstrap support.

Although the maximum-likelihood and parsimony analyses recovered the same three major mtDNA clades within *Glaucomys* (Fig. 2), intraclade relationships within *G. sabrinus* were more resolved in the maximum-likelihood tree . For example, relationships among haplotypes comprising the eastern clade of *G. sabrinus* largely were unresolved in the parsimony trees, but the maximum-likelihood tree supported clear southeast-to-northwest phylogeographic structuring. That is, those localities further to the south and east were typically basal to those found more to the north and west. Similarly, relationships within the western mtDNA clade of *G. sabrinus* were resolved slightly more in the maximum-likelihood tree than in the parsimony trees, but in all cases the haplotypes sampled from coastal Oregon were basal to haplotypes sampled from southern California and western Washington. Both methods of phylogenetic analysis recovered the same topology of relationships within the mtDNA clade corresponding to *G. volans.*

Estimates of pairwise-sequence divergence between haplotypes within each clade of *Glaucomys* were small (≤2.6% within each clade of *G. sabrinus* and ≤0.6% within *G. volans*) relative to that between the three clades (a minimum of 4.0%). The level of estimated sequence di-

TABLE 1.—*Localities and subspecies of New World flying squirrels* (Glaucomys sabrinus *and* G. volans) *sampled in this study. Assignment of subspecies was based upon distributional data presented in Hall (1981). Localities are depicted in Figs. 1 and 3. LSUMZ refers to tissues housed in the Collection of Genetic Resources at the Museum of Natural Science, Louisiana State University, and UWBM refers to tissues housed in the Collection of Genetic Resources at the Burke Museum of Natural History, University of Washington. Remaining tissues are housed at Wake Forest University and are designated by the catalog number of the author.*

Species	Sample no.	State or province	Locality	Subspecies	Museum tissue number
G. sabrinus					
	1.	West Virginia (WV)	Pendleton County	*fuscus*	LSUMZ: M-5745
	2.	West Virginia (WV)	Pendleton County	*fuscus*	LSUMZ: M-5747
	3.	West Virginia (WV)	Webster County	*fuscus*	LSUMZ: M-5722
	4.	North Carolina (NC)	Mitchell County	*coloratus*	LSUMZ: M-5748
	5.	North Carolina (NC)	Mitchell County	*coloratus*	LSUMZ: M-5750
	6.	North Carolina (NC)	Mitchell County	*coloratus*	LSUMZ: M-5753
	7.	Utah (UT)	Summit County	*lucifigus*	LSUMZ: M-3013
	8.	Utah (UT)	Summit County	*lucifigus*	LSUMZ: M-3014
	9.	Michigan (MI)	Mackinac County	*macrotis*	LSUMZ: M-5761
	10.	Michigan (MI)	Otsego County	*macrotis*	LSUMZ: M-5758
	11.	Alberta (AB)	Edmonton	*sabrinus*	LSUMZ: M-3442
	12.	Alberta (AB)	Edmonton	*sabrinus*	LSUMZ: M-3443
	13.	Alberta (AB)	Edmonton	*sabrinus*	LSUMZ: M-3444
	14.	Alaska (AK)	Fairbanks	*yukonensis*	LSUMZ: M-5756
	15.	Alaska (AK)	Fairbanks	*yukonensis*	LSUMZ: M-5757
	16.	British Columbia (BC)	Vancouver	*fuliginosus*	BSA: 230
	17.	British Columbia (BC)	Vancouver	*fuliginosus*	BSA: 231
	18.	British Columbia (BC)	Vancouver	*fuliginosus*	BSA: 232
	19.	British Columbia (BC)	Vancouver	*fuliginosus*	BSA: 233
	20.	Washington (WA)	Okanogan County	*fuliginosus*	UWBM: 49067
	21.	Washington (WA)	Okanogan County	*fuliginosus*	UWBM: 49068
	22.	Washington (WA)	Okanogan County	*fuliginosus*	UWBM: 49070
	23.	Washington (WA)	Okanogan County	*fuliginosus*	UWBM: 49071
	24.	Washington (WA)	Pierce County	*oregonensis*	LSUMZ: M-5739
	25.	Washington (WA)	Pierce County	*oregonensis*	LSUMZ: M-5740
	26.	Washington (WA)	Snohomish County	*oregonensis*	LSUMZ: M-5730
	27.	Washington (WA)	Snohomish County	*oregonensis*	LSUMZ: M-5731
	28.	Oregon (OR)	Douglas County	*oregonensis*	LSUMZ: M-5733
	29.	Oregon (OR)	Douglas County	*oregonensis*	LSUMZ: M-5734
	30.	Oregon (OR)	Douglas County	*oregonensis*	LSUMZ: M-5735
	31.	California (CA)	San Bernardino County	*californicus*	LSUMZ: M-5741
	32.	California (CA)	San Bernardino County	*californicus*	LSUMZ: M-5742

TABLE 1.—*Continued.*

Species	Sample no.	State or province	Locality	Subspecies	Museum tissue number
G. volans					
	33.	Louisiana (LA)	East Baton Rouge Parish	*saturatus*	LSUMZ: M-5768
	34.	Louisiana (LA)	East Baton Rouge Parish	*saturatus*	LSUMZ: M-1957
	35.	Tennessee (TN)	Carter County	*volans*	LSUMZ: M-5765
	36.	Tennessee (TN)	Carter County	*volans*	LSUMZ: M-5766
	37.	Tennessee (TN)	Carter County	*volans*	LSUMZ: M-5767
	38.	West Virginia (WV)	Kanawha County	*volans*	LSUMZ: M-5762
P. petaurista	39.	Sarawak	Borneo, East Malaysia		BSA: 244

vergence observed between haplotypes of the eastern and western mtDNA clades of *G. sabrinus* (4.3–7.2%) was slightly greater than that observed between the two species, *G. sabrinus* and *G. volans* (4.0–6.1%).

DISCUSSION

Phylogenetic analyses.—Both maximum-likelihood and parsimony methods of phylogenetic inference support the existence of the same three major mtDNA clades within the *Glaucomys* sampled. In addition, both methods suggest that *G. sabrinus* may be a paraphyletic species. Intraclade relationships within *G. sabrinus* were more resolved in the maximum-likelihood tree than in the parsimony trees, probably because parsimony analysis ignores branch lengths when evaluating a tree, and maximum-likelihood analysis does not. Under a maximum-likelihood approach, changes are considered to be more likely to occur along a longer branch than along a shorter branch, and, as a result, some characters that are phylogenetically uninformative under a parsimony criterion may be informative in maximum-likelihood analysis (Swofford et al., 1996). The potentially higher number of informative characters utilized by maximum-likelihood analysis relative to parsimony analysis may lead to increased phylogenetic resolution when few characters differ between taxa (as is the case between the closely related mtDNA haplotypes in this study).

In addition to the potentially higher number of informative characters recognized by maximum-likelihood analysis, two additional characteristics make maximum-likelihood analysis more appealing than parsimony analysis as a method of phylogenetic inference for this study. First, the strong base-compositional bias observed in data from *Glaucomys* can be incorporated into the underlying model of nucleotide substitution in maximum-likelihood analysis. Such base-compositional bias cannot be addressed in parsimony analysis. Second, maximum-likelihood is typically the meth-

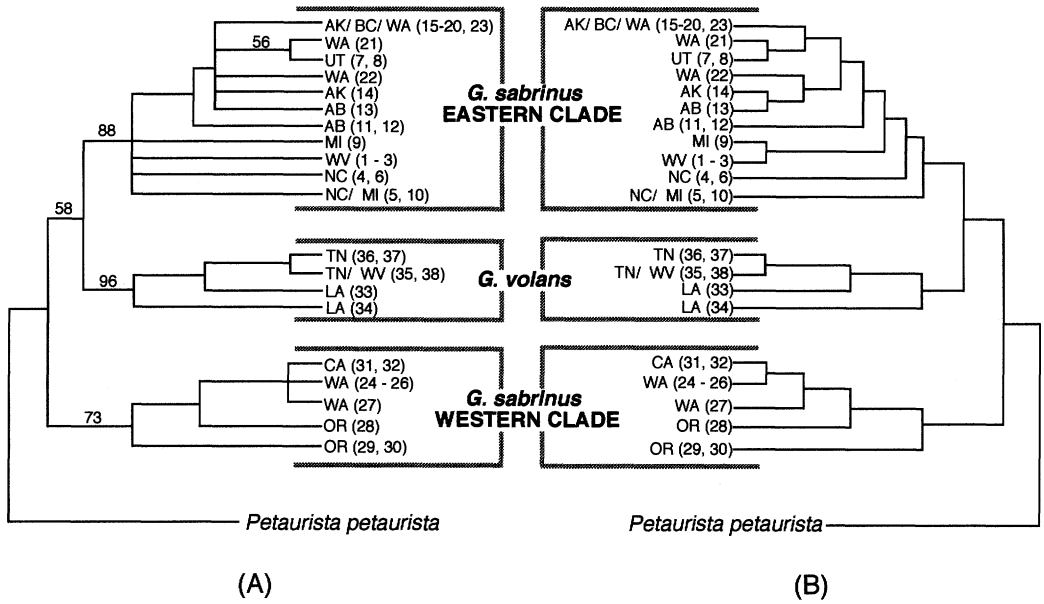

FIG. 2.—Phylogenetic trees representing relationships among mtDNA haplotypes of *Glaucomys*: A) the 50% majority-rule consensus of six most-parsimonious trees; bootstrap values >50% (based on 1,000 replicates) are shown at each node; and B) the maximum-likelihood tree (transversions weighted 3.15:1. Numbers and abbreviations of localities in Table 1.

od of phylogenetic inference with the lowest associated variance (i.e., it is the method least affected by sampling error when the number of nucleotides sampled per taxa is relatively small—Swofford et al., 1996). This quality may make maximum-likelihood analysis a better choice than parsimony analysis for intraspecific phylogeographic studies designed to maximize number of individuals and localities sampled rather than number of nucleotides sampled per individual. In this study, the two methods of phylogenetic inference largely converged on the same tree topology, differing only in the level of phylogenetic resolution recovered within the eastern and western clades of *G. sabrinus* (Fig. 2).

Taxonomic implications.—Patterns of mtDNA variation observed in this study suggest that the current taxonomic classification of the New World flying squirrels (i.e., two distinct, congeneric species) may not be an accurate reflection of evolutionary relationships within the group. Three, rather than two, distinct mtDNA lineages (one within *G. volans* and two within *G. sabrinus*) were identified within *Glaucomys*. The mtDNA discontinuity within *G. sabrinus* supports diagnoses of Bachman (1839) and Dalquest (1948) who recognized the Oregon and Washington coastal forms of *G. sabrinus* as a distinct species (*Pteromys oregonensis*) and highly distinct subspecies (*G. s. oregonensis*), respectively. However, phenotypic differences described by Bachman (1839) and Dalquest (1948) have not been quantified rigorously, and no marked differences are apparent in bacular morphology of the two clades of *G. sabrinus* (R. W. Thorington, Jr., pers. comm.; B. S. Arbogast, in litt.). Furthermore, the paraphyly within *G. sabrinus* may reflect incomplete sorting of ancestral mtDNA lineages rather than the true evolutionary relationships among clades (Avise et al., 1984; Patton and Smith, 1994). Therefore, it remains unclear if the two mtDNA clades of *G. sabrinus* exhibit species-level differentiation.

Divergence within the New World flying

TABLE 2.—*Minimum levels of sequence divergence and estimated dates of divergence among mtDNA clades of* Glaucomys. *Sequence divergence values are based on the two-parameter model of Kimura (1980). Dates of divergence are estimated based on a rate of divergence for third positions of the rodent cytochrome-*b *gene of ca. 10–15%/1.0 × 10⁶ years.*

mtDNA clades	Pairwise sequence divergence (%)		Estimated date of divergence (1.0×10^6 years)
	All positions	Third positions only	
G. volans versus *G. sabrinus*-East	4.0	12.7	0.85–1.3
G. volans versus *G. sabrinus*-West	4.2	11.5	0.77–1.2
G. sabrinus-East versus *G. sabrinus*-West	4.3	11.6	0.77–1.2

squirrels.—Pairwise comparisons reveal that levels of estimated sequence divergence among the three clades of *Glaucomys* are nearly equal (Table 2), suggesting that the clades diverged from one another relatively rapidly and contemporaneously. A quick three-way diversification within *Glaucomys* may explain the relatively low bootstrap support observed at the node defining the relationship among the three clades (Fig. 2).

Irwin et al. (1991), relying on dates of divergence estimated from fossil material for a number of mammalian taxa, estimated that the average rate of sequence divergence at third positions of the cytochrome-*b* gene of mammals is ca. 10% per 1.0×10^6 years. Thomas and Martin (1993) subsequently advocated the use of that rate in studies of sciurid evolution, showing that its application produces estimated dates of divergence within the North American ground squirrels that are consistent with Black's (1963) interpretation of the fossil record for this group. However, the relative rate of molecular evolution in rodents has been estimated to be ca. 1.5–2 times faster than that of other mammalian lineages (Britten, 1986; Dewalt et al., 1993; Li et al., 1987, 1990; Wu and Li, 1985), suggesting that the rate of divergence for the third positions of the cytochrome-*b* gene of many lineages of rodents may be considerably higher than $10\%/1.0 \times 10^6$ years. In fact, if dates of divergence in Thomas and Martin's (1993) study had been based on a rate of sequence divergence of ca. $15\%/1.0 \times 10^6$ years, rather than a rate of 10% (a 1.5-fold increase over the generalized rate for mammals), the resulting estimated dates of divergence would have been highly consistent with the alternative evolutionary time scale for North American ground squirrels proposed by Hafner (1984).

I estimated upper and lower limits of dates of divergence within *Glaucomys* based on rates of divergence for third positions of the cytochrome *b* gene of 10% and $15\%/1.0 \times 10^6$ years, respectively (Table 2). The three lineages of *Glaucomys* appear to have diverged from one another in the early-to-middle Pleistocene, ca. $0.7–1.3 \times 10^6$ years ago (Table 2). This time frame is temporally coincident with dramatic decreases in global temperatures as indicated by oxygen-isotopic, coarse-fraction, and benthoic foraminiferal records (Bergrenn et al., 1980; Peterson and Lohmann, 1982), indicating that diversification in *Glaucomys* may have been linked to the onset of major glacial cycles in the first half of the Pleistocene.

Biogeographical implications.—The distinct phylogeographic discontinuity between the eastern and western lineages of *G. sabrinus* (Fig. 3) suggests that a major environmental impediment to gene flow in the Pacific Northwest has strongly influenced the large-scale population structure of this species. In the Pacific Northwest, many vertebrate taxa, including several genera of squirrels (*Tamias, Spermophilus,*

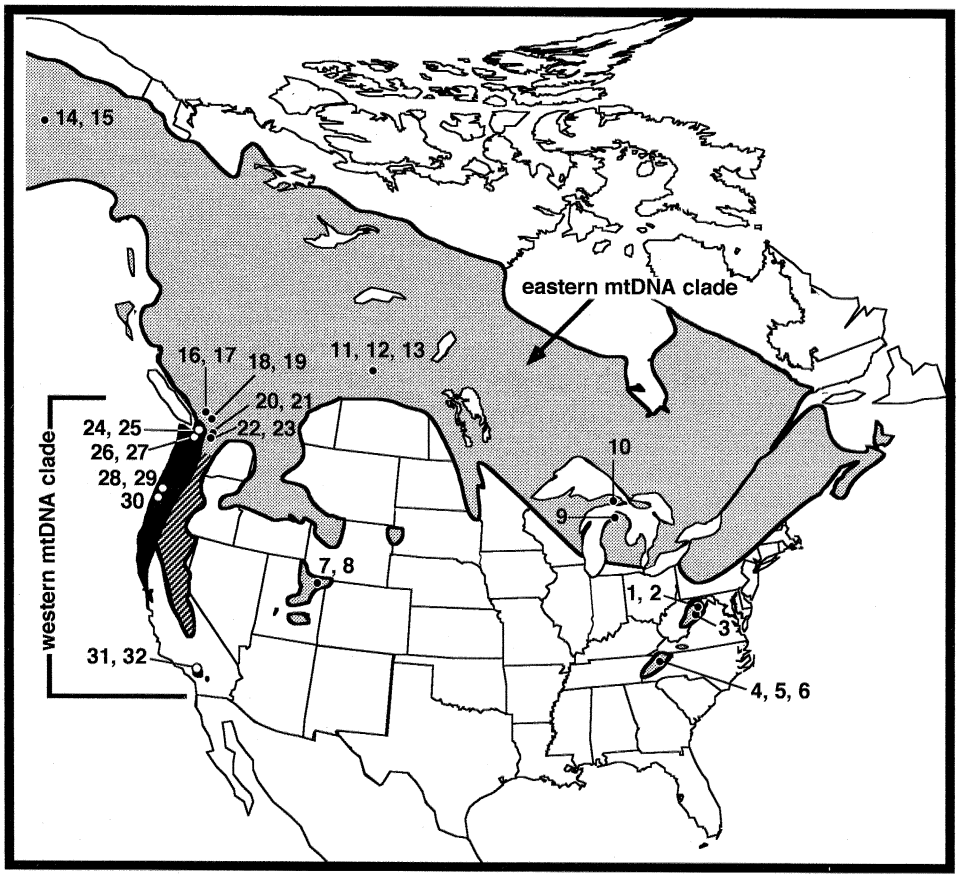

FIG. 3.—Phylogeography of *Glaucomys sabrinus* showing geographic distributions of the eastern (gray) and western (black) mtDNA clades. The location of the mtDNA discontinuity between the eastern and western mtDNA clades remains to be established within the unsampled areas of the southern Cascades and Sierra Nevadas (barred area). Locality numbers and abbreviations in Table 1 and Fig. 2.

Sciurus, and *Tamiasciurus*), show either a genetic or distributional discontinuity that aligns closely with the observed phylogeographic discontinuity of mtDNA observed in this study for *G. sabrinus.* The level of concordance between the phylogeographic patterns of *G. sabrinus* and *Tamiasciurus* is particularly striking. The present distribution of *G. sabrinus* is remarkably similar to the combined distributions of *Tamiasciurus hudsonicus* and *T. douglasii,* with a distributional discontinuity (Hall, 1981) between the two species of *Tamiasciurus* occurring in the same area as the mtDNA discontinuity observed in this study for *G. sabrinus.*

The possibility that coincident patterns between *Tamiasciurus* and *Glaucomys* were caused by a shared historical vicariant event is supported by a diverse array of vertebrate taxa that exhibit a similar genetic discontinuity in the Pacific Northwest (Arbogast, 1996). These include, among others, mule-deer (*Odocoileus hemionus*—Cronin, 1992), the American black bear (*Ursus americanus*—Byun et al., 1997; Cronin et al., 1991; Wooding and Ward, 1997), the leopard frog (*Rana pretiosa*—Green et al., 1996), giant salamanders (*Dicamptodon*—Good, 1989), the common yellowthroat, *Geothlypus trichas,* and the rufous-sided to-

whee, *Pipilo erythrophthalmus* (Ball and Avise, 1992), chickadees (*Parus*—Gill et al., 1993), and the fox sparrow (*Passerella iliaca*—Zink, 1996).

Repeated advances into Washington by the Cordilleran ice sheet and alpine glaciers, beginning ca. 1.0×10^6 years ago (Westgate et al., 1987) may have acted as major barriers to gene flow for many taxa, isolating populations along the western coast of the United States from conspecific populations in the interior of North America. The diversity of taxa that appear to have been affected by such a barrier in the Pacific Northwest suggests that a formerly contiguous North American boreal ecosystem may have been divided into two distinct communities in the early-to-middle Pleistocene. Subsequent glacial advances and large-scale flooding events (Baker, 1983; Westgate et al., 1987) may have reinforced such a division by repeatedly establishing geographic barriers to gene flow in the latter half of the Pleistocene. Following the initial divergence of the eastern and western clades of *G. sabrinus,* the two clades appear to have occupied separate refugia during periods of glacial maxima: a refugium along the western coast of the United States for the western clade, and a refugium in the south-central United States for the eastern clade (Fig. 1). Existence of boreal refugia in the south-central United States during the Pleistocene is supported by floristic reconstructions of the region and the fossil record. The paleobotanical record indicates that during the most recent (Wisconsin) glacial maximum, typical boreal tree species such as spruce (*Picea*) were restricted to the south-central United States (Davis, 1983; Ritchie, 1987). Fossil remains of *G. sabrinus* from Peccary Cave, Arkansas, dating to ca. 14,000 years ago, reveal that the distribution of *G. sabrinus* was similarly displaced south at this time (Kurtén and Anderson, 1980). Following glacial retreats, boreal forests appear to have recolonized most of northern North America quickly, with spruce reappearing in the post-Wisconsinan record of Alaska by ca. 8,000 years ago (Ritchie, 1987).

The mtDNA data support a rapid southeast-to-northwest post-Wisconsin range expansion by the eastern mtDNA clade of *G. sabrinus,* closely paralleling that of boreal forests. For example, within the eastern mtDNA clade of *G. sabrinus,* populations from Alaska and the southern Appalachians exhibit <2% sequence divergence from one another, despite being geographically separated by >8,000 km. This pattern, wherein low levels of haplotypic diversity are observed over broad portions of northern North America, has been observed in other taxa (Avise et al., 1984, 1987; Gill et al., 1993), and is characteristic of populations that are presumed to have undergone rapid, post-glacial, northward dispersal from a limited population source (Ball and Avise, 1992; Gaines et al., 1997; Gill et al., 1993). The greatest level of sequence divergence within *G. sabrinus* (7.2%) occurs between those populations found just to the east and just to the west of the crest of the Cascade mountains in Washington (populations 20–23 and 24–27 of the eastern and western clades, respectively; Fig. 3), further suggesting that the two mtDNA clades of this species have come into secondary contact in the Pacific Northwest only recently, following a rapid post-Wisconsinan range expansion by the eastern clade. Wooding and Ward (1997) recently observed a similar pattern of secondary contact among distinct eastern and western mtDNA clades of the American black bear.

Compared with the eastern mtDNA clade, the western lineage of *G. sabrinus* exhibits a greater level of intra-clade sequence variation (≤2.6%), despite ranging over a smaller geographical area. This suggests that since its divergence, the biogeographic history of the western clade of *G. sabrinus* has been more complicated than that of the eastern clade. Multiple vicariant events, resulting in repeated fragmentation of populations, may have acted to increase haplotypic diversity within the western

clade of *G. sabrinus*. In contrast to the evidence supporting a rapid post-Wisconsin range expansion of the eastern mtDNA clade of *G. sabrinus*, the western mtDNA clade appears to have expanded its range only slightly northward following glacial retreats—a pattern characteristic of those taxa comprising the contemporary Pacific Coast mammalian fauna (Dalquest, 1948).

Disjunct populations of *G. sabrinus* currently found in mountainous regions of the United States (Fig. 1) are, in all cases, closely related to nearby, more northern populations within the contiguous part of the species' range (Figs. 2 and 3). For example, populations of the two disjunct, Appalachian subspecies of *G. sabrinus, G. s. coloratus,* and *G. s. fuscus* are related closely to populations of *G. s. macrotis* from Michigan. Similarly, disjunct populations of the subspecies *G. s. lucifigus* from Utah are related closely to populations of *G. s. fuliginosus* from eastern Washington, as well as to the other, more northern subspecies (i.e., *G. s. sabrinus* and *G. s. yukonensis*) sampled from the western region of the eastern mtDNA clade (Table 1, Fig. 3). This evidence suggests that the disjunct montane populations of *G. sabrinus* are late Pleistocene relicts, isolated only recently from the more northern, continuous populations by northward movement of forest types following glacial retreats. It is unclear if the proposed south-central refugium extended far enough west during glacial maxima to allow direct recolonization of the Rocky Mountains, or if these mountains were recolonized more recently from the north as the eastern clade of *G. sabrinus* extended its range westward across northern North America.

Although not sampled as broadly as *G. sabrinus,* patterns of genetic variation in *G. volans* are consistent with Pleistocene shifts in the distribution of deciduous forests, especially forests dominated by oak (*Quercus*) and hickory (*Carya*—Davis, 1983). Populations of *G. volans* from North Carolina, West Virginia, and Louisiana exhibit a remarkably low level of sequence divergence (≤0.6%), suggesting that this species has undergone a relatively recent and severe bottleneck, followed by a rapid post-Wisconsin range expansion (Ball and Avise, 1992; Gaines et al., 1997). The paraphyly observed within populations of *G. v. saturatus* from Louisiana (Fig. 2) may be associated with similar disjunctions observed in a variety of vertebrate taxa inhabiting the southeastern United States, including the Carolina chickadee (*Parus carolinensis*—Gill et al., 1993), various freshwater fish (*Fundulus*—Wiley and Mayden, 1985), water snakes (*Nerodia*—Lawson, 1987), and rabbits (*Sylvilagus*—Chapman and Feldhamer, 1981; Chapman and Willner, 1981). Many of these southeastern discontinuities in mtDNA center near the Apalachicola River in northwestern Florida and southeastern Alabama, or along a disjunction corresponding to the Mobile Bay and Tombigbee River (Gill et al., 1993). No additional phylogeographic discontinuities are evident in the geographic area sampled (disjunct populations of Mexico and Central America were not examined).

Overall, evidence from mtDNA strongly suggests that the evolutionary history of flying squirrels in New World has been shaped profoundly by Pleistocene glacial-interglacial cycles and associated changes in geographic distributions of the boreal and deciduous forest habitats of North America. Phylogeographic discontinuities observed in both *G. sabrinus* and *G. volans* align with similar discontinuities in a variety of other North American vertebrates, suggesting that forest fragmentations during the Pleistocene may underlie a common pattern in the phylogeography of North American forest taxa (Arbogast, 1996; Wooding and Ward, 1997). In particular, the distinct Pacific Northwest discontinuity shared by *G. sabrinus* and a wide variety of taxonomically diverse, boreally distributed vertebrates argues for an evolutionary scenario wherein the boreal-forest ecosystem of North America was fundamentally

divided into two distinct communities in the early-to-middle Pleistocene.

GENBANK ACCESSION

Sequences referred to in this paper have been deposited in the GenBank sequence database under accession numbers AF063029–AF063067.

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