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MTDNA PHYLOGENY OF ANDEAN MICE: A TEST OF DIVERSIFICATION ACROSS ECOLOGICAL GRADIENTS

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Abstract.—Parapatric speciation across sharp ecological gradients is an alternative to the more usual allopatric model as both a general explanation of organismal diversification and as an explicit statement regarding differentiation of tropical forest biotas (Endler, 1977, 1982a, 1982b). The relevance of this model to species of small mammals distributed across a 3,000 meter gradient on the eastern flank of the Peruvian Andes is examined here by phylogenetic analysis of comparative mitochondrial DNA sequences, relying on both freshly collected samples and extracts taken from skins preserved in museum collections. These analyses falsify the applicability of the gradient model in this case. Moreover, the phylogenetic approach employed here provides an explicit test of the feasibility of the gradient model for any other taxon, either plant or animal, of the lowland Amazonian forest.

Key words.—*Akodon*, Andean mice, mtDNA sequences, parapatric divergence, phylogenetics.

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A general theory for the evolution of tropical biotas in paleoecological refugia has received widespread support since the early 1970s, based on a combination of geological, palynological, and plant and animal distributional analyses (see papers in Prance, 1982 and Whitmore and Prance, 1987 for general reviews). While the Refuge Theory has been enthusiastically adopted by many for both the lowland Amazon Basin and for the high Andes (Haffer, 1969; Vanzolini and Williams, 1970; Vuilleumier and Simberloff, 1980), the reconstruction of the history of the South American tropical biota has proven very difficult, for several reasons. For example, the original postulation of specific refuge areas was based either on areas of endemism or on patterns of coordinate character change across geography. As pointed out by Endler (1977, 1982a, 1982b), however, both criteria also conform to a model in which selection across present-day ecological gradients can be the primary determinant of these patterns. Moreover, while ample data supporting the requisite paleoclimatic changes of the Refuge Theory are available, even for the lowland forest of the Amazon Basin (for example, Walker and Chen, 1987; Colinvaux, 1987), historical evidence for the localization of refuges recognized on the basis of present distribution patterns of species diversity and endemism is lacking, and considerable debate continues with regard to the nature of vegetation changes within the Basin (Bush et al., 1990).

There is also little evidence that speciation in any specific group of organisms was tied to isolation in refuges, even though their present distributions were very likely influenced by Pleistocene climatic cycles. Species level diversification for some groups clearly predates the Pleistocene (see, for example, Heyer and Maxson, 1982).

The phylogenetic affinities of mice of the genus *Akodon* (Muridae; Sigmodontinae) that inhabit the forested slopes of the eastern Andes of Peru provide a test of Endler's (1977, 1982a, 1982b) ecological gradient hypothesis for the diversification of tropical biotas. These small and largely insectivorous rodents are distributed elevationally as pairs of species within drainages, with one member of a pair inhabiting high elevation (3,500–2,000 m) elfin forest and the other lower elevation (2,000–1,000 m) upper tropical forest (Cadle and Patton, 1988; Patton, 1987; Patton et al., 1990). The sharp vertical climatic and habitat shifts across this 3,000 m change in elevation present the type of steep selective gradient across which parapatric divergence might occur (Endler, 1977, 1982a, 1982b), and the zonal pattern of distribution of these small mice could be inferred to result from this mode of diversification. Such a pattern, however, might also reflect the historical opportunity for vicariant allopatric differentiation due to Pleistocene cycles of vegetation zone compression and expansion (Adams, 1977; Vuilleumier and Simberloff, 1980). Many

vicariant scenarios of faunal diversification are possible for this system of species and geographic valleys, and tests for any one of these are difficult. However, the gradient hypothesis is explicitly testable by robust phylogenetic analysis. As a model of parapatric divergence, this hypothesis requires that populations on either side of a demonstrable gradient be sister taxa (Fig. 1).

In this paper, we use the polymerase chain reaction (PCR) to amplify DNA for direct sequencing from museum specimens, and compare 401 bases of the mitochondrial (mt) DNA cytochrome *b* gene of the three species of akodont rodents distributed within and across seven adjacent Andean valleys of southern Peru. Direct sequencing following amplification by PCR (Kocher et al., 1989; Saiki et al., 1988; Wrischnik et al., 1987) has enabled the retrieval of DNA sequences from dried museum specimens (Thomas et al., 1989; Thomas et al., 1990) as well as from fresh or recently frozen tissues. This remarkable capability permits comparisons of DNA sequences between species or populations that are now extinct or currently endangered, are rare in museum collections, or come from extremely remote geographical areas from which specimens were collected in the past.

MATERIALS AND METHODS

Fresh specimens of *Akodon* were collected from five Andean river valleys (Fig. 2), and these collections were supplemented with museum specimens, so that all species known to occur in seven adjacent valley systems in southern Peru could be studied. DNA was extracted from either liver tissue frozen in the field in liquid nitrogen or from an approximately 3 mm² piece of dried skin removed from preserved museum specimens generally following the sodium dodecyl sulfate-proteinase K, phenol, RNase method (Maniatis et al., 1982). Slight modifications in the standard extraction procedure used for the skin samples are provided in Smith and Patton (1991a). Sequence was obtained for a segment of the cytochrome *b* gene (cyt *b*), one of the 13 protein-coding genes in the circular mitochondrial genome (see Brown, 1985, for review). For specimens with frozen liver available, the oligonucleotide primer pair MVZ05-MVZ04

was used to amplify a 482 base pair (pb) fragment, from which 401 bp of the cytochrome *b* sequence were read. Amplifications from skin extracts were done in three overlapping segments using primer pairs MVZ05-MVZ06, MVZ07-MVZ10, and MVZ11-MVZ04. Each of these pairs was selected to amplify a fragment of less than 200 bp, because longer sequences of old DNA have proven difficult to amplify (Pääbo, 1989; Pääbo et al., 1988). Sequences of primers MVZ04 and MVZ05 are given in Smith and Patton (1991b); those for the other primers listed here are as follows (letters in parentheses identify the light or heavy strand, and the numbers following the letters give the position of the 3' base in the complete mtDNA sequence for the house mouse (Bibb et al., 1981):

MVZ06 (H14295) 5'-GCTGTGTCTGATGTGTAGTGTAT-3'
 MVZ07 (L14230) 5'-AACCCCATCTAACATTTC(A/T)TC(C/T)TGATG-3'
 MVZ10 (H14437) 5'-TATGAGCCGTA-GTA(A/G)A(T/G)(T/G)CCTC-3'
 MVZ11 (L14350) 5'-CCTCCGTAGCCC-ACA(T/C)(A/T)TG(C/T)CG-3'

The veracity of skin sequences was confirmed by direct comparison of the 156 bp sequence obtained with primer pair MVZ05-MVZ06 from both museum skins and liver samples of a series of 10 individuals representing 6 species collected during 1984 to 1987 (Smith and Patton, 1991a). There was a perfect match between skin and liver sequences from the same individual in all cases. Conditions for both double and single-strand amplifications are given in Smith and Patton (1991a, 1991b).

Sequences were entered into BIONET (IntelliGenetics, 1988) for alignment and translation; alignment was based on direct comparison to the cytochrome *b* sequence of the house mouse (*Mus*; Bibb et al., 1981). Phylogenetic analyses of the sequences were performed using PHYLIP (version 3.2; Felsenstein, 1989), run on a VAX mainframe system, and PAUP (version 3.0 g; Swofford, 1989), run on an Apple MacIntosh IIfx. The branch-and-bound option of PAUP was used to obtain all minimal length trees based on character state matrices using parsimony

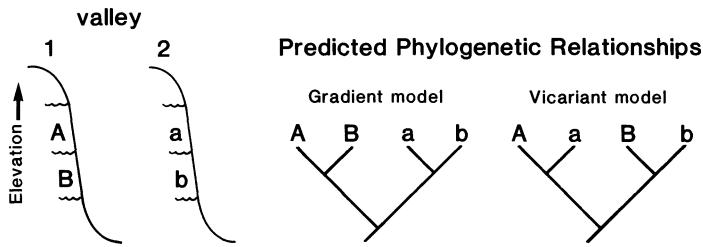


FIG. 1. Phylogenetic relationships of populations distributed within and across adjacent drainages under expectations of diversification by the ecological gradient hypothesis and one expected scenario under a vicariant model of divergence resulting from Pleistocene cycles of vegetation zone depression and expansion.

methods; the veracity of specific nodes of the most-parsimonious hypotheses of relationships was tested by a bootstrap analysis (Felsenstein, 1985) with 500 replicates using the DNABOOT subroutine in PHYLIP.

Sequence comparisons were made between the three species of akodonts that inhabit the forested Andean slopes: seven separate valley samples of *Akodon aerosus*, an inhabitant of upper tropical forest between approximately 2,000 and 1,000 m (see Patton et al., 1990), and four and two valley samples, respectively, of *A. torques* and *A.* (= *Microxus* of previous authors) *mimus*, both denizens of elfin forests at elevations between 3,500 and 2,000 m (see Fig. 2). Three other related species that are distributed in the puna grasslands above 3,500 m [*A. subfuscus* (Myers et al., 1990), *Bolomys amoenus*, and *Chroeomys jelskii*] were used as increasingly distant outgroups in phylogenetic analyses (Patton et al., 1989; Smith and Patton, 1991b). Locality data, museum collection, and catalog numbers for all individuals examined are given in the Appendix.

RESULTS AND DISCUSSION

Applicability of the Gradient Model

By current taxonomy, the gradient model appears to be falsified without benefit of the analyses completed here. That is, each of the taxa considered in this study is distributed horizontally across a series of adjacent valleys, thus supporting the vicariant hypothesis of Figure 1. However, this conclusion is premature and, therefore, in need of verification. While samples from the same elevational horizon in different valleys generally share a similar external and cranial

morphology (e.g., Patton et al., 1990), phenotypic similarity can result from selection to similar habitats as well as from common ancestry (Endler, 1977). Moreover, the taxonomy of akodont rodents in general, and of *Akodon* in particular, is in an especially primitive state. With a single exception, there have been no systematic studies of these mice, other than the original taxon descriptions, and current taxonomy of most of the group is based on the check-list compilation of Cabrera (1961).

There are several reasons to suggest that the current taxonomy of *Akodon* in general and of this complex of mice in particular is both an inadequate and an oversimplified perspective of their evolutionary dynamics. For one, the available nomenclature is more complex than that suggested by Cabrera or here. *Akodon boliviensis*, the only one of these taxa that has undergone recent revision, is a complex of at least four distinct and varying sympatric species in southern Peru, one of which is *subfuscus* (see Myers et al., 1990). Second, the samples allocated to *aerosus* here include two forms originally given specific or subspecific rank (*surdus* Thomas, from the Urubamba valley, and *baliolus* Osgood from the Limbani drainage of the Río Inambari). Because the type locality of *aerosus* is in Ecuador, several thousand kilometers to the north, the applicability of this species epithet to samples in southern Peru is questionable without appropriate documentation. Third, several of the samples of both *aerosus* and *torques* are karyotypically differentiated, suggesting that populations in separate valleys have been independent evolutionary units to some degree (Hsu and Benirschke, 1973, 1974; Patton, unpubl. data). For example, *torques*

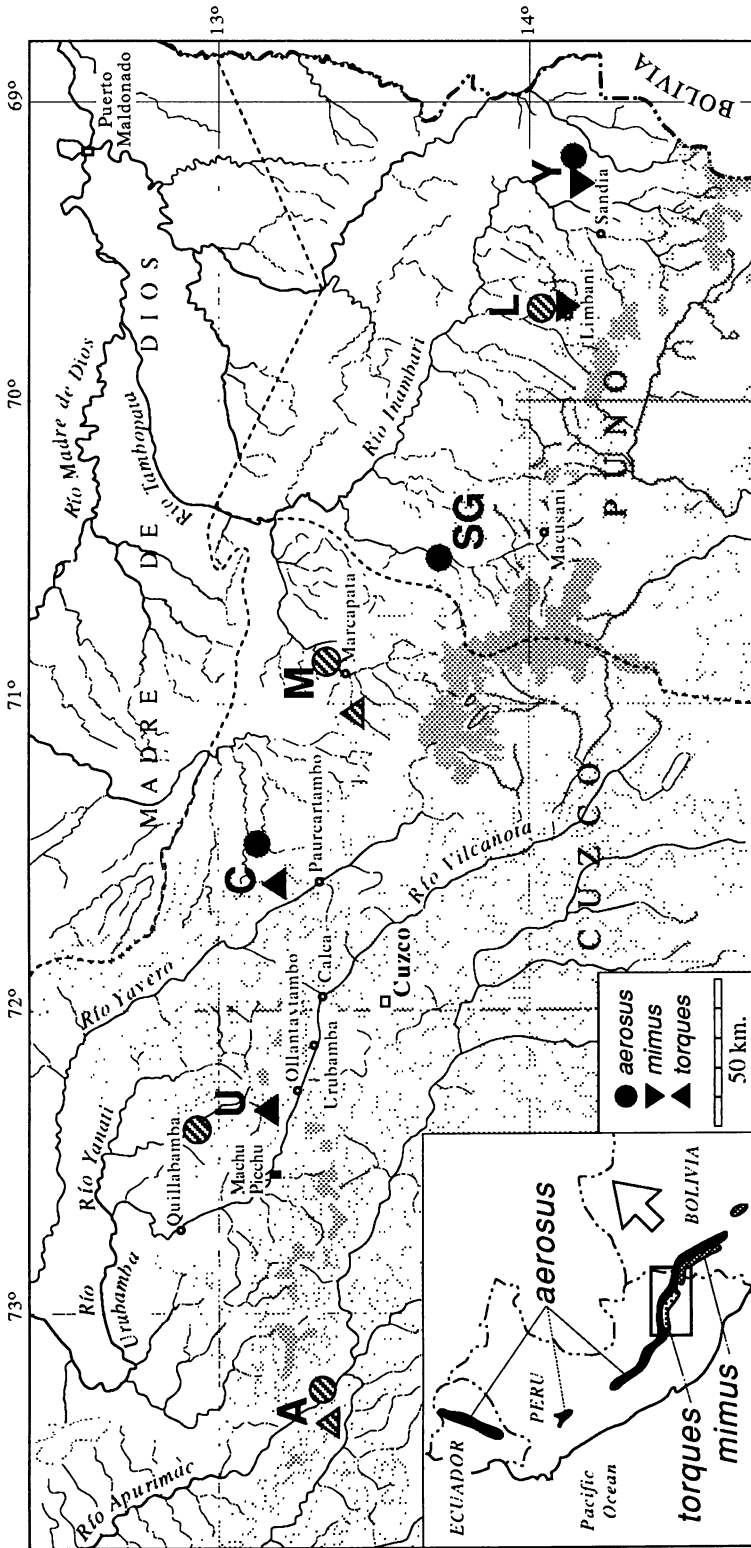


FIG. 2. Sample localities of three species of *Akodon* (including *Microxius*) of the forested eastern Andean drainages of southern Peru: A, Río Apurimac; U, Río Urubamba; C, Río Cosñipata; M, Río Marcapata; SG, Río Sangabán; L, Río Limbani; and Y, Río Sandía. Stippled patterns represent elevational contour intervals of 1,000 m, with the most dense stipple pattern delimiting areas above 4,000 m. Solid symbols represent those specimens from which DNA was extracted and sequenced from liver; diagonally hatched symbols represent localities for which only preserved skin serves as the source of DNA. The geographic ranges of the three slope species (*aerosus*, *torques*, and *mimus*) are given in the inset map.

TABLE 1. Percent sequence divergence among 6 species and 19 populations of Andean akodontine rodents, based on 401 bp of the mtDNA cytochrome *b* gene; *N* is the number of pairwise comparisons.

Comparison	Mean	SD	Range	<i>N</i>
Differentiation among genera				
<i>Chroecomys</i> to all others	0.1915	0.0075	0.180–0.204	19
<i>Bolomys</i> to <i>Akodon</i>	0.1331	0.0077	0.122–0.145	18
Within <i>Akodon</i>	0.0981	0.0093	0.073–0.117	32
Geographic variation within species				
Within <i>A. subfuscus</i>	0.0072	0.0060	0.000–0.015	6
Within <i>A. torques</i>	0.0255	0.0084	0.012–0.037	6
Within <i>A. aerosus</i>	0.0395	0.0241	0.000–0.053	28
Within <i>A. mimus</i>	0.0050	—	—	1

from the Apurimac drainage are $2n = 26$, those from the Río Urubamba are $2n = 22$, and those from the Cosñipata drainage are $2n = 24$. Similarly, *aerosus* from the Apurimac are $2n = 40$, those from the Cosñipata are $2n = 22$, and those from the Sangaban and Sandia drainages of the Río Inambari are $2n = 38$. Finally, the habitats even at the same elevation in different valley systems are radically different, some valleys being quite xeric and others very wet (see Peyton, 1980), offering opportunities for horizontal as well as vertical differentiation. Thus, despite a current taxonomy that seemingly links samples horizontally across valleys, it is not at all clear that this taxonomy reflects true phylogenetic relationships; it certainly does not preclude the applicability of gradient expectations relative to the divergence of these mice.

SEQUENCE DIVERGENCE AND PHYLETIC RELATIONSHIPS

Sequence data for 401 bp of cyt *b*, beginning with the start codon at the 5' end (position 14139 of the *Mus* genome; Bibb et al., 1981), for representatives of each of the taxa examined here are presented in Smith and Patton (1991b). Complete sequences for representatives of each of the taxa examined in the present report are available from GenBank (accession numbers M35691–M35716); these and all others are also available from the authors upon request.

The mean percent sequence divergence of all taxa relative to *Chroecomys* is 19.1% and of *Akodon* (including "*Microxus*") relative to *Bolomys* is 13.3%. Among the various sampled species of *Akodon*, the average sequence divergence is 9.8%, with a range from

7.3 to 11.7% (Table 1). Two individuals from each population sample were examined to assess the level of within-population divergence, following our previous work (Smith and Patton, 1991b). Five of 18 comparisons of individuals from the same population exhibited sequence differentiation, but the maximum number of substitutions for any of these was only 2 in the 401 bases examined (0.5% sequence divergence). Because minimal sequence divergence observed between any pair of *Akodon* species is 3% (Smith and Patton, 1991b; unpubl. data), two individuals per population are probably adequate for the purposes of documenting within-population variation relative to that among samples in the present study.

There are 129 phylogenetically informative sites (those that exhibit at least 2 states in two or more populations or species) when all 6 species and 19 populations are compared; 106 informative sites if *Chroecomys* is not included. Transitions and transversions were given equal weight as characters in the phylogenetic analyses, a decision supported by 3 arguments: First, the observed mean level of sequence divergence within *Akodon* is 9.8%, and increases to 13.3% if the outgroup *Bolomys* is included; these values are well below the theoretical threshold of 15 to 20% at which multiple substitutions at one site begin to mask unique mutational events (Nei, 1987). Second, there is no difference in the slope of the relationship between the number of transitions (TS) relative to number of transversions (TV) whether the analysis is restricted to the taxa of *Akodon* or if *Bolomys* is included; this slope, however, increases significantly if

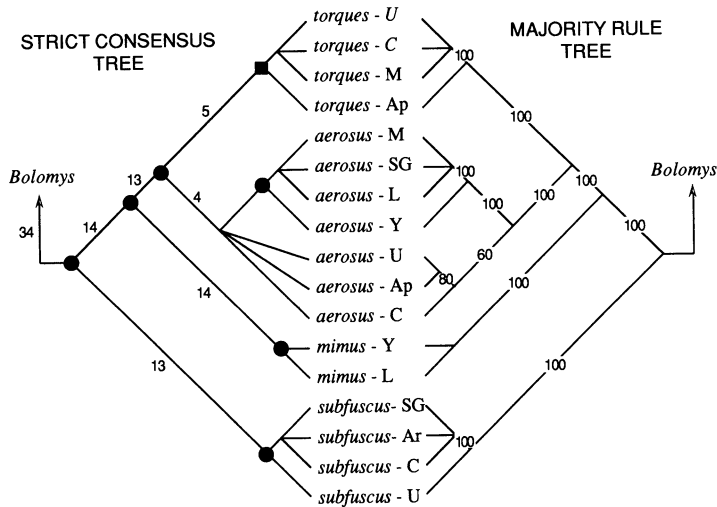


FIG. 3. Phylogenetic hypotheses for the relationship between four species and 17 populations of *Akodon* from the Andean region of southern Peru based on a 401 bp segment of the mtDNA cytochrome *b* gene and rooted by using *Bolomys amoenus* as an out-group. The tree on the left is a strict consensus of 90 equal length trees, each 171 steps in length (consistency index = 0.702). The number of informative substitutions supporting each internal branch is indicated. The solid circles identify internal nodes that were present in 95% or more of 500 bootstrap replicates, where each replicate represents a random sampling of sites with replacement, using the PHYLIP computer program (Felsenstein, 1989); the solid square identifies a node supported by more than 90% of 500 bootstrap replicates. The tree on the right is a 50% majority-rule consensus; the percentage of the 90 equal-length trees supporting a given branching sequence is indicated for each internal branch.

Chroecomys or *Mus* are added to the analysis. Finally, there is little relationship between the TS:TV ratio and the pairwise percent sequence divergence for all *Akodon* species plus *Bolomys* ($R^2 = 0.065$), but this relationship becomes significant if *Chroecomys* is included ($R^2 = 0.616$). In sum, these data indicate that site saturation has not been reached in comparisons among the various species of *Akodon* or between these and the outgroup *Bolomys amoenus*, but at the more distant relationships to the akodont *Chroecomys jelskii*, saturation is a factor. As a consequence, phylogenetic analyses were restricted to comparisons between *Bolomys* and the four *Akodon* species; *Chroecomys* was used only as an outgroup to confirm the sister group relationship between *Bolomys* and *Akodon* established by earlier studies (Patton et al., 1989; Smith and Patton, 1991b).

The branch-and-bound option of PAUP, which insures that all equally parsimonious trees will be found, generated 90 trees 171 steps in length (consistency index of 0.702) based on the 106 phylogenetically informative sites in the comparisons between

Bolomys and those species of *Akodon* that inhabit the puna grassland and forested slopes. Strict and 50% majority-rule consensus trees are illustrated in Figure 3 (left and right, respectively); these differ only in an inability to resolve dichotomous branches within the three taxa *aerosus*, *torques*, and *subfuscus*. Nodes that were identified in more than 90% of bootstrap replicates (Felsenstein, 1985) using the PHYLIP program are indicated in Figure 3 (left). While these trees are unable to define unambiguously all nodes into dichotomous pairs of sister taxa, they share the following common elements: For one, *Bolomys* has a sister group relationship to the *Akodon* clade, *Akodon mimus* is the sister taxon to *A. aerosus* and *A. torques*, and this triad of forested slope species shares a sister group relationship to the higher elevation, puna grassland *A. subfuscus*. These results mirror opinions previously presented based on morphology (Myers et al., 1990) and allozyme electrophoresis (Patton et al., 1989). The second major observation is that relationships among the taxa of the forested slopes are mostly horizontal, connecting populations from the

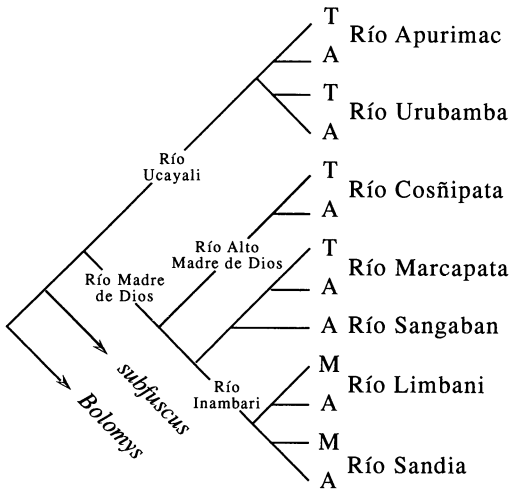


FIG. 4. The topology of a tree of relationships among Andean slope taxa based on expectations of the gradient divergence hypothesis. In this analysis, taxa (A = *aerosus*, T = *torques*, and M = *mimus*) are arranged phylogenetically by vertical position within single drainages, and the drainages are connected in the tree hierarchy in relation to their physical geographic connection (see Fig. 2). This hierarchical arrangement of taxa requires an additional 98 mutational events over the most parsimonious tree, that depicted in Figure 3, with a corresponding decrease in the consistency index to 0.446.

same elevation across adjacent valleys rather than vertically within drainages. For example, the four *torques* samples form a monophyletic clade relative to *aerosus* in 91% of the bootstrap replicates (455 out of 500). There is clearly less resolution among the samples of *aerosus*, although those of the four valley systems of the Río Inambari (see Fig. 2) group in all 500 bootstrap replicates. While these data do not support the strict monophyly of *aerosus* neither do they suggest any link between vertically distributed elements on the forested slopes. In this respect, these data answer the fundamental question of the present study; that is, the ecological gradient model for the diversification of this group of Neotropical rodents appears falsified. A similar conclusion has been reached for other Andean slope taxa of small mammals, specifically the rice rats of the *Oryzomys albigularis* complex (see Patton, 1987; Patton et al., 1990).

Additional analyses support falsification of gradient divergence for this group of mice. For one, a PAUP analysis based on the pat-

tern of sister group relationships expected from parapatric divergence within valley systems yielded a phylogenetic tree that requires an additional 98 mutational events (a 57% increase to 269 steps), with a corresponding drop in the consistency index to 0.446 (Fig. 4). This analysis built a hypothetical hierarchical relationship between population samples first by grouping each pair of samples within separate valleys and then building successive hierarchical relationships following the elevational connections of the valley systems themselves. For example, the Río Limbani and Río Sandia join at a high elevation into the Río Inambari, which then joins successively first with the Río Sangaban and then the Río Marcapata, and so forth (see Figs. 2 and 4). (Scenarios of hierarchical relationships other than this one that is described strictly by geography are, of course, possible but have not been examined.) The large increase in mutational events necessary for gradient divergence provides further confidence in a phyletic branching pattern that reflects elevationally horizontal relationships, rather than vertical ones within valleys. Second, the percent of sequence divergence among samples at the same elevational horizon increases with a decrease in elevation: the lowest elevation samples (*aerosus* from below 2,000 m) are 1.5 times as divergent among themselves (3.85%), for example, as are the *torques* samples at higher elevations in the same drainages (2.55%), and the latter are 3.5 times more differentiated than are the puna grassland *subfuscus* samples (0.72%). Because the degree of sequence divergence is, at least in part, the result of the historical cessation of gene flow and, because the origin of the genus *Akodon* is believed to be in the high elevation grasslands (Reig, 1986), the observed increase in divergence with a decrease in elevation would be expected under a vicariant model wherein taxa became progressively isolated with temporal vertical zonation shifts. There is no reason to expect this pattern under the ecological gradient model.

Although the phylogenetic relationships among populations of *Akodon* on the eastern Andean slope do not support gradient diversification within valleys, the data do not refute the applicability of that model to

the divergence of *torques* and *aerosus* on a larger geographic scale, because parapatric divergence may have proceeded prior to the segregation of their currently isolated populations in separate valley systems. This is because *torques* and *aerosus*, as groups of populations from adjacent drainages, still form a sister-taxon relationship in relation to the other species examined in this report. Clear refutation of this possibility, and thus unambiguous falsification of the gradient model, would only occur if further phylogenetic analysis indicated that the *torques* and *aerosus* clusters of taxa did not share a common ancestor relative to other *Akodon*. This possibility is being tested by our continuing studies on the systematics of this group of mice.

SUMMARY AND CONCLUSIONS

With a shift in focus to patterns of phylogenetic relationships among populations or taxa, rather than solely to patterns of endemism and species diversity, critical examination of both the Refuge Model and the opposing Gradient Model can be made, as we have done here for Andean mice of the akodontine complex. Despite the extreme ecological gradient represented by over 3,000 meters of elevational change on the eastern slope, phylogenetic relationships among these, and other mice (Patton, 1987; Patton et al., 1990), do not conform to a strict application of the gradient model of diversification. Perhaps this is not surprising because the Pleistocene glacial-interglacial impact on vegetation zones both latitudinally and elevationally in the Andean chain is still strongly evident in the limited geological and palynological records (Haffer, 1987; Wright et al., 1989; Schubert and Clapperton, 1990; Seltzer, 1990).

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APPENDIX

Specimens Examined.—Taxa, localities, and museum numbers vouchers for all specimens from which DNA was extracted and sequenced are as follows (MVZ = Museum of Vertebrate Zoology, University of California, Berkeley; FMNH = Field Museum of Natural History, Chicago; ZMUM = Zoological Museum, University of Montana, Missoula; LSUMNS = Museum of Natural Science, Louisiana State University, Baton Rouge):

Chroecomys jelskii—Peru: Depto. Junin; 22 km N La Oroya, 4,040 m (MVZ 173083, 173084); Depto. Puno; 6.5 km SW Ollachea, 3,350 m (MVZ 173073–173074).

Bolomys amoenus—Peru: Depto. Puno; 12 km S Santa Rosa, 3,960 m (MVZ 172878, 172879).

Akodon aerosus—Peru: Depto. Ayacucho; Huanhuachayo, 1,660 m (LSUMNS 16719), Hda. Luisiana, Río Apurimac (LSUMNS 16724); Depto. Cusco; Machu Picchu, 1,600 m (ZMUM 16985, ZMUM 16986); Depto. Cusco; 72 km NE Paucartambo, 1,460 m (MVZ

171679, 171680); Depto. Cusco; Hda. Cadena, Marcapata (FMNH 68612, FMNH 68614); Depto. Puno; 4 km NNE Ollachea, 2,380 m (MVZ 172818, 172819); Depto. Puno; Santo Domingo (FMNH 52535, FMNH 52537); Depto. Puno; Abra Marrancunca, 14 km W Yanahuaya, 2,210 m (MVZ 172849, 172450).

“Microxus” mimus—Peru: Depto. Puno; Agualani, 9 km N Limbani, 2,840 m (MVZ 171745, 171746); Depto. Puno; Abra Marrancunca, 14 km W Yanahuaya, 2,210 m (MVZ 171752, 171753).

Akodon subfuscus—Peru: Depto. Arequipa; 15 km S Callalli, 4,150 m (MVZ 174109, 174110); Depto.

Cusco; 26 km NW Ollantaytambo, 3,700 m (MVZ 174229, 174230); Depto. Cusco; 32 km NE Paucartambo, 3,140 m (MVZ 171573, MVZ 171574); Depto. Puno; 6.5 km SW Ollachea, 3,350 m (MVZ 172969, 172970).

Akodon torques—Peru: Depto. Ayacucho; Yuracayacu (LSUMNH 16726), Puncu, ca. 30 km NE Tambo, 3,370 m (LSUMNH 15699); Depto. Cusco; 3 km E Amaybamba, 2,200 m (MVZ 174053, 174054); Depto. Cusco; 32 km NE Paucartambo, 3,140 m (MVZ 171720, 171721); Depto. Cusco; Ccachubamba (FMNH 78705), Amacho (FMNH 78708).