Differentiation of the *Akodon mollis* species group (Rodentia: Cricetidae: Sigmodontinae) in the Andes of Peru

by

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Believing the strangest things, loving the alien…

David Bowie

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Abstract

The mechanisms responsible for the species composition of montane biotas remain poorly understood. Parapatric and allopatric speciation, in the forms of the Ecological Gradients and Montane Vicariance hypotheses respectively, have been suggested as processes to explain the diversification of Andean fauna. This dissertation reviews the possible role of these mechanisms in the speciation of mammals in Andean habitats. The soft grass mouse, *Akodon mollis*, was chosen as the test organism because of its wide elevational (500m to 4700m) and latitudinal range, and consequently, the variety of habitats and conditions its populations encounter. *A. mollis* inhabits the Andean mountains of North and Central Peru, and the Andes of Ecuador; however, this work focuses exclusively on Peruvian populations.

Geometric morphometrics and environmental analyses revealed that despite the wide elevational range of these mice, their skull morphology does not vary consistently across elevation except for a sharp change at the treeline, where montane forests end and the páramo/puna begins at around 4000m. It is hypothesized that these changes are a response to exposure to a new and severe set of biotic and abiotic conditions at high elevations. Animals living at high elevations have a slightly longer snout and a narrower braincase. Cross-correlations showed that the environment affects the rostrum and the braincase in opposite ways. Increases in temperature, precipitation and primary net productivity have the most positive influence on the rostrum, and the most negative

influence on the braincase. Partial least squares and multivariate multiple regression analyses showed a significant association between skull shape and environmental variables. The most important variables were associated with temperature, and these almost perfectly correlated with elevation. Nevertheless, a clear association between morphology and elevation was not found. This lack of local adaptation of *A. mollis* to clinal environmental conditions, at least over most of the elevational range of this species (from 500 m to 3500 m), suggests that the differentiation of populations in response to ecological gradients is unlikely to drive the diversification of this species in the Andean mountains.

Phylogenetic analyses of the mitochondrial Cytochrome b gene showed spatial structuring of *Akodon mollis* populations. Deeper lineages at the southern limit of the species coincide with the oldest part of the Andes, while shallower clades were found in the more recent northern Andes. An unambiguous test of the Ecological Gradient hypothesis was not possible; however, Montane Vicariance was suggested in two instances where mice at similar elevations on different mountains were closely related with high bootstrap support. Geographic distance did not explain patterns of genetic variation in Andean populations. When populations were defined as mice inhabiting single or adjacent mountains, significant values of genetic differentiation, F_{ST}, characterize populations of this species across its geographic range. Finally, AMOVA suggested differentiation between populations at higher southern elevations (>3500) and lower northern elevations (<3500m), but these results may be misleading. Mountains in the southern part of the range of this species are higher than those in the northern, and this analysis confounds the effects of elevation and latitude.

Further testing of Ecological Gradient and Montane Vicariance hypotheses will require additional taxa, some with ample distributional and latitudinal range like *Akodon mollis*, but also some with more moderate and restricted elevational distribution in the Andes, such as Andean endemics of the genus *Thomasomys*. More extensive sampling, including additional elevational belts and mountain replicates, is also necessary, as well as the incorporation of additional mitochondrial and nuclear molecular markers. Finally, the inclusion of isolation by resistance methods that incorporate topographic details of the Andes might help to shed further light on speciation by Andean mammals.

Chapter 1:

Hypotheses of mammalian diversification in the Andes

ABSTRACT

This chapter offers an overview of two major hypotheses to explain speciation in montane regions—the Montane Vicariance hypothesis and the Ecological Gradient hypothesis—and examines how each might be employed to explain the diversification of the mammalian fauna in the Andes. The evidence in support of and refuting each hypothesis is summarized and reviewed. The complex topographical and climatic history of the region provides a unique opportunity to test hypotheses used to explain patterns of diversification. The evidence presented here appears to support the Montane Vicariance hypothesis in the Andes most strongly, but far too few studies are available to allow any strong conclusion. Tests of the Ecological Gradient hypothesis have shown ambiguous results for Andean populations, and several studies have strongly rejected it. Tests of both hypotheses that make use of additional molecular data and theoretical advances in population genetics theory are strongly needed.

INTRODUCTION

One of the primary goals of evolutionary biologists is to explain the origins of and mechanisms behind patterns of biological diversity on earth (Cracraft, 1994). Neotropical diversity patterns, and particularly those observed in Amazonia, have been among the most highly contested areas of research, and already several major hypotheses

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have been proposed to explain patterns of diversity in this region (Haffer, 1997; da Silva and Patton, 1998; Moritz et al., 2000). Most attention has focused on eastern South America, but an important and often neglected Neotropical area is the Andean region. Patterns of diversity in the Andes may be influenced by processes that do not dominate in tropical lowlands. Yet patterns of diversity in the Andes may have influenced or been influenced by those found in the more often studied tropical lowlands (Fjeldså 1994, 1995). Fjeldså (1994, 1995) suggested that the Andes have worked as a "species pump" that populated not only high-elevation regions but also the adjacent Amazon lowlands. Along the same lines, other authors studying birds and butterflies have shown that over the past 10 million years, orogeny in the Neotropics coincide with high diversification rates in highlands and a slowdown of diversification in lowlands, corroborating Fjeldså's view of highlands as "key cradles" of diversity in the Neotropical region (Weir, 2006; Weir, 2009; Hall, 2005).

The Andes are a leading hotspot in terms of the number of endemic plants and terrestrial vertebrates (20,000 endemic plants and 1,567 endemic vertebrates) (Myers et al., 2000), and the most species-rich of any tropical mountain massif (Hughes and Eastwood, 2006). Yet to date, only a few studies of speciation have focused on Andean mammals, and most of those have concentrated primarily on lower montane or rainforest faunas. The lack of such studies means that a full understanding of the processes underlying the rich number of species in the Neotropics is not currently possible. A recent comparison of the number of species of mammals in Andean versus Amazonian habitats made by Pacheco (2002) nicely illustrates the contrast between these regions, using rodents as an example. He calculates that there are 358 mammal species in the Andes vs. 301 in Amazonia. Of those, 153 are rodents in the Andes (vs. 67 rodent species in Amazonia). Fifty-seven rodent species are endemic to the Andes vs. 8 to Amazonia. Although impressive, this is still a conservative number, as species are still being discovered during field expeditions in Andean habitats (e.g., Emmons, 1999; Luna and Pacheco, 2002, in prep.; Luna and Patterson, 2003; Gardner and Romo, 1993; Leo and Gardner, 1993; Patterson and Velazco, 2008; and Velazco, 2005). Why is this region

so diverse? The origin of Andean diversity is a critical question not only because its answer should help biologists address fundamental questions concerning the origin of species and adaptation, but because knowledge of how diversity is generated and spread could help us do a better job of designing reserves and parks to protect present and future biotas.

Two major hypotheses have been proposed to explain diversification of the mammalian fauna in the Andes: the Montane Vicariance hypothesis (see Coyne and Orr, 2004 for Vicariant speciation; see Haffer, 1967, 1969, 1982, 1990, 1993 for the related Pleistocene Refuge Hypothesis); and the Ecological Gradient hypothesis (Endler, 1977; 1982a, 1982b, see also Coyne and Orr, 2004 concerning clinal speciation). Few studies have tested both hypotheses using mammals, and most of those have concentrated primarily on lower montane or rainforest faunas (e.g., da Silva and Patton, 1998; Moritz et al., 2000). Moreover, the role of the Andes in speciation events, while the object of much speculation, has seldom been addressed empirically. Pioneering efforts to test these hypotheses in the Andes include those of Patton and colleagues (e.g., Patton et al., 1990; Patton and Smith, 1992a, 1992b). Although a few other studies (e.g., Patterson et al., 1992; Patterson and Velazco, 2008; Velazco and Patterson, 2008) have made suggestions or drawn conclusions about biogeographic patterns of Andean mammal species, they were not designed to test speciation hypotheses.

The present chapter summarizes the evidence for or against each of the abovementioned hypotheses, evaluates the evidence from research on lowland diversification, and considers the implications and relevance of this research to Andean diversification. The hypotheses are compared and their importance in explaining observed patterns of mammalian diversity in the Andes is discussed.

The Andes: history and geography

The Andes are Earth's second largest mountains and the dominant component of the Neotropical highlands. They extend 5000 km along the western side of South America. Their greatest width E-W is approximately 700 km and is reached at the level of the Central Andes of Bolivia. They have extensive land area above 4000 m, mostly in central and southern areas from Peru through northern Chile and Argentina (Garzione et al., 2008; Gregory-Wodzicki, 2000; Ribas et al., 2007; Weir, 2009).

Understanding the Andes uplift has become important for climate studies, as it is postulated to have affected global circulation (Gregory-Wodzicki, 2000). The Andes act as the only barrier to atmospheric circulation in the Southern Hemisphere, increasing rain along the eastern slope by holding condensation originated from the Amazon Basin. As a result, the environment is increasingly arid from east to west (Gregory-Wodzicki, 2000).

The Andes came into existence much before the Mesozoic (Zeil, 1979), and Paleozoic structures within the range prove that the orogenic history of the western edge of South America dates back to the Precambrian. Their orogeny is the result of the subduction processes operating between the Nazca and South American tectonic plates (Gregory-Wodzicki, 2000). While their bases were established long before their elevations, the modern Andes resulted from orogeny that was under way during the Cenozoic. By 45 million years ago, during the middle Eocene, the Andean Western Cordillera may have been as high as 5000 m (Bush, 1994). More recent studies, however, suggest that (1) by 25 Ma, the Western Cordillera was at not more than half its modern elevation; (2) the uplift of the Altiplano and Eastern Cordillera has occurred since 10 Ma at rates or 0.2-0.3 mm/yr, and (3) the final uplift of the uplift of the Northern Andes occurred 3-5 Ma (Gregory-Wodzicki, 2000).

The Andes chain is divided in Northern, Central, and Southern Andes. At around 4000m, the Central Andes are the highest and most mountainous section of the Andes. The Central Andes are divided into the northern Central Andes, the Central Andean Orocline (with the largest area of the entire Andes), and the southern Central Andes (Picard et al., 2008; Fig. 1). The molecular phylogenetics of well-sampled Andean species such as the potato and its nematode parasite suggest that the elevation of the Peruvian Andes occurred from south to north, with the Central Andean Orocline the first to reach high elevations (Picard et al., 2008).

Vuilleumier and Simberloff (1980) and Vuilleumier (1986) described three provinces in the Andean Domain: páramo, puna, and Patagonia. Páramo habitats, found in the northern Andes, are generally wetter than puna habitats, which have a clearly marked dry season. The physiognomic diversity of páramo formations is greater than that of puna formations (Vuilleumier and Simberloff, 1980, Troll, 1959). Páramo vegetation is present in the tropical Andes of Venezuela, Colombia, and Ecuador, while puna vegetation covers the tropical Andes of Peru (excluding the northern part), Bolivia, northern Argentina, and northern Chile. Because of the relief of the Andes, the punas and páramos are distributed in patches (Simpson, 1975). At slightly lower elevations are wet montane forest, with several kinds of moist, cool, forested environments from about 2000 m to the upper limit of continuous forests at 3000 to 3800 m in the tropical Andes from Venezuela, and Colombia, southwards to northwestern Argentina along the eastern slope of the Andes, and northern Peru along the western slope (Vuilleumier, 1986).

Because of their diverse habitats, tremendous elevational gradients with mountain tops and valleys often characterized by different vegetation types depending on elevation and age, the Andes are an ideal system in which to study speciation, providing multiple avenues for population isolation and, consequently, reduced gene flow (Futuyma, 1998; Weir, 2009). This diversity creates conditions in which hypotheses of speciation can be

tested and population genetic methods applied in order to shed light on the origin of Andean diversity and its relationship to Amazonian diversity.

HYPOTHESIS OF MAMMALIAN DIVERSIFICATION

Three main hypotheses have been proposed to explain diversification in the Neotropics. These include the Montane Vicariance hypothesis (Haffer, 1967, 1969, 1982, 1990, 1993, Haffer, 1997; Coyne and Orr, 2004), the Ecological Gradient hypothesis (Endler, 1977, 1982a, 1982b, Haffer, 1997; Coyne and Orr, 2004), and the Riverine Barrier hypothesis (Wallace, 1852; Ayres and Clutton-Brock, 1992, Haffer, 1997; Patton et al., 1994). Both the Montane Vicariance hypothesis and the Riverine Barrier hypothesis rely on allopatric speciation to explain patterns of diversity. Parapatric speciation mechanisms are the basis of the Ecological Gradient hypothesis and, to some extent they also play a role in the Riverine Barrier hypothesis. Explanations for each hypothesis are not mutually exclusive, and it would be a mistake to expect that any one hypothesis could explain all diversity patterns in any region. Indeed, Haffer (1997) offers a list of six hypotheses that could account for vertebrate speciation in Amazonia. These are combinations of the three main hypotheses (e.g. River-Refuge and Disturbance-Vicariance hypotheses) and in addition, a paleogeographic one. In the case of birds, complex scenarios have been drawn, in which for example highland populations of parrots were originally distributed at low elevations, and were gradually uplifted and vicariantly isolated from adjacent lowland populations during the orogeny of the Andes (Ribas, et l., 2007).

In this chapter, I will focus on the Montane Vicariance hypothesis and the Ecological Gradient hypothesis, both because the Riverine Barrier hypothesis is primarily applicable to lowland Amazonia, and because it has been convincingly rejected for

mammals by empirical studies (Patton, 1994). Below, the evidence for and against each hypothesis is considered.

The "Vicariance" hypotheses – the Refuge Hypothesis and Montane Vicariance Hypothesis

First developed by biologists studying the distributional history of the Palearctic and Nearctic biotas during the climatically unstable Pleistocene period, the vicariance hypothesis, in the form of the refuge hypothesis, argues that glaciations have cyclically isolated groups of species in refuges of suitable habitat, thus promoting allopatric speciation. The effect of glacial cycles on speciation in western North America has been and continues to be studied extensively (e.g., Knowles, 2000; 2001; Pielou, 1991; and Sullivan, 1985, 1994, 1996). This hypothesis has also been applied to the tropical biotas of Australia, Africa, and South America ("Refuge theory"; e.g., Haffer, 1982; Mayr and O'Hara, 1986). In both temperate and tropical areas, research has centered on identifying isolated areas or refugia where plants and animals survived glacial periods (Pielou, 1991, Willis and Whittaker, 2000). However, studies in the Neotropics have also focused on how refugia have behaved as "species pumps" or "cradles" for populations immediate to refuges (Willis and Whittaker, 2000; Fjeldså, 1994, Weir, 2006; 2009).

This hypothesis is driven by allopatric speciation. Allopatric speciation is accomplished by a physical barrier extrinsic to the organism, and it is often subdivided into two modes: *vicariant speciation*, in which reproductive isolation evolves after the geographic range of a species splits in two or more reasonably large, isolated populations; and *peripatric speciation*, in which reproductive isolation evolves after a small population becomes geographically isolated from a much larger one (Coyne and Orr, 2004). In this paper, I will use the term vicariance when referring to allopatric processes generally, since the size of the populations that resulted from the split of the parent population is

rarely or never known for Neotropical faunas. Allopatric speciation occurring in populations inhabiting mountaintops is specifically referred to as "montane vicariance" (Jansa, 1998; Gorog, 2003; Sullivan, 1985, 1994, 1996).

Vicariance hypotheses were widely used to explain tropical lowland diversity patterns throughout the 1980's (as "Refuge theory"). They remained attractive for some time, but they have not been examined critically (Endler, 1982a, 1982b) until recently. Now, as a result of the increased availability of molecular tools for investigating patterns and age of speciation, investigators have determined that at least some conditions of vicariance hypotheses appear to be met. Nevertheless, in South America almost all evidence concerning these hypotheses has come from the Amazonian lowland rainforest; little is known about potential refuges in the Andes. Despite the criticisms and evidence against them (below), and the fact that their predictions have not been tested, Vicariance hypotheses are widely cited in studies concerning diversity in lowland Amazonia.

Refuge theory, a particular form of Vicariance hypothesis, was first proposed by Jürgen Haffer (1967) as an explanation for the "patchy" distribution of the bird fauna of South America. In Haffer's view, a refuge is "a comparatively restricted area where a particular biome such as forest or savanna remained more or less constant during periods of vegetational shifts, thus preserving their mosaic of habitats and serving as a region of survival for at least part of the forest's or savanna's animal and plant populations." While studying toucans of the genus *Pteroglossus* and passerines of the genus *Manacus*, Haffer identified four refuge areas: the Chiriquí and Caribbean refuges in Central America, and the Nechí and Chocó refuges on the western side of the Colombian Andes (Haffer, 1967). Two years later, he identified seven additional refuges in South America based on indirect evidence from rainfall inequalities and patterns of avian distribution (Haffer, 1969). Subsequent studies have shown that these same areas may have served as refuges for other organisms as well, including some groups of plants (Vanzolini and Williams, 1970), butterflies (Brown, 1982), scorpions (Lourenço, 1986), lizards of the *Anolis*

chrysolepis group (Vanzolini and Williams, 1970), frogs (Duellman, 1982), and mammals (Kinzey, 1982), though with variation in refuge number and degree of overlap among them.

Haffer reconstructed refuges by superimposing two sources of evidence: (1) uneven distribution of rainfall over the Amazonian lowlands; (2) the current distribution patterns of Amazonian birds (e.g., chachalacas, curassows, and guans [Cracidae]), toucans (Ramphastidae), antbirds (Formicariidae), cotingas (Cotingidae), manakins (Pipridae), and others. Haffer argued that disjunct centers of lowland species endemism in diverse plant and animal groups often appear to correlate with mid-elevational hilltops. He further proposed that these hilltops captured orographic rainfall and supported isolated refugia throughout dry glacial periods. The successive expansion and contraction of populations to and from these refugia is proposed to have provided a "species pump" by isolating populations of wet forest animals by arid lowlands during glacial intervals, thus restricting gene flow between refugia and encouraging allopatric speciation (Morley, 2000, following Haffer, 1969, 1987).

Haffer's conclusions have been criticized for assuming that current variation in rainfall across the Amazon basin corresponds with Pleistocene variation in rainfall over the same region. In addition, he relied more heavily on modern plant and animal distribution patterns and climatic extrapolations than on geomorphology and paleobotany (Simpson and Haffer, 1978). While refugialists have cited data for pollen deposits in Colombia and Venezuela to support this hypothesis, the same data have been used by others to contest it (Endler, 1982; Colinvaux *et al.*; 1996, Colinvaux *et al.*, 2000; Colinvaux and Oliveira, 2001).

Andean faunas and floras have certainly been affected by glacial cycling, and apparently more dramatically so than faunas found in lowland areas. Geological studies

conducted throughout the North and Central Andes (including Venezuela, Ecuador, Peru, western Bolivia, northwestern Argentina and adjacent Chile) have shown that glacial climates caused the descent of both snowlines and timberlines (Simpson Vuilleumier, 1971), in addition to increased habitat patchiness. Indeed, puna (grassland) vegetation was fragmented, separated by areas of páramo vegetation. This form of patchiness could only have developed after the Andes had reached their present height at the Plio-Pleistocene boundary (Simpson Vuilleumier, 1971). Habitat patchiness in the Andes is reminiscent of islands or "refuges." The hypothesis that isolation in these kinds of habitat patches on mountains resulted in differentiation and speciation is referred to as the "Montane Vicariance" hypothesis. But can the topography and isolation of habitats in the Andes in and of themselves provide sufficient evidence to test the Montane Vicariance Hypothesis predictions here?

Predictions of the Vicariance Hypotheses

Because Haffer did not articulate clear predictions for the refuge hypothesis (Haffer, 1969; 1982; Simpson and Haffer, 1978), the hypothesis has not been unequivocally tested (Endler, 1982a). In attempting to refute the refuge hypothesis, Endler (1982a) synthesized the predictions as follows: (1) there should be more species, semispecies, and subspecies, and more endemics, in the sites of former refugia than in other areas; (2) for those subspecies, semispecies, and species pairs showing secondary contact, the positions of the centers of the contact zones should be concentrated between pairs of former refugia. Contact zones can be defined as a zone or band where two subspecies, semispecies, or species meet, possibly with intergradation (clines) or limited overlap. If present-day distributional patterns resulted from secondary contact of species that were formerly isolated in refuges, we would expect to find most of the contact zones between the postulated refuges, perhaps concentrated midway between them (Endler, 1982b). Finally, prediction (3) establishes that the widths of contact zones should be broader for species with shorter generation times and/or faster dispersal rates. In the

discussion that follows, I will focus primarily on the Montane Vicariance hypothesis, as it is the most relevant of the Vicariance Hypotheses to Andean faunas.

Evidence supporting the Vicariance hypotheses

Despite recent evidence challenging this hypothesis as an explanation for mammalian diversity in South America (Lessa et al., 2003), Vicariance hypotheses remain the favorite among South American biogeographers. If true, the implications of the Montane Vicariance hypothesis in particular for the Andes are significant. Lowland tropical forest in Amazonia has undergone episodes of shrinkage and expansion (Simpson and Haffer, 1978), creating a patchy environment during the last glacial with replacement of rain forest by savanna or savanna forest during dry climatic intervals. Direct evidence of this process was provided by pollen data from Brazil that suggested a savanna-type environment (van der Hammen, 1972; 1974). For the Andes, inferences about the climate in the Quaternary using the present distribution of vegetation were provided by Simpson (1975). She concluded that arid-humid cycles were present during glacial periods in the Andes of South America and that refuges indeed existed. However, Salo (1993) claimed that there is insufficient evidence showing that South America forests went through changes during the Pleistocene, including a fragmentation process. A more complete picture was provided by van der Hammen and Hooghiemstra (2000) who, based on pollen data, concluded that during the last glacial maxima (LGM) Amazonian and Cordilleran lakes dried up, and the dry rainforest was locally replaced by savanna, savanna forest, or cerrado-type vegetation. Later, the remaining dry rainforest, savanna forest and pure savanna were locally replaced by extensive semi-desert dune formations. Hooghiemstra and van der Hammen (1998) also concluded that patchy and homogeneous rainforests might have occurred simultaneously in different parts of the Amazon basin. This suggests that extinction and speciation in isolation under precipitation and temperature stress may have taken place in refuges at the same time that there were continuous forests in other areas.

Criticized for his views regarding the role of refuges in the divergence of species during the Quaternary (Endler, 1977), Haffer argued that the hypothesis does not propose that all speciation has taken place in refuges, or that all extant species are Quaternary in age. Rather, it attempts to explain the latest and perhaps most effective of the series of differentiation events beginning during the Mesozoic that contributed to the development of the modern biotas of the world (Simpson and Haffer, 1978; Haffer, 1982).

Evidence against the Vicariance Hypotheses

The "refugial debate" has gained intensity in recent years (Lessa et al., 2003, Bush, 1994; Endler 1982a, 1982b; Cracraft and Prum, 1988; Lynch, 1988; Moritz, et al., 2000) based mainly on potential flaws in its spatial and temporal components.

One problem pointed out by some critics of the theory concern a sampling bias favoring one of the Brazilian "refuges" near Belém (Bush, 1994). Indeed, the herbarium specimens presumably collected from Manaus, Trombetas, Belém, and the Rio Jari region outnumber those collected from other regions by a factor of >300 (Bush, 1994, Nelson et al., 1990).

Considering the predictions of the hypothesis (above), Endler (1982a) pointed out that only the first had been tested. He argued, however, that even the tests of prediction (1) were flawed, because sites of refugia were identified by animal distributions. This leads to a tautology caused by testing the existence of distributions by searching for the patterns on which they were defined. Endler falsified prediction (2) because only nine percent of the contact zones studied occurred between proposed refuges. Prediction (3) was falsified based on the observation that approximately the same gene flow rate has

been estimated for Neotropical birds and butterflies, despite the extremely different generation times (Endler, 1982a).

Further, recent molecular evidence from phylogeographic studies in some Amazonian and Andean species suggests pre-Pleistocene divergence is more common than divergence around the LGM (Moritz, et al., 2000). These phylogeographic studies focused primarily on birds, including flycatchers of the genus *Leptopogon* (Bates and Zink, 1994); parrots of the genus *Pionopsitta*, and toucans of the genus *Selenidera* (Cracraft and Prum, 1988); and chat-tyrants *Ochthoeca*, tapaculos *Scytalopus*, tanagers, *Metallura* hummingbirds, and cotingas (García-Moreno and Fjeldså, 2000; García-Moreno et al., 1999b; García-Moreno and Arctander, 1998). A similar pattern is seen in frogs of the genus *Hyla* (Check et al., 2001) and frogs of the genus *Leptodactylus* (Heyer and Maxon, 1982), and a probable Late Miocene and Pliocene divergence has been suggested for old lineages of mammals (Lara et al., 1996; Lara and Patton, 2000; da Silva and Patton, 1998; and Leite and Patton, 2002). These examples point to a higher degree of vertebrate divergence in South America during the Tertiary than the Quaternary. In addition, paleontological evidence suggests that Amazonian and Andean plant diversity arose mainly during the Tertiary (van der Hammen and Hooghiemstra 2000). Similar patterns are seen in hystricognath rodents and support the possibility of a role of pre-Pleistocene climate cycling in their diversification (Vucetich et al., 1999).

With respect to the paleoecological data, Bush (1994), following the studies of Salo (1987) and Colinvaux (1987), suggested that while precipitation fell below modern levels in Pleistocene Amazonia, this alone does not demonstrate forest fragmentation, as all sites that show drying were marginal to the Amazon basin, or edaphically sensitive to precipitation changes. On the other hand, Bush (1994) agreed that cooling in the lowlands is completely consistent with pollen evidence, suggesting that the Amazon basin became cooler, but not generally drier, during glacial maxima.

Adopting a different approach, Beven et al, (1984) considered 430 species and subspecies of birds in the Amazon basin, together with the refuges proposed by Haffer in 1969, and sought to determine if species' boundaries are independent of refuges. The authors found that some refugia and centers of endemism do have an unusually low number of species' boundaries traversing them, while others have too many, or as many as expected. This diversity of patterns led them to conclude that modern-day distributions of birds in the Amazon basin are the product of multiple processes, not simply a result of isolation in refugia.

An alternative model for Amazonian endemics developed by Colinvaux (1998) proposed the "negative image" of the Montane Vicariance model. Instead of forest patches or "refuges" standing like islands in a sea of savanna or cerrado vegetation, he suggested that the opposite was true: "ice-aged climatic change actually raised an archipelago of islands while the forest sea remained intact" (Colinvaux, 1997 cited by Colinvaux, 1998).

Colinvaux (1998) called into question whether the Amazon was ever arid enough for the forest to be fragmented as Haffer suggested (see also Bush, 1994). He based this view on the only continuous and well-dated pollen record of lowland Amazon rainforest, from Lake Pata in Colombia, as well as the evidence of reduced CO_2 concentration and lowered temperatures inferred from the ice core of Greenland and Antarctic. These data demonstrate that closed forests persisted in a region required by most refugia hypothesis maps to have been part of the savanna sea. The hypothesis contends that the effects of lowered temperatures and decreased levels of CO_2 might have influenced invasions of the lowland forest by species now restricted to higher elevations, with these latter habitats the ones that produced endemics. According to Colinvaux, the dispersal of species from high to low elevations would have been favored by the more critical effects of low temperature and CO_2 concentration as they changed with altitude. Therefore if Haffer's endemic isolates are to be found in the elevated areas proposed as refuges (see above), these are

the regions that have changed, rather than the lowlands, which remained the same throughout the Pleistocene. This hypothesis however was not discussed by others and the conclusions seem speculative without data from Andean montane forests. Indeed, this hypothesis will not be tested until there is an adequate pollen record for all Amazonia (Colinvaux, 1996, 1998, Salo, 1993; Tuomisto and Ruokolainen, 1997).

While rainforest refuges have been postulated for many rainforest areas, the molecular data to test whether such refuges existed, and whether their existence is associated with speciation, have been collected for only a few systems (Moritz, et al., 2000). The most recent test of the origin of diversity in Amazonia was proposed by Lessa *et al.* (2003). This study set out to address the controversy concerning the causes and significance of speciation by using coalescence theory to examine the genetic evidence for and against Montane Vicariance in boreal North American vs. tropical Amazonian mammals. Lessa *et al.* (2003) predicted that, if species had been restricted to refuges during the LGM and expanded from those refuges when climatic conditions ameliorated, then phylogeographic studies of those species should reveal the genetic patterns typical of rapid expansion (Epperson, 2003). For the neotropics, the authors tested species of small nonvolant mammals (*Proechimys simonsi, P. stereei, P. brevicauda, P. cuvieri, Oecomys bicolor, Oryzomys macconnelli, O. perenensis, Neacomys spinosus, Isothrix bistriata,* and *Micoureus demerarae*) in the lowlands close to two of the refuges in South America, the Napo refuge, and the East Peruvian refuges (eastern slopes of Andes). They also examined populations from close to the Juruá river. For North America, they examined the rodents *Microtus longicaudus* and *Glaucomys volans*; the shrew *Sorex monticolus,* and the bear *Ursus americanus.* They found significant phylogeographic structure in both boreal and Amazonian mammals, with Amazonian species showing high levels of variation within populations. North American species clearly showed the genetic imprint of rapid post-LGM expansion. However, they found that Neotropical populations had either remained stable since the last glaciation, or, alternatively, had experienced only modest demographic expansion. They concluded that, in contradiction to the Refuge Hypothesis, greater demographic stability may have been a key factor in the

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accumulation of species diversity in the tropics (Lessa et al., 2003). This valuable study, however, considered only a handful of nonvolant mammals in two of the refuge areas proposed for South America. If indeed such stability can be shown for other proposed refuges, the refuge hypothesis will appear to be substantially falsified.

Application of the Vicariance Hypotheses to the Andes

In order to apply the Vicariance hypothesis to the Andes, one must think of mountaintops as refuges where species populations are isolated and exposed to little or no gene flow (Hughes and Eastwood, 2006). Studies in the Andes of Colombia have shown that during Pleistocene glacial periods, temperatures at high elevations in Colombia were lower, with a maximal decrease estimated at 6-7ºC below present temperatures. Precipitation was also reduced (van der Hammen, 1974; van der Hammen and Hooghiemstra 2000; Clapperton, 1993). As a result, vegetation belts descended and expanded horizontally at lower elevations (van der Hammen, 1974, van der Hammen and Hooghiemstra, 2000; see also Simpson, 1975 fig. 3). This displacement of vegetation might have created conditions for allopatric speciation, henceforth referred to as "montane vicariance," a term used to describe the pattern of allopatric speciation on isolated mountain ranges during glacial cycles (Jansa, 1998, Gorog, 2003, Sullivan, 1985, 1994, and 1996). In mountainous habitats of western North America, discontinuities in montane vegetation during interglacials are known to have influenced patterns of differentiation and local extinction of terrestrial mammals (Sullivan, 1985; Sullivan, 1994; 1996; Knowles, 2000, 2001). The prediction for Andean populations is that they also were influenced by "montane vicariance."

The Andes are the result of a recent orographic event, with its last uplift occurring during the Pliocene-Pleistocene, compared to Amazonia, which is much older. If, as was found by Lessa *et al.* (2003), greater demographic stability (*sensu* Fjeldså, 1995) is

confirmed in Amazonian populations, it would be interesting to compare this with the stability of populations in the presumably younger Andes (Bates and Zink, 1994; da Silva and Patton, 1998; García-Moreno et al., 1999a; Hackett, 1993; Moritz, et al., 2000; Smith and Patton, 1999). If Andean species are, in fact, younger than their Amazonian counterparts, then they would be expected to be undergoing expansion across Andean territories or at least to show the imprint of recent expansion in their population structure. And if it is true that the Andean faunas are younger than their Amazonian counterparts, that conclusion would contradict the idea that the Andes worked as a "species pump" contributing to the diversity of Amazonia (Fjeldså, 1994, 1995).

Conclusions

For some time, the Refuge hypothesis has been broadly accepted as an explanation for the high diversity in Amazonia. Evidence for this hypothesis came primarily from recent distributional data, rainfall patterns, and controversial pollen data. However, a consensus concerning how refuges actually mapped onto communities in those habitats was never reached. Perhaps more importantly, with the inclusion of molecular tools, it has been shown, at least for non-volant mammals, that populations in Amazonia have a history of demographic stability, not one of rapid expansion that would be expected if they were shaped by the Pleistocene/Recent refuges (Lessa et al., 2003). This observation appears to falsify the Refuge form of the Vicariance Hypothesis, at least for those populations for which long-term stability has been shown. On the other hand, evidence about the age of Andean fauna with respect to the Amazonian fauna is still incomplete. Some Andean species are hypothesized to be younger than Amazonian species (Bates and Zink, 1994; Hackett, 1993; García-Moreno et al., 1999a; Lessa et al., 2003; Moritz, et al., 2000; Patton and Smith, 1992; Pearson and Smith, 1999; Smith and Patton, 1993; 1999; Fjeldså, 1994; 1995), but no general conclusion is possible given the limited evidence available.

The diversification in the Andes more likely did not result from vicariance exactly as stated by Haffer's hypothesis for Amazonia, but from montane vicariant events in mountaintops and along elevational gradients, while vegetation zones descended and expanded horizontally during the last glaciation in the Pleistocene. If mountaintops functioned as "refuges," isolating populations and reducing gene flow between them, it should be possible to build sets of predictions based on the phylogenetic relationships of species in these vegetation zones. This approach has been attempted in only a few instances for Andean faunas (Patton and Smith, 1992; Patton et al., 1990; Pearson and Smith, 1999; Smith and Patton, 1991, 1993; 1999); the results are described below under the Ecological Gradients hypothesis.

The Ecological Gradient Hypothesis

This hypothesis states that speciation is the result of isolation along ecological gradients (clines) via parapatric speciation. Spatial separation initiates the process and leads to the evolution of isolating mechanisms between groups of geographically distinct but contiguous populations. In this case, the isolation is determined by distance rather than by geographical, ecological, or temporal factors, although these may also play a part (Endler, 1977). Endler (1977, 1982a, 1982b) proposed this hypothesis as an alternative to the Montane Vicariance hypothesis via allopatric speciation. He agreed with the refuge hypothesis that more species are found in areas of present day forest and fewer in savannas. However, Endler (1982b) focused on the "neglected" predictions of Haffer's hypothesis, which he described as the distributions of the positions of refuges and the width of contact zones after the species have been separated by some period of time, and argued that they were falsified by evidence (Endler, 1982b).

Under the ecological gradient hypothesis, populations become reproductively isolated via parapatric speciation. This involves the evolution of reproductive isolation

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between two populations that exchange genes but not freely. There are two types of parapatric speciation. The first of these, the "*clinal model*," occurs when a single species is continuously distributed across a variable environment, such as an altitudinal gradient, and subpopulations became adapted to their local habitats. The second form of parapatric speciation is the *"stepping-stone model,"* in which discrete populations have restricted gene exchange (Coyne and Orr, 2004). Neither the clinal model nor the stepping-stone model has explicitly been applied to Andean mammalian populations.

Predictions of the Ecological Gradient Hypothesis

If the present distribution of the species in a region is the result of secondary contact between populations isolated in refuges, the contact zones are predicted to lie between the refuges. Endler formulated two equations to calculate the position and width of the contact zone. For a group of species with approximately the same dispersal ability, if the refuge hypothesis were correct then the width of the contact zones of species with short generation times should be larger than those with long generation times, because the time (years) since contact should be the same for all species. If the gradient model were correct or predominant in its effect, the width of the contact zone should be independent of generation time, and should depend upon the strength of the gradients. Because all species in a region are responding to the same environmental gradient, we would expect the width to be similar among related species with very different generation times.

Endler (1977) modeled these equations after Fisher's theory of clinal speciation, a model that involves populations locally adapted to an environmental gradient (Coyne and Orr, 2004). This theory predicts that gene and phenotypic frequencies will tend to remain relatively uniform in areas where there is little environmental change, and that they will vary across a region where the environment changes (Endler, 1977, 1982b) facilitating parapatric speciation.

More recently, a phylogenetic test was proposed by Patton *et al.* (1990) for Andean faunas. If two taxa inhabiting two separate "island" mountaintops in the Andes were the result of an allopatric process of evolution (mountain vicariance hypothesis), we would expect them to be sister taxa. However, if they were the result of a parapatric (ecological gradients) process, we would expect their sister taxa to be found adjacent to them along the altitudinal gradient. Some caution in interpreting these results is necessary, however; species' ranges change substantially over short periods of time and the pattern distribution depicted by a phylogeny is probably just the "last stage" in the movement of the species analyzed (Losos and Glor, 2003). Hypotheses of relationship might be unable to rigorously test speciation models because of the lability of geographical ranges and the lack of correlation between the role of adaptive processes and geographical mode of speciation (Losos and Glor, 2003). If that is the case, additional data that provide information on how the populations have moved throughout time and space are needed to corroborate mechanisms of speciation. This could be accomplished by testing the population data in light of predictions provided by population genetic analysis to record the expansion, shrinkage, dispersal or migration of the populations. The population genetic predictions for the ecological gradient hypothesis are for relatively stable population sizes, since environmental changes are gradual (Lessa et al., 2003).

Evidence supporting the Ecological Gradient Hypothesis

Endler (1982b) concluded that observed biogeographic patterns could be explained by parapatric divergence along current ecological gradients as well as by Montane Vicariance; that is, there is no need to postulate Montane Vicariance to explain patterns of distribution that are currently observable. In fact, using this reasoning Endler rejected the formation of African Montane Vicariance for birds and butterflies species (Endler, 1982b).

Smith et al. (2001) tested the hypotheses of divergence by allopatry in Montane Vicariance versus postglacial diversification across ecological gradients for populations of mice of the genus *Abrothrix* in Chile and Argentina. They used genetic data to determine the phylogenetic pattern of relationship of members of this genus. The results support the gradient hypothesis of diversification. In addition, there were indications that the ancestral population was originally isolated in Chile due to a glacial maximum; once the glaciers melted, the population began to diverge in a manner consistent with the Ecological Gradients hypothesis with dispersal of populations toward Argentina (Smith et al., 2001).

Evidence against the Ecological Gradient Hypothesis

Aside from Endler's work, explicit tests of the role of elevational ecological gradients in speciation are scarce (Moritz, et al., 2000). Patton and colleagues (Patton, et al., 1990; Patton and Smith, 1992b), Gorog (2003), and Jansa (1998) have applied a phylogenetic approach to taxa in mountains in South America, Southeast Asia and Madagascar to test ecological gradients and montane vicariance hypotheses. In the Andes, Patton and colleagues (Patton, et al., 1990; Patton and Smith, 1992b) tested these hypotheses with species of sigmodontine rodents distributed across geographically adjacent elevational transects. They studied Andean species of the genera *Akodon, Thomasomys*, and *Oryzomys.* Populations of morphologically similar mice that belong to species of each of these genera are distributed across large areas of the east slope of the Andes. Mice in populations living in similar habitats (elevational zones) on different mountain are very similar in appearance, and in fact current taxonomy is uncritically based on this similarity. Thus mice of the genus *Akodon* living in upper montane forest on one mountain are often given the same name as mice in the same zone on neighboring mountains, resulting in a pattern where "species" are distributed in horizontally narrow bands extending across many separated drainages at the same elevation in the eastern slope of the Andes (Patton, et al., 1990, see Fig. 1.1 here). But is this morphological

similarity based on phylogenetic similarity, or is it simply the result of convergent evolution due to selection on different animals living in similar habitats? If the populations or species followed the vicariant model, taxa in similar zones on different mountains are predicted by be sister species (Fig. 1.1). On the other hand, if populations on these mountains diverged under the ecological gradient model, taxa would be expected to have their phylogenetic relationships vertically within drainages rather than horizontally among them (Fig 1.2); that is, a taxon's sister species would likely be immediately upslope or downslope on the same mountain (Patton et al., 1990; Patton and Smith, 1992).

In the study by Patton *et al.* (1990), no strong conclusion was drawn from studies of the genus *Thomasomys* due to scarce sampling and poor taxonomic knowledge of the two species tested. For *Oryzomys*, the Ecological Gradient hypothesis was rejected in favor of the montane vicariance model of diversification. In the case of *Akodon*, the same team spent years studying this group of mice (Smith and Patton, 1991; 1993; 2007; Patton and Smith, 1992b; Geise et al., 2001; Myers et al., 1990; Myers, 1989; Myers and Patton, 1989a; 1989b). Chromosomal and electromorphic characters suggest that the taxa had followed the montane vicariance model of speciation, based on the fact that horizontally distributed pairs of samples of two populations differ by significantly fewer base substitutions than vertically distributed pairs (Patton et al., 1990). The three species of *Akodon* examined fit the allopatric model (Fig. 1.1). These results were later corroborated by Patton and Smith (1992) using cytochrome b for the same species of *Akodon*.

The same conclusion (rejection of the ecological gradients hypothesis) was reached by Arctander and Fjeldså (1994) while studying the Andean tapaculos *Scytalopus* (a purely terrestrial and agoraphobic bird of the family Rhinocryptidae). After analyzing morphological, molecular and bioacoustic, and habitat evidence, the authors concluded that the speciation mechanism operating in tapaculos population is allopatric,

with disjunct isolates in parts of the Andes of Ecuador and Peru (Arctander and Fjeldså, 1994).

Conclusions

Studies looking at this hypothesis, by itself or in contrast with the refuge hypothesis, have shown ambiguous results in Andean populations, and several studies have rejected it. These studies, however, have mainly used phylogenetic inference to reconstruct the mechanisms of speciation. Phylogenetic hypotheses of relationship have recently been criticized as being unable to rigorously test speciation (Losos and Glor, 2003). If that is the case, additional data that provide information on how populations have moved through time and space are necessary to corroborate the mechanism of speciation. This could be accomplished by testing the population data in light of the predictions provide by population genetic analysis to record the expansion, shrinkage, dispersal or migration of the populations. The population genetic predictions for the vicariance hypotheses predict population expansion following climate amelioration, while in contrast, the gradient hypothesis predicts a history of more stable populations (Lessa et al., 2003).

DISCUSSION

The Andes is an orographic structure that has held multiple potential refuges throughout its history. In contrast to those in the lowland, habitats in the Andes were probably never continuous (Fjeldså, 1994, Hughes and Eastwood, 2006). It is a region of great spatial heterogeneity, where differences in relief and altitude result in a great diversity of habitats that have changed in nature and position during the episodes of uplifting (Reig, 1986). These numerous fractionated habitats promoted speciation, and

the resulting biodiversity is probably maintained by the topographic and climatic complexity created by the uplift of the central and northern Andes since the Miocene (Moritz, et al., 2000).

The observation of unusual endemism at high elevation in the Andes is not new (Fjeldså, 1995; Reig, 1986; Moritz, et al., 2000, Pacheco, 2002) and supports the idea of isolated populations responding to evolutionary forces favoring speciation in isolation. In recent years, for example, even though mammals are relatively well known, one genus and four species of rodents have been described from high elevations in the Peruvian Andes, and systematists suspect that a great number of unnamed species can be found in collections from that region. Some recent examples showing endemism in isolated environments in the Andes include Emmons (1999), and Luna and Pacheco (2002), who described the genus *Cuscomys* and the species *Thomasomys onkiro* respectively, from the Southeast Andes at 3600 m. of elevation. Luna and Patterson (2003) described a rodent from the southeastern Andes of Peru at 2000 m, while Gardner and Romo (1993), and Leo and Gardner (1993) described two more species from the northern Central Andes at 3280 m. In most cases, and despite collecting efforts, these species are known only from the collecting locality, suggesting that they are indeed isolated.

The two hypotheses that may explain diversification in the Andes, Montane Vicariance and Ecological Gradient, are explicitly testable by robust phylogenetic analysis using morphology and/or molecular data, though the latter would appear to yield stronger evidence because the direction of a population's migration can be traced using population genetic techniques (Avise et al., 1987; Avise, 2000; Smith et al., 2001). However, molecular data are scarce for taxa inhabiting the Andean region, and phylogeographic tests of proposed refuges have yet to be performed (Moritz, *et al.*, 2000). There is a need for phylogeographic tests that include mitochondrial and nuclear DNA. Genes can show accumulated differences due to populations having been separated for long periods of time by zoogeographic barriers. These barriers, if strong, should mold

the genetic structures of independently evolving species in concordant fashion (Avise et al., 1987). The action of barriers should also be seen in phylogenetic analysis based on morphological data. One example of this was provided by Cracraft and Prum (1988), who conducted morphological phylogenetic analyses of parrots of the genus *Pionopsitta* and toucans of the genus *Selenidera*, and concluded that ancestral populations had been partitioned first into Chocó, Central America, and Amazonian components. The authors suggested that the Sierra of Perijá—a result of Oligocene uplift—became a barrier between Chocó and Amazonian populations. The inclusion of molecular data, and a thorough evaluation of the predictions of population genetics, could provide further indications as to the validity of the barrier hypothesis.

An alternative theory of long-term paleoclimatic shifts (Patton and da Silva, 1998), suggests that Amazonian speciation cannot be explained entirely by any single model of vicariance or climate change. Bush (1994) argued that Quaternary speciation is a result of a combination of glacial age cooling, reduced atmospheric CO_2, and moderate reductions in precipitation. Areas with maximum species movement, such as the flanks of highlands, are assumed to have the greatest likelihood of isolation and speciation, and hence the peripheral areas of the Amazon and the slopes of the Andes are expected to be especially rich in endemic taxa. Meanwhile, the central portion of the Amazon basin, too remote from the highlands to allow the migration of cold-tolerant competitors, would be relatively poor in endemic species even though they wet enough to maintain a forest (Bush, 1994). Bush's hypothesis is based mainly on the ideas of Cracraft and Prum (1988), Colinvaux (1988), and Salo (1988), but Bush offers little additional evidence to support it, other than the fact that the mountainous areas surrounding the Andes are rich in endemic taxa (Reig, 1986;Vuilleumier and Simberloff, 1980).

It seems reasonable that Montane Vicariance and Ecological Gradient hypotheses should be re-evaluated, incorporating molecular data in the phylogenetic analysis. In addition, the studies favoring the refuge hypothesis (e.g. Patton and Smith, 1992b) should

be re-evaluated in light of population genetics expectations in order to trace the history of change in populations at different elevations. At the same time, results obtained by Arctander and Fjeldså (1994) should be re-evaluated in light of additional molecular evidence, since they employ a very small number of base pairs of cytochrome b.

The combination of phylogenetics and population genetic approaches could provide more insights into the population dynamics throughout time. We may, for example, discover that speciation events in the Andes have been neither solely sympatric nor allopatric in origin, but rather a result of a combination of these two processes acting at different rates and times (Jiggins and Bridle 2004, Chaves et al., 2006).

The next obvious question will be how to test these hypotheses in Andean populations. This will include choosing Andean populations of related taxa, whose distributions include several mountaintops and at the same time whose members occupy a wide range of elevations. The perfect candidate group will include species that are sensitive to habitat discontinuities, and with limited vagility. This is the case of rodents of the genus *Akodon*. Only by subjecting the species or groups that meet these criteria to a variety of phylogenetic and descriptive analyses will we begin to understand the relative importance of hypotheses that attempt to explain diversification of Andean faunas. In the following chapters, I attempt to test both hypotheses of diversification using populations of one group of Andean mice, the soft grass mouse species complex *Akodon mollis*.

GENERAL CONCLUSIONS

The aim of this chapter was to explore potential hypotheses of mechanisms responsible for the mammalian diversity of the Andes. I focused mainly in the Vicariance hypothesis, for which we have the most evidence. I also examined the Ecological Gradient hypothesis, as applied to the Andes.

Potential mechanisms that could explain diversification in puna and páramo communities include Montane Vicariance and the Ecological Gradient hypotheses. For the first hypothesis, the environment resulting from Andean spatial heterogeneity potentially makes each mountaintop and each delimited altitudinal zones act as a "refuge," pre-conditioning the fauna to allopatric speciation. From the point of view of the ecological gradient hypothesis, different environmental conditions at different elevations in the Andes create habitat gradients along which parapatric speciation might occur. The existing evidence appears to support the Montane Vicariance hypothesis events most strongly, but far too few studies are available to allow any strong conclusion. Studies looking at the ecological gradient hypothesis for Andean populations have shown ambiguous results; some corroborating and some rejecting it. We are far from knowing whether or not one of these hypotheses has dominated speciation in the Andes, or whether Andean diversification is a result of a combination of processes acting at different rates and times.

FIGURES
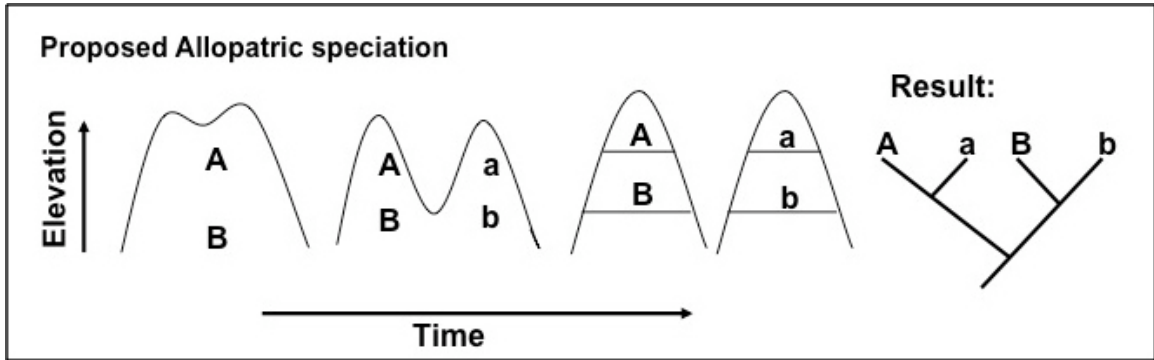
Figure 1.1. Proposed model of speciation for the Montane Vicariance hypothesis.
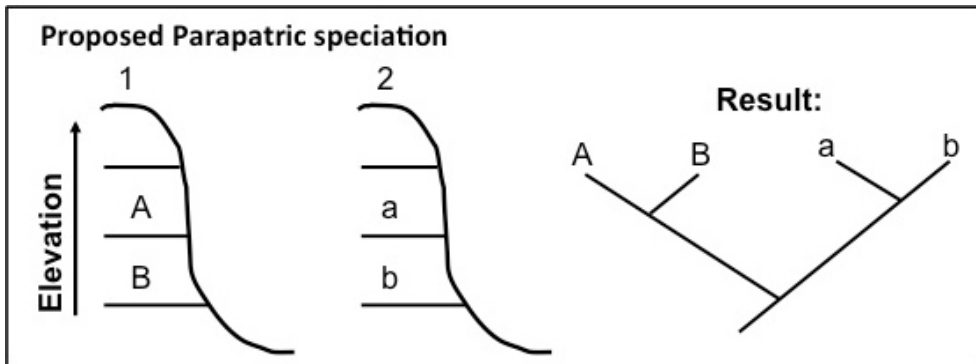
Figure 1.2. Proposed model of speciation for the Ecological Gradient hypothesis. Modified from Patton and Smith, 1992a

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Chapter 2:

How general is a generalist? The influence of environmental gradients on intraspecific phenotypic variation

ABSTRACT

This work provides the first look into the influence of altitudinal environmental gradients on intraspecific phenotypic variation in a Neotropical mountain setting (500 - 4500m), using a species with a wide elevational range, the soft grass mouse *Akodon mollis*. We tested two hypotheses of how this species deals with the wide range of environmental conditions throughout its range, whether using a *true-generalist* strategy along its range or a *locally-specialized* strategy at different elevational ranges according to environmental conditions. Using geometric morphometric techniques to analyze 153 skulls and 21 environmental variables, we discovered a morphological break between populations at 4000m - 4500m of elevation and the remaining populations (from 500 - 3500m). This differentiation coincided with the forest-grassland transition (montane forest - páramo) in the Andean mountains, and it may be the result of animals in high-elevation populations being exposed to severe biotic and abiotic conditions that impose physiological and dietary constraints. Partial least square (RV coefficient: 0.275, p-value: <0.0001) and multivariate multiple regression analyses showed a moderate and significant association between shape and environment, the most important variables being the mean temperatures of the coldest quarter and of the driest quarters, both of them almost perfectly correlated with elevation. Despite this strong correlation, we did

not observed a clear association between morphology and elevation, as shape principal component values scatter widely along elevational ranges.

Preliminary average pairwise nucleotide comparisons (1123 bp of cytochrome b) between individuals at 4000m, 4500m, and at lower elevations, showed that despite the fact that 4000 and 4500m individuals share similar skull morphology, their average pairwise nucleotide difference is high, which reinforces the idea that highland conditions are likely the driving force of skull shape.

At lower elevations from 500 to 3500m, *A. mollis* populations are similar in cranial morphology, suggesting that they cope with the wide range of environmental conditions by being a *true-generalist*, changing only when reaching the top of the forested zone at 4000 and 4500m elevation. This pattern of differentiation can neither corroborate nor falsify the Ecological Gradient hypothesis (Chapter 1).

INTRODUCTION

Phenotypic variation along environmental gradients in closely related species is well documented (e.g., Majerus and Mundy, 2003; Luxbacher and Knouft, 2009; Binning et al., 2010). Classic examples of this variation are illustrated by Bergmann's rule, the tendency of body size to be negatively correlated with environmental temperature (Ashton et al., 2000) or in the precipitation-hypsodonty association seen in mammals, in which higher crowned-teeth are found in species inhabiting arid and grassy regions where the plants they consume are more abrasive (Eronen et al., 2010; Polly et al., 2011). Yet, much less studied is the extent to which environmental gradients shape intraspecific phenotypic variation. In particular, it remains unclear how phenotypic variation is geographically structured in species with wide distribution ranges, and the extent to which such structure is conditioned by local environmental conditions. Two alternative scenarios are possible. There could be the expression of a common generalist phenotype throughout the species' range capable of thriving in a wide variety of conditions.

Alternatively, there could be locally-variable phenotypes in different portions of the species range owing to phenotypic plasticity (West-Eberhard, 2003; West-Eberhard, 2005), strong local selection in spite of gene flow (Hoekstra and Nachman, 2003) or a by-product of genetic drift (Grieco and Rizk, 2010; Jourdan-Pineau, et al. 2012).

Elevational gradients represent an ideal system to uncover the role of environmental gradients in shaping intraspecific phenotypic variation because organisms with ample elevational ranges face substantial and often dramatic environmental variation. For example, with elevation, temperature, air pressure, solar radiation, precipitation, area, cover cloud, soil quality, and productivity considerably change (McCain and Grytnes, 2010). Tropical mountains, in particular, are characterized by steep environmental gradients and encompass a wide array of habitats (Terborgh, 1970; Patterson, et al. 1998). Along these mountains, abiotic conditions tend to change gradually with elevation (e.g., temperature cooling rate with elevation is around 10˚C/km in dry areas or 6˚C/km in wet areas; MacArthur, 1972). In contrast, vegetation formations tend to replace each other less uniformly (Terborgh, 1970; Presley, et al. 2012). Boundaries between vegetation types are blurred as the result of topographic and climatic heterogeneity (Patterson, et al. 1998; McCain and Grytnes, 2010). Despite representing the most evident axes of climatic and ecological variation in the tropics (Sarmiento, 1986), tropical elevational gradients have not been the subject of studies of environmental influences on intraspecific phenotypic variation. Instead, most studies of elevational gradients in the tropics have focused on changes in species diversity, distribution, and abundance (e.g., Pearson and Ralph, 1978, Solari, et al. 2006).

One of the few tropical species distributed across a dramatic elevational gradient, and hence an ideal taxon to address the question of how phenotypic variability is structured in widely distributed species, is the soft grass mouse, *Akodon mollis*. This species (or species complex, Patton and Smith, 1992), ranges from approximately 500 to 4800m in the Andes of Ecuador and northwestern Peru (Wilson and Reeder, 2005).

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Ecological studies suggest that it is a predominantly nocturnal, widespread and an abundant generalist with relatively small individual dispersal capabilities relative to other small terrestrial mammals (Patton and Smith, 1992; Barnett, 1999; Alvarado-Serrano 2005). The diet of this species is similar to other members of the genus, comprising plant and insect material in varying proportions (Barnett, 1999; Alvarado-Serrano, unpublished data). Its distribution encompasses several steep ecological transitions including the abrupt transition between tree-dominated formations on the Andean slopes and grassland-dominated formations in the highlands. In the central-north and southeast Peruvian Andes, this abrupt transition occurs when the humid Andean cloud forest is replaced by elfin forest (Terborgh, 1970; 1977), which is itself replaced by grasslands called páramos at higher elevations. These grasslands are characterized by high atmospheric humidity, high rainfall, and a continuous covering of short vegetation, dwarf shrubs, and wetlands (Quintanilla, 1983; Sklenár and Ramsay, 2001; Mena Vásconez and Hofstede, 2006). On the central-south and southwest Peruvian Andes, this abrupt transition occurs between the xeric habitats characteristic of this region. There, a short forest dominated by quenoa trees (*Polylepis* spp.) is replaced by puna at higher elevations (Simpson, 1983). The puna ecosystem is characterized by steppes of isolated grasses and shrubs, low productivity, and an annual precipitation markedly seasonal and lower than that of the páramo (Tovar Serpa, 1957; Cabrera, 1968; Kuentz, et al. 2007).

This ability of *Akodon mollis* to thrive in contrasting habitats, together with its diet versatility and generalist habits, poses the question of how phenotypic variability within this species responds to the broad range of environmental conditions its populations experience. Is this wide tolerance associated with individuals having a general phenotype adapted to an ample range of conditions (hereafter, **true-generalist strategy**), or is it attained through phenotypically-specialized populations adapted to local conditions that maintain cohesiveness through gene flow (hereafter, **locally-specialized strategy**). To evaluate this question, we analyze intraspecific shape variability in the skull of *A. mollis* and test how this variation is related to environmental gradients in the Peruvian Andes. We follow a geometric morphometric approach and

perform ecomorphological analyses to test the prediction that if *A. mollis* employs a true-generalist strategy, no morphological differentiation across elevation or environmental gradients will be observed between populations. Alternatively, if *A. mollis* employs a locally-specialized strategy, we expect to find consistent morphological individual differentiation across elevations and a strong association between skull morphology and environment. Distinguishing between these two strategies has important implications for our understanding of the evolution of diversity in this region and its conservation. True-generalists species, for example, might be expected to be more resilient to environmental changes because individuals would be less likely to diversify given the versatility of its phenotype, whereas environmental changes might be expected to exert significant effects on the evolutionary trajectories of locally-specialized species. These two different strategies would have differing consequences for the generation and maintenance of species in the Andes, a biodiversity hotspot (Olson and Dinerstein, 1998; Myers, et al. 2000), such as the general effectiveness of specific mechanisms like the "species pump," which is used to explain diversity patterns across not only high-elevation regions but also the adjacent Amazon lowlands (Fjeldså, 1994; 1995). True-generalist species are unlikely to diversify as predicted under the Ecological Gradients model (Chapter 1), and even their diversification under Montane Vicariance is apt to be less than would be expected for locally-specialized species, as they are less likely to be affected by barriers presented by intermontane valleys. Locally-specialized species, in contrast, might be expected to display rapid diversification under both Montane Vicariance and Ecological Gradients scenarios.

MATERIALS AND METHODS

Data acquisition

Morphological variation was assessed in complete skulls of 153 *Akodon mollis* collected along elevational gradients across the entire geographic range of the species in Peru (Fig. 2.1). Specimens were obtained from the Museo de Historia Natural "Javier

Prado", Universidad Nacional Mayor de San Marcos (UNMSM) in Lima, Peru and the Field Museum of Natural History in Chicago (FMNH), US, and collected by L.L. in northern Peru (Appendix 2.1). Given the taxonomic complexity of the genus (D'Elía, 2003; Smith and Patton, 2007; Jayat et al., 2010) and the poorly defined diagnosis available for *A. mollis*, we based our taxonomic identification of specimens on a combination of external and internal morphological characters that others have found useful (e.g., a robust braincase, short rostrum, zygomatic plate broad and not inclined; Alvarado-Serrano, in prep.; Hershkovitz, 1940; Myers and Patton, 1989; Voss, 2003). Only adult specimens were used to minimize the effects of ontogenetic growth (de Oliveira et al., 1998; Alvarado-Serrano, 2005); adults were defined as individuals with all three molars completely erupted and with the posteroloph in the third molar eroded (which corresponds to ages 3 to 5, Myers, 1989). Sex and locality information were recorded for all 153 specimens from skin tags and collector's field notes. When available, the locality's latitude and longitude were also recorded. Otherwise, specimens were georeferenced based on the collector's notes following a point-radius method (Wieczorek et al., 2004). We assessed the confidence of our georeferencing by contrasting the elevation given in the field and museum notes with one we obtained from a Digital Elevation Model (DEM) provided by Shuttle Radar Topography Mission (SRTM, Farr, et al. 2007) based on the coordinates estimated.

For each locality we compiled information from 21 ecogeographical variables (Table 2.1) that have been shown to be of biological relevance in a wide variety of vertebrates (e.g., Graham, et al. 2010; McCormack, et al. 2010). These variables summarize temperature and precipitation patterns (Hijmans, et al. 2005), vegetation (Friedl, et al. 2002), and productivity (Imhoff, et al. 2004), both means, maximum and minimum values. Because of the resolution of the ecogeographical data (Table 2.1), localities in close proximity (i.e., less than 1 km apart) were treated as a single locality, resulting in 26 unique localities (Appendix 2.1). All geographic analyses were performed in ArcGIS v.9.3 (ESRI, 2008).

Morphological analyses

  Ventral images of these 153 skulls were taken using a digital photographic camera Nikon D80 under standard conditions. For all images, the camera was kept at a standard height of 30cm using a photographic stand. The focus of the image was always set at the posterior margin of the palate, while keeping the molars of both toothrows in focus and at the same plane. In all photographs, a scale was placed next to the skull. The images were analyzed using geometric morphometrics techniques. Specifically, 54 two-dimensional, X, Y landmarks (Fig. 2.2) were digitized on each skull image using tpsDig, version 2.16 (Rohlf, 2010). The 54 landmarks were selected according to the standards proposed by Zelditch and collaborators (2004) and after an analysis of digitizing and photographic errors. For this analysis, five specimens were randomly selected, photographed twice, and digitized four times in random order by the same person. These replicates were compared using univariate analyses of variance (ANOVA) under a block design with individual as fixed factor. Landmarks with significant differences between replicates were not used in the study.

  The outputs from tpsDig v.2.16 (Rohlf, 2010) were standardized with a full Procrustes orthogonal projection (Dryden and Mardia, 1998; Klingenberg, 2011) to remove the effects of position, size and orientation (Rohlf and Slice, 1990; Zelditch et al., 2004). All outliers in this dataset were redigitized to assure they were not the product of error. Sexual dimorphism was assessed using an ANOVA on the $\log_{10}$-transformed coordinates in SPSS v.18.0 (SPSS, 2009). However, no sexual dimorphism was detected (ANOVA: F=1.02; p=0.48), and hence, all specimens were pooled for further analyses.

Ecogeographic analyses

  To evaluate the hypothesis that shape variation in skull is structured in response to environmental gradients that covary with elevation, we performed a Canonical Variate Analysis (CVA) on the specimens grouped into elevation bins separated by 500m

intervals. Using this analysis, we identified linear combinations of variables that maximized the differentiation between the bins, as indicated by the ratio of between groups to within groups variance (Hotelling, 1936; Holt et al., 1995), and assessed significance by a permutation test over 10000 replicates on the Mahalanobis and Procrustes distances separating the groups. In addition, the strength of the association between environment and skull morphology was assessed visually by running cross-correlations between all morphological (i.e., landmark coordinates) and environmental data, as well as analytically, by performing a two-block Partial Least Squares analysis (PLS) between all landmarks and the set of standardized 21 environmental variables compiled (Table 2.1). The PLS analysis establishes the strength of the association between two blocks of variables (morphological and environmental variables in our case) by constructing a set of orthogonal latent variables that maximizes the covariance between the two blocks (i.e., each latent variable in each block is maximally correlated with the corresponding latent variable of the other block, but uncorrelated with the other latent variables in its own block) (Sampson et al., 1989; Bookstein et al., 1990; Klingenberg and Ekau, 1996). We summarized the overall strength of the association using the RV coefficient, which is a multivariate statistic analog to the squared correlation and ranges from 0 (uncorrelated) to 1 (completely correlated) (Escoufier, 1973; Abdi, 2007; Klingenberg, 2011). A multivariate multiple regression (MMR) was also run on the same data to complement the PLS analysis by identifying the amount of variation in skull shape explained by different environmental factors. In order to be able to compare the MMR with the PLS results, all 21 environmental variables were used to fit the MMR regression despite autocorrelation among them, (in contrast to PLS, MMR is not robust to highly correlated variables; Quinn and Keough, 2002). Still, to assure consistency of the results of the MMR, the same analysis was redone using only a set of 8 less autocorrelated variables (Table 2.1). All analyses were run in MorphJ (Klingenberg, 2011) with the exception of the cross correlation, which was run in R (R Development Team, 2011) using the CCA package (Gonzalez et al., 2008). In all analyses, the symmetric component of the morphological variation was used to avoid redundancy (Klingenberg et al., 2002).

RESULTS

The *a priori* grouping of individuals according to elevation resulted in 9 highly unbalanced bins (Table 2.2), with most specimens concentrated in the bins between 2000 and 4000m, as expected based on the biology of *Akodon mollis*. The CVA identified 14 of the 36 pairwise distances to be significant in both multivariate measures (i.e., Procrustes and Mahalanobis distances; Table 2.2). Nine of these 14 significant distances identified by both measures separated the combined highest two bins (4000 and 4500m), which were significantly different from each other only in Mahalanobis distance, from the rest of the lower elevation localities. The differentiation between these two groups was also evident when plotting the individuals according to their scores on the first and second CV axes (Fig. 2.3a), which together accounted for almost 60% of the variance. The first CV axis predominantly summarized differences in the ventral perimeter of the braincase (hereafter, braincase) and the position of the narrowest portion of the wall dividing the incisive foramina (i.e., landmark 8), and the second CV axis predominantly summarized differences in the position of the masseteric tubercle and the shape of the squamosal and sphenoid regions (including the structures associated with the anterior branch of the carotid arterial supply system). Similar results were obtained when the CVA was rerun excluding all elevation bins with fewer than 5 individuals (Table 2.3, Fig. 2.4).

When the ecosystem of each individual is depicted in the CV plot (Fig. 2.3b), it become apparent that the distinct high elevation assemblage (i.e., individuals from 4000 and 4500m) corresponded to individuals from the páramo and puna habitats (Fig. 2.5e,f). However, six additional páramo individuals were morphologically similar to the lower elevation group; it is noteworthy that these six individuals were collected at lower elevation than those from páramo and puna among the southern localities (Figs. 2.1, 2.4).

Of the 21 environmental variables analyzed, 15 were significantly and strongly correlated with elevation, 4 were marginally significantly correlated, and only 2 (temperature seasonality and net primary productivity) were not significantly correlated (Table 2.1). Visual exploration of the cross-correlation between skull morphology and environment showed that the rostrum and the braincase have opposite correlations with the environmental variables (Fig. 2.8). The rostrum was highly and positively correlated with most environmental variables, whereas the braincase was highly but negatively correlated with the same variables. Among these variables, the most strongly correlated were temperature variables, followed by precipitation of the driest and coldest seasons and net primary productivity (Fig. 2.8). The PLS analysis showed a moderate but significant association between skull morphology and environment (RV coefficient: 0.275, p-value: <0.0001). Similarly, the MMR identified environment as a moderate, and significant, predictor of skull morphology. The regression explained 34.94% (p-value: <0.0001) and 20.96% (p-value: <0.0001) of the variation in skull shape when 21 and 8 less autocorrelated environmental variables were used, respectively. When all 21 environmental variables are considered, the two variables with the greatest average absolute regression coefficients were the mean temperature of the coldest quarter and the mean temperature of the driest quarter, which were both very highly correlated with elevation (Table 2.1). In the analyses with the 8 less autocorrelated variables, annual and diurnal temperature ranges, had the greatest average absolute regression coefficients. These later variables were also significantly, but less highly, correlated with elevation (Table 2.1). The estimated effect of all these four variables on shape morphology is summarized in Figure 2.8.

A morphological comparison between the average skull shapes of populations at the extremes of the elevation gradient showed several morphological differences among them, with high elevation individuals exhibiting a narrower cranium and a slightly longer rostrum than low elevation individuals (i.e., localities at 4500m and at 500m, respectively; Fig. 2.6d). However, no clear association between morphology and elevation was observed (Fig. 2.6a), despite the significant association identified by the

PLS and MMR analyses between skull morphology and environmental variables that covary with elevation (Table 2.1). This lack of association may be in part caused by the opposing effects of environment on the rostrum and braincase regions (Figs. 2.6b,c). Additionally, the large morphological variation within each elevation and sampling locality likely contributes to this poor association between elevation and skull morphology (Fig. 2.6a).

DISCUSSION

Intraspecific morphological variation is commonly associated with microhabitat use, resource consumption, or physiological constraints, and is well documented among vertebrates (e.g., Mullen et al., 2009; Kaliontzopoulou et al., 2010). In particular, vertebrate skulls are especially sensitive to environmental changes and commonly vary within species (Fadda and Corti, 2001; Caumul and Polly, 2005; Márchan-Rivadeneira et al., 2012). Through the skull, the animal perceives sensory information that allows it to interact with its environment, and together with the mandible and associated musculature, the skull is essential for food search and mastication (Grieco and Rizk, 2010). Studies addressing the effect of environment on mammalian skull morphology have uncovered considerable variation due to diet, latitude, temperature, elevation, precipitation, seasonality and/or major productivity, or combinations of these factors. Yet, few studies have explicitly addressed the effect of environmental changes along elevational gradients on the skull morphology of small mammals. Among the few exceptions, a recent study on an elevational gradient in the Sierra Nevada Mountains in North America uncovered a significant but subtle influence of elevation on skull shape variation in deer mice (*Peromyscus*; Grieco and Rizk, 2010). Accordingly, our study of skull morphological variation along environmental gradients in tropical mountains in Peru found some morphological differentiation among populations of *Akodon mollis*. Most notably, the results of the CVA indicate that populations inhabiting highland wet grassland habitats in the southern portion of the range of *A. mollis* (i.e., puna and páramo ecosystems) differ

from the rest of populations of this species, including populations at similar latitudes but from lower elevations. These wet grassland populations present a less robust braincase and a slightly longer snout. Populations from habitats at lower elevations (lower than 3500m) are less differentiated among themselves, with most differences concentrated in the masseteric tubercle and the squamosal and sphenoid regions. Indeed, the differentiation between these lower-elevation populations was constrained to the second canonical variate axis, which accounted only for 18.41% of the variance. No consistent morphological change with elevation was identified, even after analyzing the response of rostrum and braincase independently in consideration of developmental modularity (Leamy et al., 1999; Hallgrimsson et al., 2004), even though both the PLS and MMR analyses identified a significant association between environmental conditions of individuals' habitats and skull morphology. Furthermore, as seen in Grieco and Risk's (2010) study on Gambel's white footed mice, the amount of relative morphological differentiation within populations is fairly high and comparable to that seen across elevations. Taken together, these results indicate that low and mid elevation populations of *A. mollis* fits better a true-generalist scenario, which proposes the existence of individuals with a general common morphology across a wide range of environmental conditions.

The morphological differentiation seen in populations from highland grasslands in central Peru suggests that the forest-grassland transition represents a morphological break for *A. mollis.* Although this conclusion seems to be contradicted by the presence of individuals in northern Peru with a skull morphology similar to that of lower elevation individuals that fell in our analyses within a páramo ecosystem (Olson et al., 2001), it is important to note that all these specimens come from localities located below 3000m (Appendix 2.1). Indeed, the classification of these northern localities as páramo seems misleading as they are dominated by upper montane pluvial vegetation (Fig. 2.5d) not páramo vegetation (Fig. 2.5e) (cf. Tosi, 1960). This classification inconsistency likely results from the broad scale nature of the ecosystems dataset used (Olson et al., 2001). While finer-scale ecosystem or vegetation zone maps could have been used to resolve

these apparent inaccuracies, the availability of samples for this study limited our comparison to broad-scale ecosystem classifications. The morphological patterns and environmental associations found, however, are independent of the ecosystem map used, as these low-resolution maps were used only for visual summarizing purposes; all statistical analyses were based on high-resolution environmental information (Table 2.1). Moreover, the topographic nature of the Peruvian Andes prevents the formation of analog forest-páramo transitions in the north of the country similar to those seen in Central and South Peru (Tovar Serpa, 1957). This is the result of the Peruvian Andes decreasing in elevation from south to north, with their lowest points in the frontier with Ecuador, from the Huancabamba depression to the Loja Knot (Church, 1901, Fig. 1) (Fig. 2.1). Thus, this apparent contradiction does not negate the conclusion that environmental changes associated with the forest-grassland transition are the main contributor to the patterns of differentiation observed.

The morphological break identified in this study at the forest-grassland transition in Central Peru may be the result of the severe ecological changes associated with high elevation environments. The progressive disappearance of tree coverage at high elevations diminishes the buffering effect of vegetation on microclimatic conditions (Sarmiento, 1986). Further, the physical conditions with which organisms have to cope at high elevations become harsher as average temperatures, atmospheric pressure, atmospheric concentration of oxygen, and water vapor pressure decrease, and daily temperature fluctuations considerably increase (Mani, 1968; Hammond et al., 2001; Kuentz et al., 2007; Stork et al., 2007). At the same time and as a result, there is a significant turnover of resources that likely impacts the diet of *Akodon mollis*. Indeed, dietary flexibility has been observed in páramo populations of *A. mollis* in Ecuador (Barnett, 1999). A previous study of the diet of other species of *Akodon* in southwestern Peru revealed a significant relationship between the proportion of insects in the diet and elevation (Pizzimenti and Desalle 1980). In accordance with this slight elongation of the snout on skulls of the highland populations (>4000m), strongly insectivorous mice from other Neotropical genera have elongated snouts. Similar changes in diet and associated

morphological traits with elevation have also been observed in other cricetid mice, and in particular, strongly insectivorous mice in South America tend to have narrow, elongated skulls similar in many respects to those of high-elevation *A. mollis* (Hammond et al., 1999; Hammond et al., 2001; Hershkovitz, 1994). Thus, it is likely that the differences in skull morphology observed in the high-elevation populations of *Akodon mollis* result from the interaction between direct physiological constraints imposed by the challenging environmental conditions of tropical highlands and indirect pressures imposed by dietary constraints, as both these factors have been shown to influence skull morphology in cricetid mice (Myers et al., 1996; Grieco and Rizk, 2010).

    While the environmental pressures associated with tropical highlands (Mani, 1968; Sarmiento, 1986) seem to impose unique morphological constraints on the elsewhere common skull phenotype of *Akodon mollis*, alternative explanations are also possible. Patton and Smith's (1992) suggested that *A. mollis* is a species complex, and it is possible that the morphological differences seen at high elevations are the result of genetic independence of these highland populations. While testing this possibility requires a complete phylogenetic assessment beyond the goals of this paper, a preliminary exploration of levels of population genetic differentiation seems to partially support this possibility. Average pairwise nucleotide differences in 1123bp of the cytochrome b gene between individuals from several of the populations used in this study (Fig. 2.7, Luna et al., in prep.) showed marked differences between high and low elevation populations. Average pairwise nucleotide differences between highland populations (those from 4000 and 4500m) were also high, suggesting that members of these populations may not be closely related, even though no consistent morphological differentiation among these populations was found. This preliminary observation supports the hypothesis that the morphological differentiation of highland populations compared to lower-elevation ones is likely associated with the unique conditions of tropical highlands and not the result of these populations being genetically close to each other. Although further studies are needed to identify the mechanistic causes for the differentiation observed, these results point to the effect of strong selection on individuals

to cope with the prevalent conditions in tropical highlands as a driver of morphological differentiation in *A. mollis*.

Regardless of the exact forces behind the morphological differences observed between highland and lower-elevation populations of *Akodon mollis*, the fact that the morphological distinction of the highland forms was limited to these populations highlights that local environmental matching (i.e., congruent morphological differentiation across elevations, expected under the locally-specialized strategy) is not a main driver of skull morphology in this species. Thus, the results of this study suggest that the ample altitudinal range and broad ecological tolerance of *A. mollis* may be accomplished by the expression of a variable generalist phenotype throughout most of its geographic range (true-generalist strategy). Even within the highland populations no obvious morphological skull differences were found between individuals from páramo and puna despite the ample ecological differences between these two ecosystems (Tovar Serpa, 1957; Cabrera, 1968; Quintanilla, 1983) (Fig. 2.5). Such broad ecological tolerance contrasts with other vertebrates (including small mammals) in the region, as evidenced by sharp species turnover at the forest-grassland transition (Pearson and Ralph, 1978; Patterson et al., 1998).

Similar absence of marked cranial differentiation across elevational gradients has been observed in other generalist rodents (e.g., Ríos and Álvarez-Castañeda, 2007; Grieco and Rizk, 2010). Yet, all these studies come from temperate regions where strong seasonality likely promotes the evolution of populations with wide environmental tolerances (Janzen, 1967; Ghalambor et al., 2006; Kozak and Wiens, 2010). Thus, generalist species comprising locally specialized populations should be exceptional in temperate regions. It is not clear if these findings are applicable to the tropics where environmental constancy likely promotes environmental specialization (Janzen, 1967). This study, which to the extent of our knowledge constitutes the first to look at skull variation along elevational gradients in a tropical generalist, suggests that the true-

generalist strategy (i.e., a generalist phenotype thorough a species range) may be common in the tropics as well. This result has important implications for understanding biological resilience to environmental change and diversification processes in tropical mountains. While tropical mountain species are believed to be disproportionally susceptible to ongoing ecosystem changes (Williams and Jackson, 2007; Deutsch et al., 2008), our findings suggest that such generalization may be inaccurate for generalist species following a true-generalist strategy as these species can likely accommodate an ample range of conditions without major phenotypic changes. On the other hand, the ample range of phenotypic variation, high adaptability, and wide geographic range of "true" generalist species seems to contradict the thought-to-be general pattern of diversifying selection as a major driver of tropical diversification (Schneider et al., 1999; Moritz et al., 2000). Although such diversification mechanism may be common in other species, the lack of local specialization seen in *A. mollis* suggests that other evolutionary forces such as drift may play a greater role in generalist species. Then, forces that limit gene flow among allopatric populations such as climate-driven distribution shifts into refugia are likely of major importance in the evolution of these species (Alvarado-Serrano, in prep.).

If local adaptation to environmental conditions is not a significant aspect of the biology of *Akodon mollis*, then it is unlikely that evolution along Ecological Gradients (Chapter 1) plays an important role in the speciation of this species.
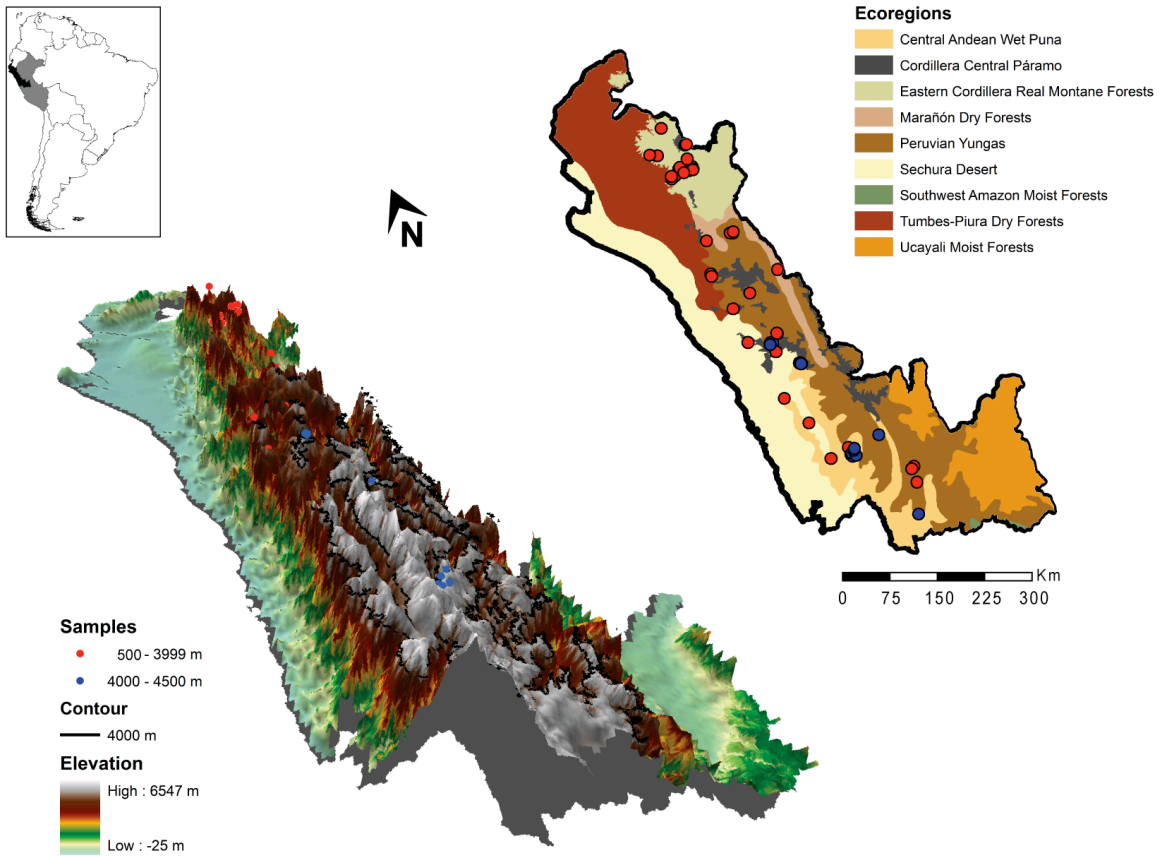
Figure 2.1. Topographic (left) and ecosystem (right) maps of the study region in northwestern Peru indicating the location of samples of populations of *Akodon mollis* used in the analyses. The inset on the left shows the position of Peru (in grey) and of the study region (in black) within South America. Samples are colored according to the elevation at which they were sampled (see legend).
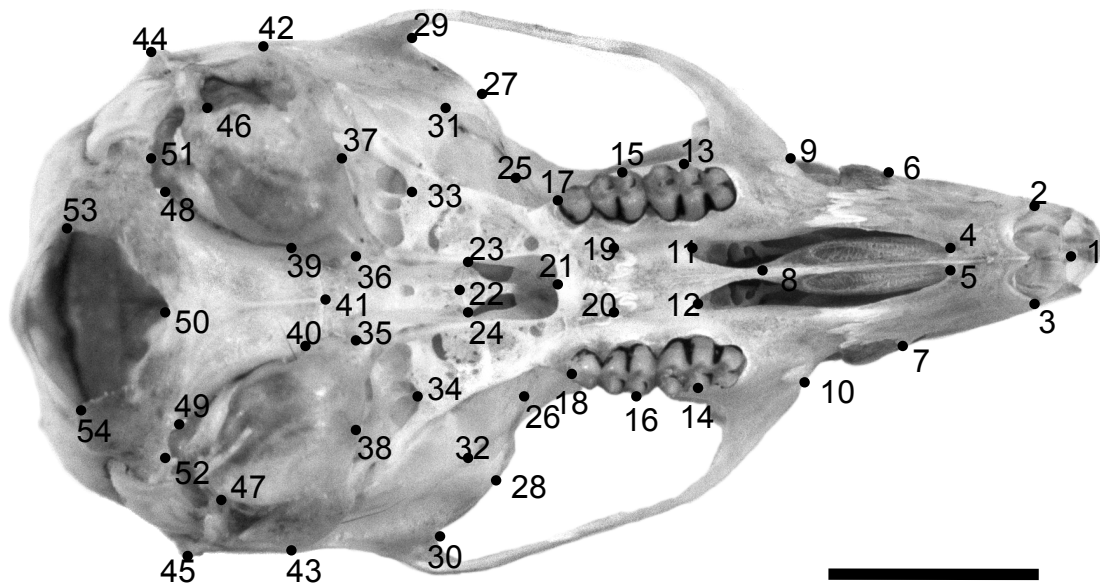
Figure 2.2. Positions of the 54 landmarks used to characterize the ventral skull of *Akodon mollis* (L1: most anterior border of the gnathic process; L2, 3: outermost curvature of the alveolus of the incisor; L4, 5: Most anterior border of the incisor foramen; L6, 7: outermost suture between the premaxilla and maxilla; L8: point of maximum width of the incisor foramina at the ventral nasal concha; L9, 10: base of the zygomatic plate at the level of the masseteric tubercle; L11, 12: most posterior border of the incisor foramen; L13, 14: paracone of first upper molar; L15, 16: paracone of right upper molar; L17, 18: most posterior border of third upper molar; L19, 20: center of the posterior palatine foramen; L21: medial anterior border of the mesopterygoid fossa; L22: medial suture between the presphenoid and basisphenoid; L23, 24: outermost suture between presphenoid and basisphenoid; L25, 26: suture of the alisphenoid and the frontal at the level of the squamosal-alisphenoid groove, L27, 28: groove at suture between the frontal and squamosal; L29, 30: most anterior curvature of the squamosal projection that creates the orbit; L31, 32: suture of the alisphenoid and squamosal at the level of the squamosal-alisphenoid groove; L33, 34: most anterior border of the foramen ovale; L35, 36: center of the posterior curvature of the Eustachian tube; L37, 38: most anterior border of the tympanic thickening of the bulla; L39, 40: anterior border of the carotid canal; L41: Most anterior medial margin of the basioccipital; L42, 43: most anterior external border of the ectotympanic; L44, 45: most posterior border of the masseteric tubercle; L46, 47: innermost opening of the auditory meatus; L48, 49: opening of the basioccipital at the level of the occipital condyle; L50: most anterior medial border of the foramen magnum; L51, 52: most anterior border of the paramastoid process; L53, 54: most external border of the occipital condyle). Scale bar = 5 mm.
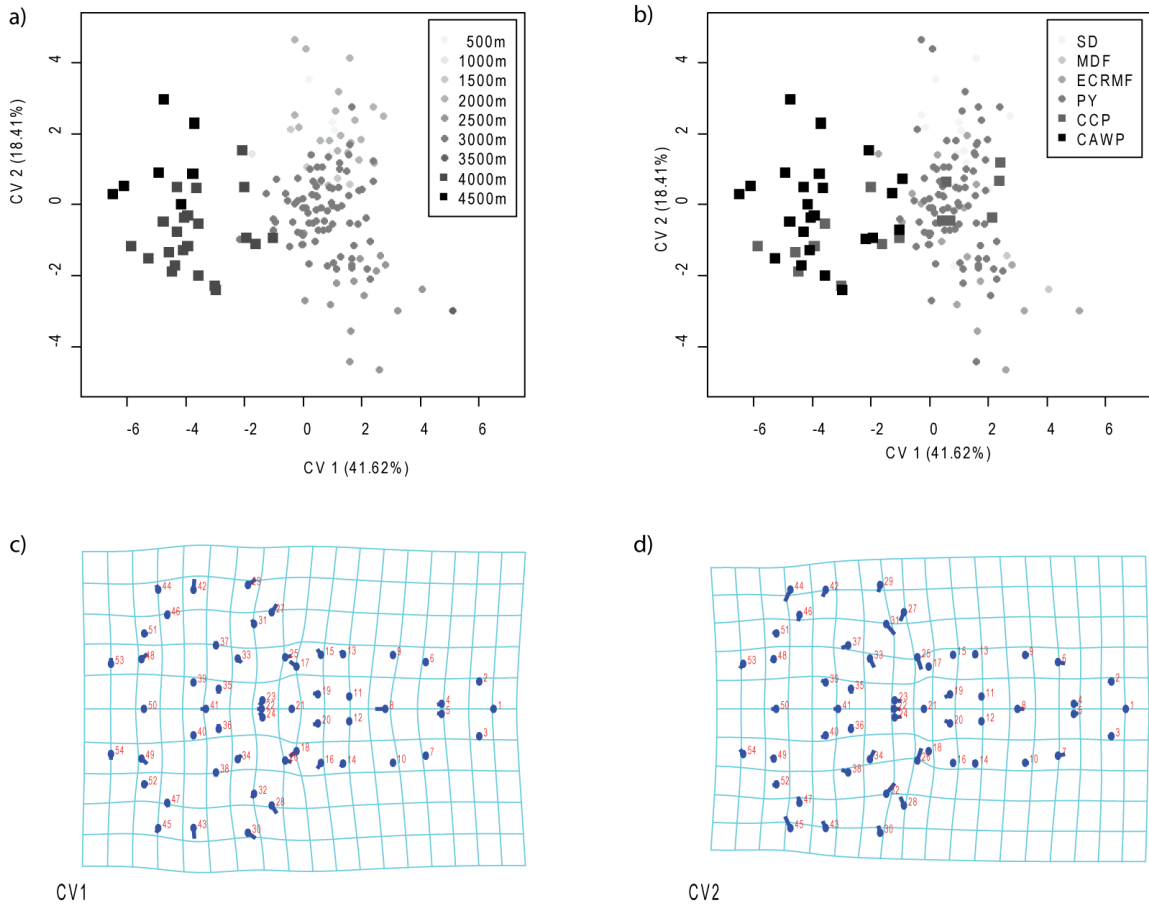
Figure 2.3. Results of a CVA analysis on all individuals grouped *a priori* by the elevation at which they were sampled. Scatterplots of analyzed individuals according to their scores on the first two canonical variate axes are shown with plot symbols defined according to the elevation bin they were group in (a) or according to the ecosystem where they were sampled (b). Ecosystems definition follows WWF's Terrestrial Ecoregions GIS Database (Olson *et al.* 2001): Sechura Desert (SD), Marañón Dry Forest (MDF), Eastern Cordillera Real Montane Forest (ECRMF), Peruvian Yungas (PY), Cordillera Central Páramo (CCP), and Central Andean Wet Puna (CAWP). Deformation grids of the skull showing the morphological variation associated with the first (c) and second (d) CV axes.
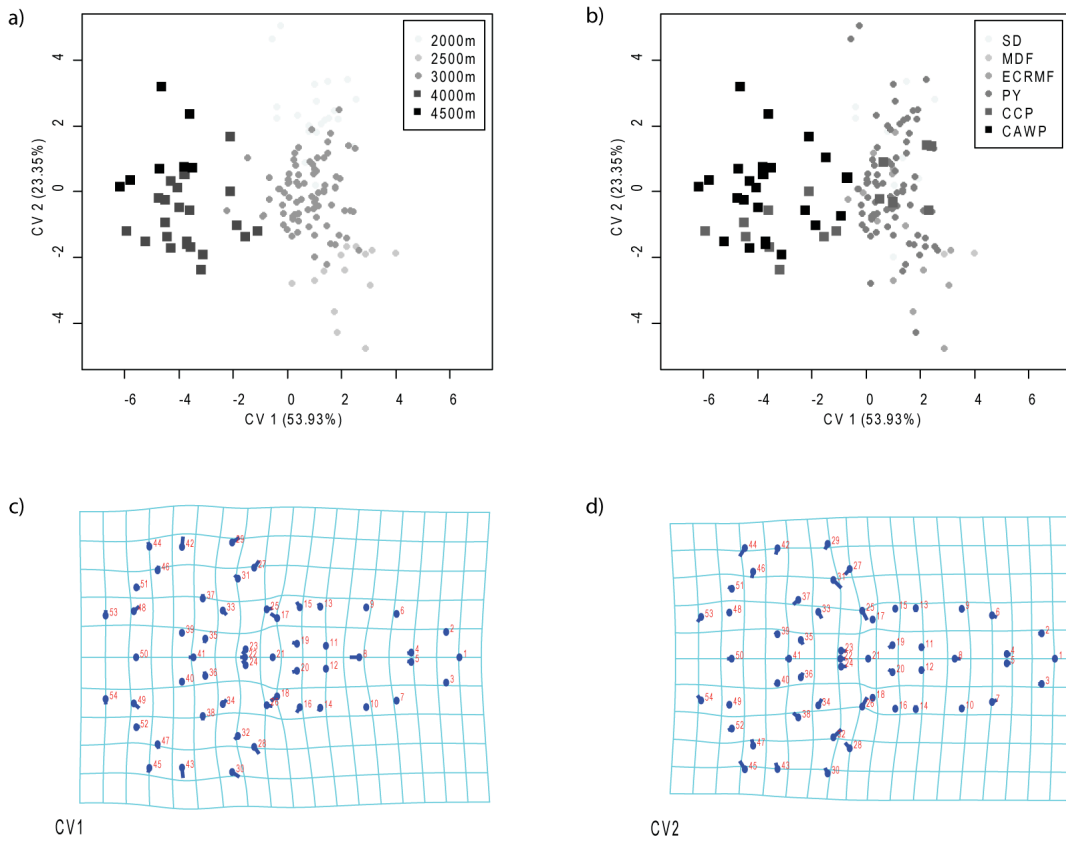
Figure 2.4. Results of a CVA analysis on Peruvian population of the *Akodon mollis* using only *a priori* defined elevation groups that contain more than 5 individuals. Scatterplots of analyzed individuals according to their scores on the first two canonical variate axes are shown with plot symbols defined according to the elevation bin they were group in (a) or according to the ecosystem where they were sampled (b). Ecosystems definition follows WWF's Terrestrial Ecoregions GIS Database (Olson *et al.* 2001). Transformation grids of the skull showing the morphological variation associated with the first (c) and second (d) CV axes.

a)

@ Field Museum

Trujillo, Menocucho, 500m

b)

Huancabamba, Canchaque, 1190m

c)
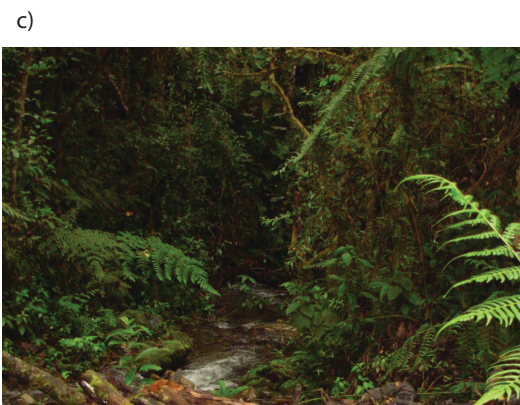
Tabaconas, Cerro La Viuda, 2140m

d)

Tabaconas, Cerro La Viuda, 2880m

e)

Santiago de Chuco, Callacuyán, 4000m

f)

Huari, Canrash, 4470m

Figure 2.5. Examples of some of the habitats occupy by *Akodon mollis* in Peru along its ample elevation range.
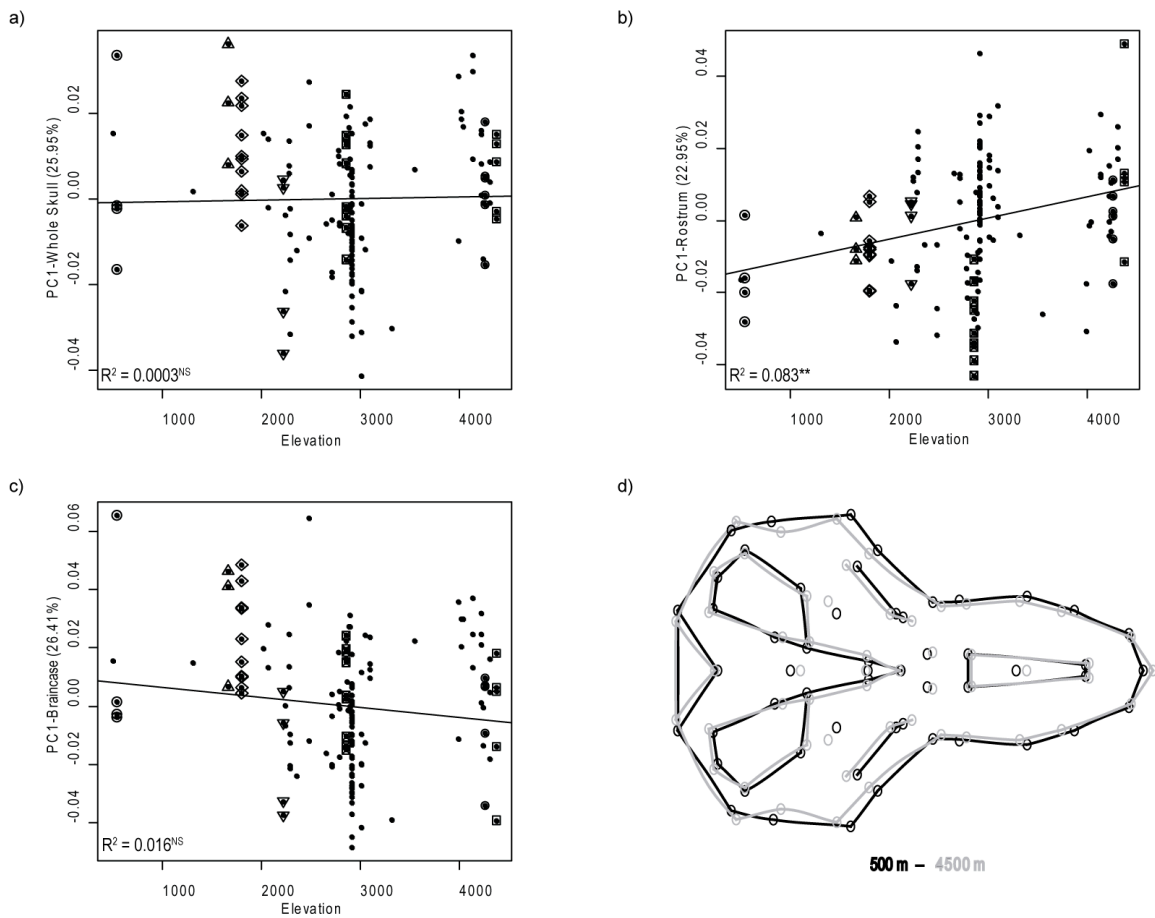
Figure 2.6. Morphological changes in *Akodon mollis* associated with elevation. Shape changes are summarized by the first principal component of a PCA from all skull landmark data (a), only rostrum data (i.e., landmarks 1-21), and only braincase data (i.e., landmarks 22-54). The average morphological configuration from populations at the extremes of the gradient, 500m and 4500m, is contrasted in d). For each elevation bin in the scatterplots, one population was selected, and individuals of each of these populations are depicted using unique symbols to indicate the extent of local variability in the sample.

64
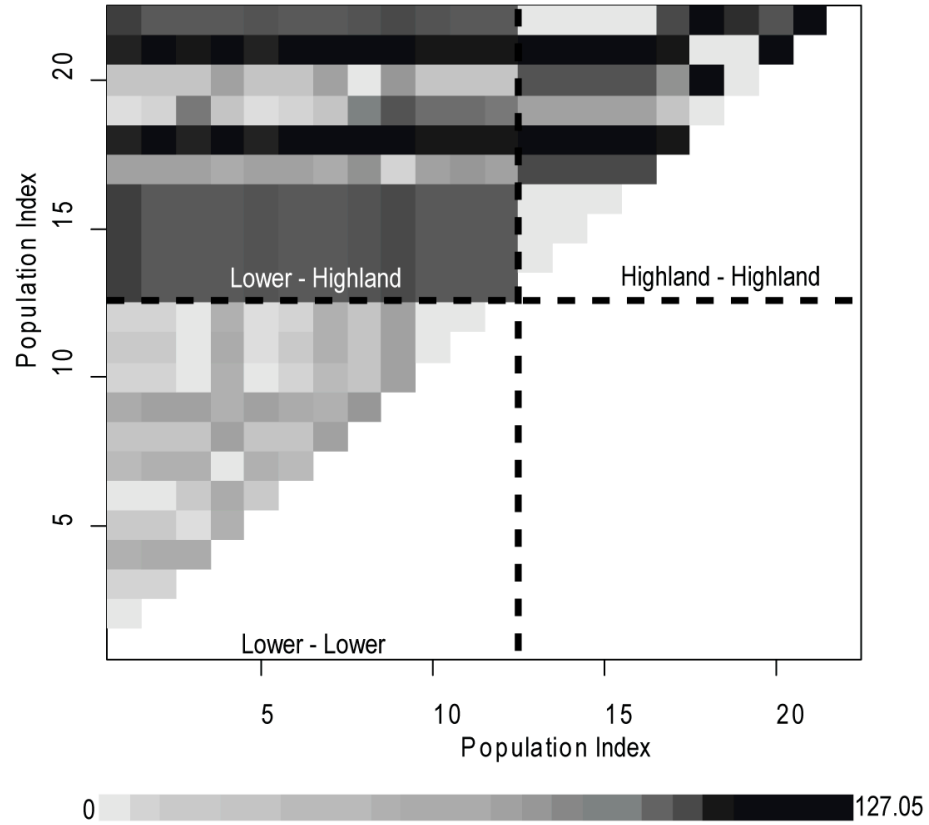
Figure 2.7. Gray-scale matrix of pairwise genetic distances between some of the *Akodon mollis* populations used in the analyses. The dotted lines point to the separation between the morphologically similar populations of the highlands and the rest of the populations. Population numbers in the x and y axes follow appendix
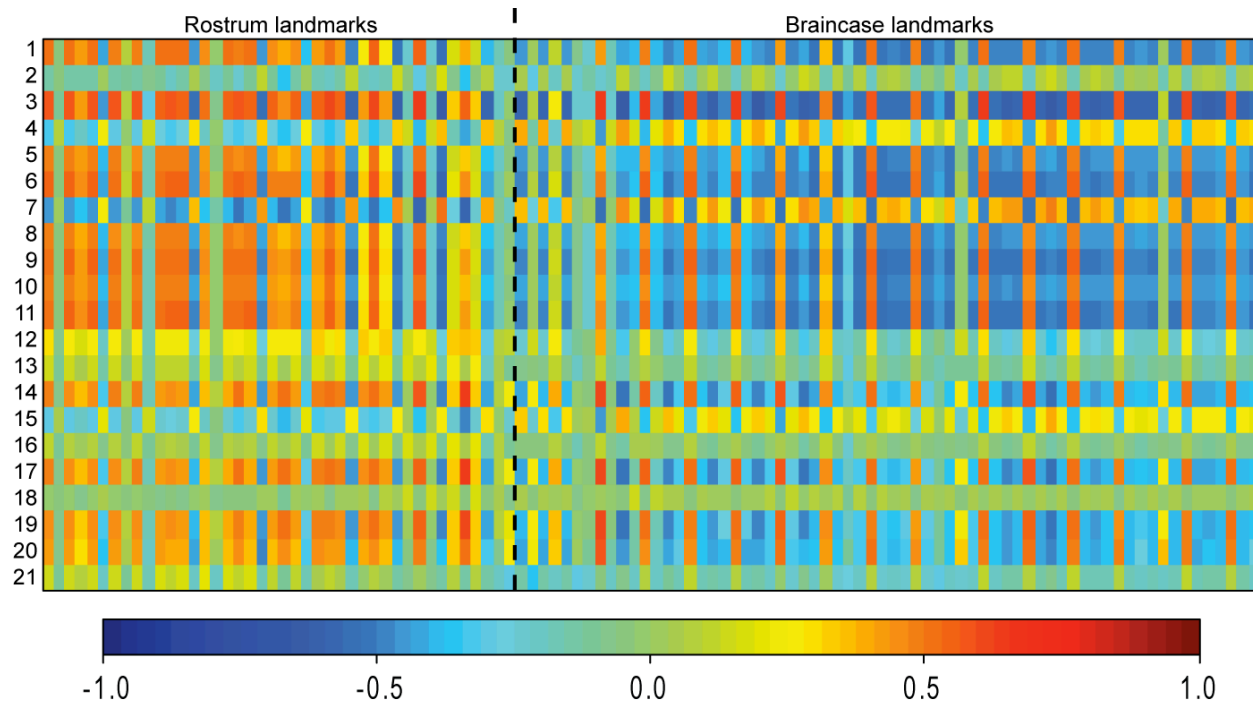
Figure 2.8. Cross-correlation heat chart of skull morphology against environment for *Akodon mollis*. The 108 X-Y coordinates (54 landmarks) are organized in increasing order as columns, while the 21 environmental variables used in the analysis are organized as rows: 1) annual mean temperature, 2) mean diurnal temperature range, 3) isothermality, 4) temperature seasonality, 5) maximum temperature of warmest month, 6) minimum temperature of warmest month, 7) temperature annual range, 8) mean temperature of wettest quarter, 9) mean temperature of driest quarter, 10) mean temperature of warmest quarter, 11) mean temperature of coldest quarter, 12) annual precipitation, 13) precipitation of wettest month, 14) precipitation of driest month, 15) precipitation seasonality, 16) precipitation of wettest quarter, 17) precipitation of driest quarter, 18) precipitation of warmest quarter, 19) precipitation of coldest quarter, 20) net primary productivity, 21) normalized difference vegetation index. Color spectrum at the bottom represents the value of the correlation coefficient for each pairwise comparison.

Table 2.1. Environmental variables used in this study. Pearson correlation coefficients between SRTM-based elevation and all 21 variables used in the analyses are shown in the last column. Symbols used: ^: less autocorrelated variables that were used for the reduced-dimensionality multiple multivariate regression (see text for details); NS: p-value > 0.10; (*): 0.10 > p-value > 0.05; *: 0.05 > p-value > 0.01; **: p-value < 0.01.

Variable	Source	Resolution	Pearson's r
Annual Mean Temperature	Worldclim	30 seconds	-0.969 **
Mean Diurnal Temperature Range^	Worldclim	30 seconds	0.550 **
Isothermality	Worldclim	30 seconds	-0.175 *
Temperature Seasonality	Worldclim	30 seconds	-0.060 NS
Maximum Temperature of Warmest Month	Worldclim	30 seconds	-0.964 **
Minimum Temperature of Warmest Month	Worldclim	30 seconds	-0.954 **
Temperature Annual Range^	Worldclim	30 seconds	0.573 **
Mean Temperature of Wettest Quarter	Worldclim	30 seconds	-0.981 **
Mean Temperature of Driest Quarter	Worldclim	30 seconds	-0.956 **
Mean Temperature of Warmest Quarter	Worldclim	30 seconds	-0.981 **
Mean Temperature of Coldest Quarter^	Worldclim	30 seconds	-0.956 **
Annual Precipitation	Worldclim	30 seconds	0.360 **
Precipitation of Wettest Month^	Worldclim	30 seconds	0.374 **
Precipitation of Driest Month	Worldclim	30 seconds	-0.137 (*)
Precipitation Seasonality^	Worldclim	30 seconds	-0.184 *
Precipitation of Wettest Quarter	Worldclim	30 seconds	0.445 **
Precipitation of Driest Quarter	Worldclim	30 seconds	-0.140 (*)
Precipitation of Warmest Quarter	Worldclim	30 seconds	0.554 **
Precipitation of Coldest Quarter^	Worldclim	30 seconds	-0.156 (*)
Net Primary Productivity^	HANPP	15 minutes	-0.032 NS
Normalized Difference Vegetation Index^	MODIS	30 seconds	-0.205 *

Table 2.2 Multivariate distances among elevation-based groups of Peruvian populations of *Akodon mollis* identified *a priori* for the Canonical Variate Analysis on all individuals sampled (see text for details). Mahalanobis distances are above the diagonal and Procrustes distances are below the diagonal. Permutation-based significance is reported (NS: non-significant, *: 0.05 < p-value < 0.01, and **: p-value < 0.01) with significance results consistent between both distances underlined. Sample size for each group is given in the second row.

n	500m (4)	1000m (1)	1500m (4)	2000m (21)	2500m (15)	3000m (78)	3500m (1)	4000m (22)	4500m (7)
500m		7.376 NS	6.996 *	5.302 **	6.788 **	5.462 **	12.127 NS	7.060 **	8.608 *
1000m	0.025 NS		9.295 NS	8.926 *	9.550 *	8.427 NS	15.310 NS	8.312 NS	10.018 NS
1500m	0.022 NS	0.021 NS		5.180 **	5.900 **	5.255 **	12.942 NS	6.721 **	8.192 **
2000m	0.015 NS	0.025 NS	0.015 NS		5.118 **	3.271 **	10.645 **	5.863 **	7.252 **
2500m	0.021 NS	0.031 NS	0.026 *	0.020 **		3.584 **	10.001 *	6.161 **	8.379 **
3000m	0.020 NS	0.029 NS	0.024 **	0.018 **	0.009 NS		10.533 *	4.786 **	6.461 **
3500m	0.045 NS	0.057 NS	0.055 NS	0.045 *	0.034 NS	0.035 NS		12.337 **	14.413 NS
4000m	0.024 *	0.026 NS	0.019 NS	0.016 **	0.022 **	0.019 **	0.045 NS		5.144 **
4500m	0.030 **	0.033 NS	0.027 **	0.024 **	0.025 **	0.021 **	0.047 **	0.016 NS	

Table 2.3. Multivariate distances among elevation-based groups of Peruvian populations of *Akodon mollis* identified *a priori* for the Canonical Variate Analysis on all individuals sampled (see text for details). Mahalanobis distances are above the diagonal and Procrustes distances are below the diagonal. Permutation-based significance is reported (NS: non-significant, *: 0.05 < p-value < 0.01, and **: p-value < 0.01) with significance results consistent between both distances are underlined. Sample size for each group is given in the second row.

n	2000m (21)	2500m (15)	3000m (78)	4000m (22)	4500m (7)
2000m		5.178 **	3.320 **	5.766 **	7.153 **
2500m	0.020 **		3.569 **	6.226 **	8.329 **
3000m	0.018 **	0.009 NS		4.814 **	6.376 **
4000m	0.016 **	0.022 **	0.019 **		5.122 **
4500m	0.024 **	0.025 **	0.021 **	0.016 NS	

Appendix 2.1. Specimens used in the analyses, 153 as listed below. Geographical coordinates and elevations were recovered from georeferencing collector's notes using point-radius method described in the Material and methods section. Abbreviations: FMNH (Field Museum of Natural History, Chicago, Illinois), and collectors' initials as in field and catalog notes.

ANCASH: *Huaraz*, Tullparaju, 4500m, -9.0344; -77669 (FMNH 81371, 81373); *Huari*, Canrash, 4500m, -9.676042; -77.05375 (mcp 54, mcp 55, mcp 56, mcp 59, mcp 63); *Huari*, Chacacmonte, 4000m, -9.684715; -77.105255 (mcp 34, mcp 35, mcp 36, mcp 42); *Huari*, Jupro, 4000m, -9.588604; -77.084414 (mcp 67, mcp 68); Huari, Paccha, 4000 m, -9.63179; -77.119506 (mcp 04, mcp 05, mcp 11, mcp 15, mcp 18, mcp 20); *Huari*, Pumahuain, 4000m, -9.663556; -77.130111 (mcp 24); *Huari*, Río Mosna, between Chavín and San Marcos, 3000m, -9.55; -77.166667 (FMNH 129213, 129215, 129219, 129225); *Santa*, Macate, 2000m, -8.766667; -78.083333 (FMNH 20899, 20905, 20909).

CAJAMARCA: *Cajamarca*, Cajamarca, 3000m, -7.166667; -78.516667 (FMNH 19275, 19277, 19281, 19283), *Celendín*, Hacienda Limón, 2500m, -6.833333; -78.083333 (FMNH 19285, 19287); *Contumaza*, Bosque Cachil, entre Cascas y Contumaza, 3000 m, -7.38925; -78.78188 (jaa 178, vpt 1674); *Cutervo*, San Andrés de Cutervo, 2000m, -6.235533; -78.71885 (llw 1197, llw 1212); *Cutervo*, San Andrés de Cutervo, Cutervo National Park, 100m over El Tragadero, 3000m, -6.249972; -78.766528 (llw 1085, llw 1086, llw 1087, llw 1088, llw 1089, llw 1093, llw 1094, lw 1095, llw 1096, llw 1098, llw 1100, llw 1102, llw 1106, llw 1108, llw 1109, llw 1112, llw 1114, llw 1115, llw 1120, llw 1122, llw 1126, llw 1131, llw 1132, llw 1136, llw 1140, llw 1143, llw 1145, llw 1148, llw 1151, llw 1153, llw 1154, llw 1155, llw 1161, llw 1164, llw 1165, llw 1166, llw 1167, llw 1169, llw 1173, llw 1174, llw 1179, llw 1180, llw 1181, llw 1183; *Cutervo*, San Andrés de Cutervo, 4 km W San Andrés de Cutervo, 2500m, -6.25674; -78.7246 (jaa, 135, jaa 139, jlm 175, vpt 1597); *San Ignacio*, Tabaconas, Cerro Coyona (Tabaconas-Namballe National Sanctuary), 3500m, -5.23344; -79.27817 (jaa 805); *San Ignacio*, Tabaconas, Piedra Cueva in Cerro Coyona (Tabaconas-Namballe National Sanctuary), 3000m, -5.268429; -79.269934 (llw 926, llw 929, llw 930, llw 946, llw 967, llw976, llw 995); *San Ignacio*, Tabaconas, Cerro La Viuda (Tabaconas-Namballe National Sanctuary' Buffer Zone), 3000m, -5.290794; -79.336961 (llw 1003, llw 1004, llw 1013, llw 1023); *San Ignacio*, Tabaconas, Cerro La Viuda (Tabaconas-Namballe National Sanctuary' Buffer Zone), 2000m, -5.284432; -79.321697 (llw 1048, llw 1082); *San Miguel*, La Florida, Agua Azul, 1500m, -6.88504; -79.07512 (llw 491, llw 503, llw 511); *Santa Cruz*, 2 km E Monteseco, 2000m, -6.847342; -79.088503 (jlm 208, vpt 1636); *Santa Cruz*, Cata 3.81 km NE from Monteseco, 2000m, -6.847342; -79.088503 (lhl 92, lhl 93, lhl 116, llw 1241, llw 1248, llw 1249, llw 1273, llw 1274).

LA LIBERTAD: *Otuzco*, Hacienda Llagueda, 2000m, -7.766667; -78.583333 (FMNH 19317, 19321, 19325); *Otuzco*, Summit between Otuzco and Llagueda, 3000m, -7.9; -78.583333 (FMNH 19849, 19851); *Sánchez Carrión*, Sanagorán, 3000m, -7.792068; -78.138445 (lhl 85, llw 1219, llw 1220, llw 1221, llw 1222, llw 1225, llw 1226, llw 1227, llw 1232); *Sánchez Carrión*, Sanagorán, 2500m, -7.783123; -78.146387 (vpt 2251, vpt, 2252, vpt 2263); *Santiago de Chuco*, Cachicadán, 3000m, -8.064632; -78.17321 (vpt

2277); *Santiago de Chuco*, Camp. Callacuyán, Quebrada Quishuara Sur, 4000m, -7.938683; -78.23468 (avg 143); *Santiago de Chuco*, Campamento Callacuyán, 4000m, -7.95126; -78.237412 (mvc 313, mvc 323); *Santiago de Chuco*, Campamento Callacuyán, 4000m, -7.921906; -78.249845 (mvc 333); *Santiago de Chuco,* Campamento Callacuyán, 4000m, -7.952933; -78.252512 (vpt 2380, vpt 2387, vpt 2390); *Trujillo*, Menocucho, 500m, -8.016667; -78.833333 (FMNH 19329, 19333, 19335, 19343);

PIURA: *Ayabaca*, Ayabaca, 3000m, -4.633333; -79.716667 (FM 81379); *Huancabamba*, Canchaque, 1000m, -5.5; -78.6 (FM 81357); *Huancabamba*, Canchaque, 1500m, -5.4; -79.6 (FM 83441); *Huancabamba*, El Carmen de la Frontera, Carmen de la Frontera, Alto Samaniego, 2500m, -5.11017; -79.35269 (ucf 43); *Huancabamba*, Huancabamba, 2000m, -5.233333; -79.466666 (FM 84203); *Huancabamba*, Huancabamba, km 30 on road to San Ignacio, 2500m, -5.25; -79.483333 (FMNH 81353, 81363, 81365, 81367); *Huancabamba*, Tambo, 2500m, -5.35; -79.55 (FMNH 81359)

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Chapter 3:

Differentiation of the *Akodon mollis* species group (Rodentia:
Cricetidae: Sigmodontinae) in the Andes of Peru: testing models of
diversification in the Andes

INTRODUCTION

The mechanisms responsible for the species assemblages of montane biotas are
still poorly understood (Ribas et al., 2007). This is surprising as one of the conservation
hotspots, and the world's highest biodiversity center, is the Tropical Andes (Brehm, et al.,
2005; Myers et al., 2000; Picard et al., 2008). Due to their extreme heterogeneity in
elevation, biotic and abiotic factors, the Andes have been postulated to play an important
role in diversification in tropical South America (Fjeldså, J.1994; García-Moreno, et al.,
1999). This conclusion, however, was based mostly on studies of plants and birds, few of
which adopted a phylogenetic approach (Chesser, 2000). Two of the mechanisms
postulated as responsible for the diversification of flora and fauna in the Andes are
allopatric and parapatric speciation (Coyne and Orr, 2004) via two important hypotheses
tailored for the Andean mountains: the Montane Vicariance hypothesis (Patton et al.,
1990; Patton and Smith 1982a) and the Ecological Gradient hypotheses (Endler, 1977,
Patton and Smith, 1982a, b). These are explained at length in Chapter 1. Under the
Montane Vicariance hypothesis it is expected that closely related lineages will be found
in adjacent drainages at the same elevation; therefore populations on mountains will not
form clades, but elevational ranges will. Under the Ecological Gradient hypotheses it is
expected that geographically contiguous lineages should be more closely related to one

another than to lineages on different slopes; therefore populations on mountains will form clades, and individuals from same elevations on different mountains will appear in different clades (Figure 3.1).

In order to test these hypotheses, as well as to investigate the nature of the genetic differentiation of a mammalian group along latitudinal and elevational gradients, a species with a moderately extensive latitudinal distribution as well as a wide elevational gradient was chosen, the Andean soft grass mouse, *Akodon mollis.*

The genus *Akodon* (Rodentia: Cricetidae: Sigmodontinae) is one of the largest, most complex, and least understood groups of South America muroid rodents (Myers et al., 1990; Smith and Patton, 2007). This genus represents an important challenge for taxonomists due to the similar cranial and external morphology presented in most of the Andean species that make up the bulk of the genus (Myers, 1989; Myers and Patton, 1989; Myers et al., 1990; Patton and Smith, 1992a, b). Recent advances at the molecular level have shed some light on the relationships of *Akodon* with other sigmodontine rodents, as well as corroborated the monophyly of the tribe Akodontini to which this genus belongs to (D'Elía, 2003; see also Smith and Patton, 2007); however, the relationship of the tribe with others in the Sigmodontinae has remained unresolved (D'Elía et al., 2006). One the largest and most complicated groups in this genus is *Akodon mollis,* a taxon that for long has been regarded as a complex of closely related species and subspecies that exhibit some differentiation across their range (Patton and Smith, 1992); it has, however, never been systematically reviewed. The species occupies the montane and highland regions of northern and central Peru northward through the central highlands of Ecuador, from the 500 to 4700 m of elevation (Smith and Patton, 1992), and its members are among the most common rodents in montane habitats (Osgood, 1914). This level of taxonomic and morphologic complexity, combined with the wide range of elevations and environmental conditions occupied by this species (see Patterson et al., 1998; Pearson and Pearson Ralph, 1978), make it a potential subject for

investigations of speciation, diversification, and morphological and molecular variation of vertebrate species in the Andean region.

This chapter explores the role of the distance among populations and their pattern of elevational distribution in determining patterns of genetic variation of this taxon along its distribution in the Andes of Peru in an attempt to provide insight into the speciation mechanisms responsible for the diversification of this species along and across the Andes.

In order to test Ecological Gradients and Montane Vicariance hypotheses, as well as explore the role of distance and elevation on the genetic structure of populations of *A. mollis*, I provide a phylogenetic hypothesis base on the highly variable mitochondrial gene Cytochrome b (cyt-b). Mitochondrial DNA has been widely used in mammalian phylogenetics, due to existing primers (Patton and Smith, 1992a), high mutation rates (tenfold faster than nuclear DNA), and extensive intraspecific polymorphism. For those reasons mitochondrial DNA has been and continues to be an important tool in molecular population genetics and systematics (Avise, 2000; Page and Holmes, 1998). However, its limitations are also well known. Using a single gene for the construction of a phylogenetic hypothesis provides a single representation of divergence history even if multiple mtDNA loci are sequenced (Belfiore, 2010). Further, mitochondria are inherited maternally, so only maternal lineages can be revealed.

This study employs patterns of genetic relationships to reveal the history of dispersal among populations of *Akodon mollis* along the distribution of the species in the Andes of Peru by. In particular, I focus on two basic questions. First, is the genetic difference between two individuals simply a function of the geographic distance separating them ("isolation by distance"), or are other factors at play in determining the pattern of genetic relationship (Wright, 1943, see also Coulon, et al., 2004)? Second, are individuals living at similar elevations but separated on different peaks more closely related to each other than to individuals at other elevations on the same peaks, as

predicted by the Montane Vicariance hypothesis, or are individuals living at different elevational belts on the same mountains more closely related to each other than other individuals living in the same zones on another mountain, as predicted by the Ecological Gradient hypothesis?

MATERIAL AND METHODS

Sampling

The *Akodon mollis* complex of species occupies the montane and highland regions of northern and central Peru northward through the central highlands of Ecuador from 500 to 4700 m of elevation (Smith and Patton, 1992). We sampled 132 specimens of the Andean soft grass mouse *A. mollis*, assigned to 8 populations, along the distribution of the species on the northern Peruvian Andes (Fig 3.2. and 3.5). In order to replicate observations across altitudes and latitudes, I collected 51 out of those 133 specimens following a systematic sampling design that included collections on three mountains higher than 3000 m of elevation. Each mountain was sampled at around 2000 m and around 3000 m with various intensities of sampling totaling an effort of 15900 trap/nights. Generally, intermontane valleys were appropriate habitats for *A. mollis,* and therefore did not likely serve as a barrier for dispersal. These collections were complemented by stored samples at the Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos (MUSM), Lima, Peru. These later specimens came from localities around 1000 to 4000 m and close to 5000 m. A list of specimens with their collector's numbers and localities is given in Appendix 3.1 (see also Figure 3.2). Genetic data were also collected for two outgroups, *A. cf. subfuscus* (LLW 505), and an undetermined species of *Akodon* (CT 397). Three additional outgroup taxa were obtained from GenBank: *Chroeomys jelskii*, *Geoxus valdivianus*, and *Abrothrix longipilis.* These three species are members from the recently defined sigmodontine tribe Abrotrichini

(D'Elía et al. 2007, see also Smith and Patton, 1999). Although the Subfamily Sigmodontinae has been proved monophyletic, relationships among tribes remain unclear (i.e. D'Elía et al., 2006); therefore, the selection of any other sigmodontine tribe is valid for as outgroup.

Genomic DNA was extracted from liver tissue (~ 25mg) that was preserved in absolute ethanol during fieldwork. DNA extractions were performed using Qiagen DNeasy Tissue kit and protocols therein. DNA isolates were used as templates for polymerase chain reaction (PCR) to amplify double-stranded DNA products from mitochondrial gene cytochrome b (cyt-b). Each PCR had a reaction volume of 25μL and contained 1μL of DNA stock, 12.5μL of Go Taq Green Master Mix (Promega), 1.25μL of forward primer, and 1.25μL of reverse primer (primers explained below). PCR amplifications were performed using the Eppendorf Mastercycler gradient thermal cycler. A 1124-base pair (bp) de cyt-b were sequenced using the external combination of primers LU284F (L5'CYAATGACATGAAAAATCATCG) and LU2228R (H5'GAGATGTCTTATTTAAGGGGAACG). PCR included an initial denaturation at 94ºC for 2-3 min and 30 s, followed by 35 cycles of PCR. Each cycle involved denaturation at 94ºC for 30 s, annealing at 45-50ºC for 35s, and a final extension at 72ºC for 1 min 20 s.

The target PCR products were purified by gel electrophoresis using 1.5% low-melting point agarose (Fisher Biotech) and cleaned of agarose using the Qiagen QIAquick Gel Extraction Kit and protocols therein, regardless of if multiple bands were shown in the gels. In some cases PCR products were cleaned using the Qiagen QIAquick PCR Purification Kit and protocols therein.

Sequencing reactions were performed using the facilities at The University of Michigan DNA Sequencing Core according to protocols for Applied Biosystems DNA Sequencers (Model 3730 XL sequencer). Sequencing primers used for the 1124 bp of cyt-b were designed to be used in combination with the PCR primers (see above). LU284F was used in combination with LU928R (H5'GGGTCTCCGAGRATRTCTGG) for the

first portion of cyt-b; LU183F (L5'AGCATTCTCNTCAGTNRCMCA) and LU1007R (H5'GYTGVCCHCCRATTCAKG) for the middle part, and LU1176F (L5'ACATGAATYGGRGGNCARC) for the final part LU2228R. There was substantial overlap between the pieces of DNA generated; only five of the six pieces generated were necessary in most cases to reconstruct the entire segment (Fig. 3.3). Sequences were aligned using Sequencher® 4.8 software (Gene Codes). After alignment and trimming of sequences, the cyt-b gene data set for the *Akodon mollis* complex contained 1124 characters. MacClade version 4.08 (Maddison and Maddison, 2005) was used to assign 1st, 2nd and 3rd codon position for later analyses. In several cases, identical haplotypes were found in many individual sampled, these redundant data were merge into a single sequence. Seventy haplotypes remained after the exclusion of redundant information. BBEdit version 9.6.2 (Bare Bones) was used to edit matrixes.

Phylogenetic analyses

Maximum parsimony

Maximum parsimony analyses (MP) were conducted using PAUP* version 4.0b10 (Swofford, 2003). For these analyses, missing data were identified by '?', and gap characters were identified by '-'. The saturation plot of the raw sequence divergence (uncorrected *p* distance) *vs.* the number of substitutions for all pairwise comparisons among individuals did not reach an asymptote (Fig. 3.4), and so characters were considered unordered and no weighting scheme was employed. Heuristic searches for minimum length topologies were performed, because the exact methods of exhaustive search would have required prohibitively long computer runs. Starting trees were obtained via random stepwise addition with 500 replicates. Only the best trees were saved. The branch-swapping algorithm was tree-bisection-reconnection (TBR). In those cases in which multiple hypotheses of relationships were made, strict and 50% majority-

rule consensuses were computed. To evaluate the robustness of the phylogenetic hypotheses, bootstrap sensitivity were also performed with 400 replicates with full heuristic search. Groups with frequency greater than 50% were retained for the consensus tree, and branches with less than 50% of support were allowed to collapse. For all analyses, no topological constraints were made and the resulting trees were rooted making the ingroup monophyletic.

Maximum likelihood

Model selection was performed using the Akaike Information criterion (AIC; Akaike, 1974) as implemented in MrModeltest version 2.3 (Nylander, 2004). MrModeltest was preferred over Modeltest because the former considers only 24 models of nucleotide substitution versus the 56 considered by the later, many of which are redundant as they are variations from the 24 models. Maximum likelihood (ML) analyses were partitioned by codon position, first and third codon positions following the GTR+G model, while the 2nd codon position following HKY+I.

The ML analyses were performed using RAxML HPC version 7.2.0 (Stamatakis, 2006). The analysis was performed using the linked version of RAxML since it had the best likelihood for the data (27944.003732) in comparison with the unlinked version (28190.368942). The analysis was performed using a GTR gamma model and run for 50 replicates from random start trees with the default rapid hill-climbing algorithm. Later, 1000 bootstrap replicates were run from random start trees with the default rapid hill-climbing algorithm. FigTree (Rambaut, 2006-2008) was used to visualize trees generated by the program, and PAUP* version 4.0b10 (Swofford, 2003) was used to create ML strict consensus as well as majority rule consensus trees. Phyutility (Smith and Dunn, 2008) was used to visualize Bootstrap support values generated by PAUP* version 4.0b10 (Swofford, 2003).

Bayesian inference

Bayesian inference analyses (BI) were conducted in MrBayes version 3.1.2. (Huelsenbeck and Ronquist, 2001). For the analyses, model selection was performed using the Akaike Information criterion (AIC; Akaike, 1974) as implemented in MrModeltest version 2.3 (Nylander, 2004). BI analyses were partitioned by codon position, first and third codon position following the GTR+G model, while the 2nd codon position following the HKY+I model. Two runs of four simultaneous Markov chains were run for 25,000,000 generations using the CBSU Web Computing Resources, a web interface of the Computational Biology Service Unit at Cornell University (http://cbsuapps.tc.cornell.edu/index.aspx). Trees were sampled every 1000 generations resulting in 25000 trees saved. The two independent runs of the same data resulted in similar topologies. After the runs, -ln *L* was plotted against the generation using Tracer version 1.4 (Rambaut and Drummond, 2003-2007). This was done to identify the convergence point, and the first 2500 trees were discarded as "burn-in". Finally, a 50% majority rule consensus tree was constructed. Posterior probabilities greater than 95% were considered significant support for a clade (Ronquist and Huelsenbeck, 2003)

Test of Isolation by distance (IBD) and population genetic models

Isolation by distance (IBD) describes the accumulation of local genetic differences under geographically restricted dispersal (Slatkin, 1993). A first test of IBD was based on pairwise nucleotide differences for all 132 specimens and pairwise difference of their geographic coordinates (UTM) converted to kilometers. Pairwise differences for the genetic and geographical components were performed using GenAlEx 6.41 (Peakall and Smouse, 2006), these pairwise differences were then assembled by hand to created a genetic-geographic matrix. IBD analyses were performed by using

IBDWS v. 3.23 (Jensen et al., 2005. http://ibdws.sdsu.edu/~ibdws/) with statistical significance assessed using 10000 randomizations.

Levels of gene flow and population differentiation were estimated by calculating F_{ST} and Slatkin linearized F_{ST} (Slatkin, 1993), with localities grouped into 8 populations based on geographical proximity and elevation (Figure 3.5, Table 3.1). In order to see if there is an effect of geographic distance on the genetics differentiation of these 8 populations, a genetic and geographic matrix was assembled by hand with the pairwise differences generated by GenAlEx 6.41 (Peakall and Smouse, 2006). Slatkin linearized F_{ST} for each pairwise population comparison was done on Arlequin 3.5 (Excoffier and Lischer, 2010). For each population a single geographic coordinate was generated by averaging the specimen's coordinates in each population. The average number of pairwise differences between and within populations was calculated using Arlequin 3.5 (Excoffier and Lischer, 2010). IBD for the populations along geography was tested using Mantel test in IBDWS v. 3.23 (Jensen et al., 2005. http://ibdws.sdsu.edu/~ibdws/) with statistical significance assessed using 1000 randomizations. Finally, Slatkin linearized F_{ST} was plotted against log geographic distance as proposed by Rousset (1997) (see also McRae and Beier, 2007).

Specimens were first divided into "populations" on the slopes of single mountains, except for the red [VII] and pink (V) populations, which each included two adjacent peaks. The valleys here (red [VII]: ~1500m; pink [V]: 2500m) were likely not low enough to deter the dispersal of *A. mollis* between mountains. These populations are color coded and assigned roman numerals in Fig 3.5. Specimens were next divided into two elevational bins, **High** (specimens collected above 3500m), and **Low** (specimens collected under 3500m); the 3500m cut was established based on the results from the previous chapter showing a break in cranial morphology in specimens from 3500m upwards. To test the null hypothesis of no significant differences in genetic distances between these two elevational bins or among populations occupying the slopes of single

mountains (color-coded areas in Fig. 3.5 nested within elevational ranges; an analysis of molecular variance (AMOVA; Excoffier et al., 1992) was performed. The pink population (V) was divided in two because it contained individuals crossing the 3500m break point. As a result, I analyzed a total of 9 mountain populations. These analyses were carried out using Arlequin 3.5 (Excoffier and Lischer, 2010).

RESULTS

All of the color-coded mountain populations (I-VIII) are represented by localities along elevational gradients encompassing an average range of 500m - 1000m. Most of the localities were located between ~2000m and ~3000m; two mountain populations (pink [V]; Fig. 3.5 and yellow [VI]) range from ~3000 to ~4000m; one mountain population (blue [IV]) covers from ~1000m to ~2000m; and in only one mountain population were animals collected between 4000m and ~4500m (light blue [I]) (Table 3.1).

The 132 specimens analyzed represented 66 haplotypes from these 8 populations (Fig. 3.5). Phylogenetic trees recovered from the MP, ML, and BI algorithms coincide in basic topology; however, MP bootstrapping shows poor resolution in several branches.

The molecular data show spatial structuring with most of the clades corresponding to mountain populations (Fig. 3.5 and Table 3.1). The tree also shows a basal dichotomy with two well supported clades: 1) all the northern and most of the central mountain populations including some members of the pink (V) mountain population; 2) a clade formed by the light blue mountain population (I) and part of the pink mountain population (V). The first clade has the most highly nested clades with shallow branch lengths and corresponds to northern localities, while the second clade is

made up of deeper lineages and corresponds to central and southern localities in the Peruvian Andes (Fig. 3.5).

Mountain populations color-coded as gray (VIII), red (VII), and orange (III) are made up of well-supported lineages that occupy on average ~2000m to ~3000m elevation. The light blue (I) mountain population is highly differentiated from others and is also well supported. It is made up of individuals collected around ~4000m. Specimens from the dark blue (IV) and green (II) mountain populations form a cluster with moderate support. The dark blue (IV) clade is nested inside the green lineage with moderate support. It comprises individuals inhabiting a gradient of ~1000m (from 1300 to 2400m). Similarly, specimens in the green clade inhabit elevations ranging from ~2000m to 2500m. Individuals from the pink mountain population (IV) sort into three well differentiated lineages: 1) a highly differentiated clade that includes only individuals from around 4000m elevation; 2) a strongly supported group that includes individuals from 2775m and some individuals from around 4000m; 3) a strongly supported group comprising the yellow (VI) mountain population and a few from the pink population, inhabiting ~3500 and ~4000m.

The total length of cyt-b sequenced included 1124 bp. The average number of pairwise differences between populations was greater (60.79) than the average pairwise differences within populations (24.88). Pairwise F_{ST} depicts large and significant population structuring (Table 3.3) with an average F_{ST} of 0.589, (Hartl and Clark, 1997). The highest F_{ST} values came from the pairwise comparisons of the light blue (I) population with the rest of populations, and the lowest F_{ST}, 0.065, came from two populations in close proximity and elevational range, gray (VIII) and blue (IV).

Mantel tests showed no significant correlations between the genetic distance and geographic distance of all the individuals (p=1) (Fig. 3.6), indicating that simple isolation

by distance is not an explanation for the differentiation of the mountain populations of *A. mollis* along the Andes (Fig. 3.6). The same was true for the Mantel test carried out for the Slatkin linearized F_{ST} and the logarithmic geographic distance of each population (p=0.868) (Fig. 3.7).

AMOVA of the molecular variation within and between mountain populations grouped in elevational ranges (higher than 3500m and lower than 3500m) suggested that both within and among-population variation is significant (p=0.01-0.00) and homogeneously partitioned. The variation between groups (mountain populations living higher than 3500m vs. populations living lower than 3500m) represent 36.1% of the total variance; the variation within color-coded mountain populations regardless of elevational grouping represents almost 25% of the total, and the variation among color-coded mountain populations within each elevational grouping (high and low) represent 39.17% of the total (Table 3.2).

DISCUSSION

Maximum likelihood, maximum parsimony, and Bayesian inference phylogenetic analyses recovered a similar set of relationships among mountain populations. Interestingly, the deeper lineages correspond to the southern populations, while the shallower clades were found in the northern populations. This is in accordance with recent reports of an evolutionary pattern of evolution of species in the Peruvian Andes, where deeper lineages are found in Andean southern Peru, and shallower lineages occur progressively northwards (Chaves, et al., 2011; Picard, et al., 2008). This pattern has been found in wild potatoes (*Solanum*) and their nematode parasite (*Globodera pallida*), in another tuber crop (*Oxalis tuberosa*) (Picard, et al., 2008), and also in other vertebrates including two Andean hummingbirds (*Adelomyia* and *Metallura*) (Chaves, et al., 2011; Picard, et al., 2008) and lizards (*Proctoporus*) (Picard, et al., 2008). Indeed, these data

suggest that altitude in the Peruvian Andes was acquired longitudinally from south to north starting at the Central Andean Orocline, slightly south of where our southernmost population is located (Fig. 3.8). Reig (1986), based on areas of major generic richness for Sigmodontinae, suggested that the area of deep cladogenesis for the tribe Akodontini–to which the genus *Akodon* belongs–was also coincident with the southern part of the Central Andean Orocline.

Although our results are in agreement with the abovementioned studies, they are preliminary because they are based on a single gene (cyt-b), which can provide only a single representation of divergence history. A reliable depiction of a species tree requires multiple genes per species (Knowles and Kubatko, 2010), since the species tree is made out of the variance among all gene trees included (Knowles, 2010; Maddison, 1997). Nevertheless, the results presented here represent the first report of cladogenesis within a mammalian species that is partially consistent with this south-to-north diversification of the Peruvian Andes.

Gray (VIII), red (VII), and orange (III) populations each cluster in well-supported clades that consist of montane samples from ~2000 and ~300m elevation on single mountains. The same is also true for the dark blue (IV) clade, which is found in a similar, although lower, elevational range (~1000 to ~2000). Within each mountain population, individuals do not appear to sort by elevation. This is perhaps a result of the limited elevational ranges occupied by this species on each of these northern mountains. That is, it is possible that little or no differentiation has occurred on these slopes because habitats are similar over the range of elevations available. Further, it is probable that populations of mice can be found in lowlands between mountain ranges. Thus, these data do not allow a test of the Ecological Gradients hypothesis, and the limited elevational ranges available suggest that neither Ecological Gradient nor Montane Vicariance hypotheses may be important in the differentiation of northern Peruvian populations of this species.

Members of one pair of high-elevation mountain populations appear to be each other's closest relatives (pink [part of V] and light blue [I]). In the case of the pink mountain population (V), individuals belonging to the high-elevation clade are genetically distinct from individuals downslope from them (above and Fig. 3.5; information on populations downslope from the high elevations on the light blue mountain population [I] is not available). This pattern is that predicted by the Montane Vicariance hypothesis.

The pink (V) population is divided among 3 clades in the molecular phylogeny. One is as a well-supported deep lineage of high elevation individuals (~4000m) linked to the light blue mountain population (I, described above). In a second, individuals from the pink population ranging from ~3000 and ~4000m formed a well-supported clade composed of individuals of the gray (VIII), and red (VII) populations also collected at ~3000 and ~4000m, in accordance with the Montane Vicariance hypothesis. Finally, two members of the pink (V) population are nested inside the well-supported yellow (VI) population. Members of the yellow population were collected between ~3500 and ~4000m. One of the pink (V) population specimens was taken slightly above this range at ~4000m; the other was from somewhat below, at 3100m, therefore this is also consistent with the Montane Vicariance hypothesis.

It appears that the phylogenetic relationships of these mice, however, offer little strong support for either the Ecological Gradients or the Montane Vicariance hypotheses, and neither hypothesis provides a general explanation of the genetic relationships of populations of *Akodon mollis*.   Lack of support for either hypothesis might be due to one or more of several problems. First, it may be an artifact of small sample size, and the fact that few samples from single mountains cover broader elevational ranges. While overall, *A. mollis* has an exceptionally broad elevational range, most mountains produced samples from a range of no more than 1500m, which may not be sufficient for these tests to be meaningful. Second, additional genes should be sampled; the gene tree obtained

with the cyt-b marker may not reveal the species tree of *A. mollis.* Third, despite the suggestion that *A. mollis* is a complex of species, its populations appear to be at best weakly differentiated, at least in the northern two-thirds of its range in Peru. It is possible that differentiation is too recent or too poorly developed for either incipient montane vicariance or evolution along ecological gradients to be detectable.

Southern mountain populations represented by light blue (I) and some individuals from the pink (V) population are well differentiated from northern mountain populations (II-IV, VI-VIII), and some individuals of the pink (V). The yellow mountain population (VI), with its associated two pink individuals, may represent an area of secondary contact of clades that evolved independently in isolation in the northern and southern Andes. In this case, with only two taxa and small samples, the importance of neither the Montane Vicariance nor the Ecological Gradients hypothesis can be tested. The distinctiveness of these taxa and their distributions requires further examination.

Akodon mollis did not shown morphological or molecular differentiation along most of its elevational range. Differentiation was found only at the 3500m of elevation, which is the transition from forest to páramo/puna habitats (Chapter 2). This taxon is enough of a generalist across most of its range of elevations that it is not appropriate for testing these hypotheses. Inclusion of additional taxa and perhaps additional elevational ranges would be necessary to permit testing of the relative importance of Montane Vicariance, Ecological Gradients, or other hypotheses concerning differentiation and speciation of small mammals in this region.

Nevertheless, the absence of an isolation by distance pattern suggests that the distribution of genetic variation might be due to local adaptation acting on populations inhabiting isolated areas, in this case mountains, perhaps related to elevational ranges and the habitats that they encompass. The AMOVA results (Table 3.2) reveal very significant

variance attributed to the high (above 4000m) and low (below 4000m) elevational bins, suggesting that the elevational break encountered at 4000m could be important and that elevation along the Andean mountains had an effect on the differentiation of this taxon. Populations in the High bin (light blue [I], yellow [VI], and pink [V]) are all southern, and the Andes reach greater heights in this part of Peru than they do in the north. Thus it is impossible to separate the effect of elevation and the effect of latitude in the AMOVA.

Significant variation is also observed among populations within high and low groups; as well as within all the mountain populations (Table 3.2). This combined with the fact that *A. mollis* is an animal with low vagility (Alvarado-Serrano, 2005), increases the possibility of its genetic variation being shaped by local adaptation to different environments in the Andes. The Central Andes, where most of this work is being done, is the largest and most mountainous segment of the Andes (Picard et al., 2008) (Fig. 3.8), therefore the most heterogeneous, with marked vegetational ranges along elevations and latitudes, and varying biotic and abiotic factors (e.g. Patterson et al., 1998; Pearson and Pearson Ralph, 1978; Terborgh, 1971) that could be shaping differentiation of the populations of this species.

The effect of geography on the variation of populations can also be more complex, and can be more appropriately tested. In this paper we used simple Euclidean distance to evaluate isolation by distance in populations of *Akodon mollis*; however a more intricate pattern might be revealed by isolation by resistance analysis (McRae, 2006; McRae et al., 2007; see also Coulon, et al., 2004; Zellmer and Knowles, 2009). Such an analysis would add landscape structure to the analysis via the addition of ecogeographical variables of relevance to the study organism. In the case of a diurnal rodent like *A. mollis*, primary productivity of the area, as well as patterns of precipitation and temperature can be added to the analysis. Adding these other factors can shed light on the most important factors and/or mechanisms shaping the evolution of these populations.
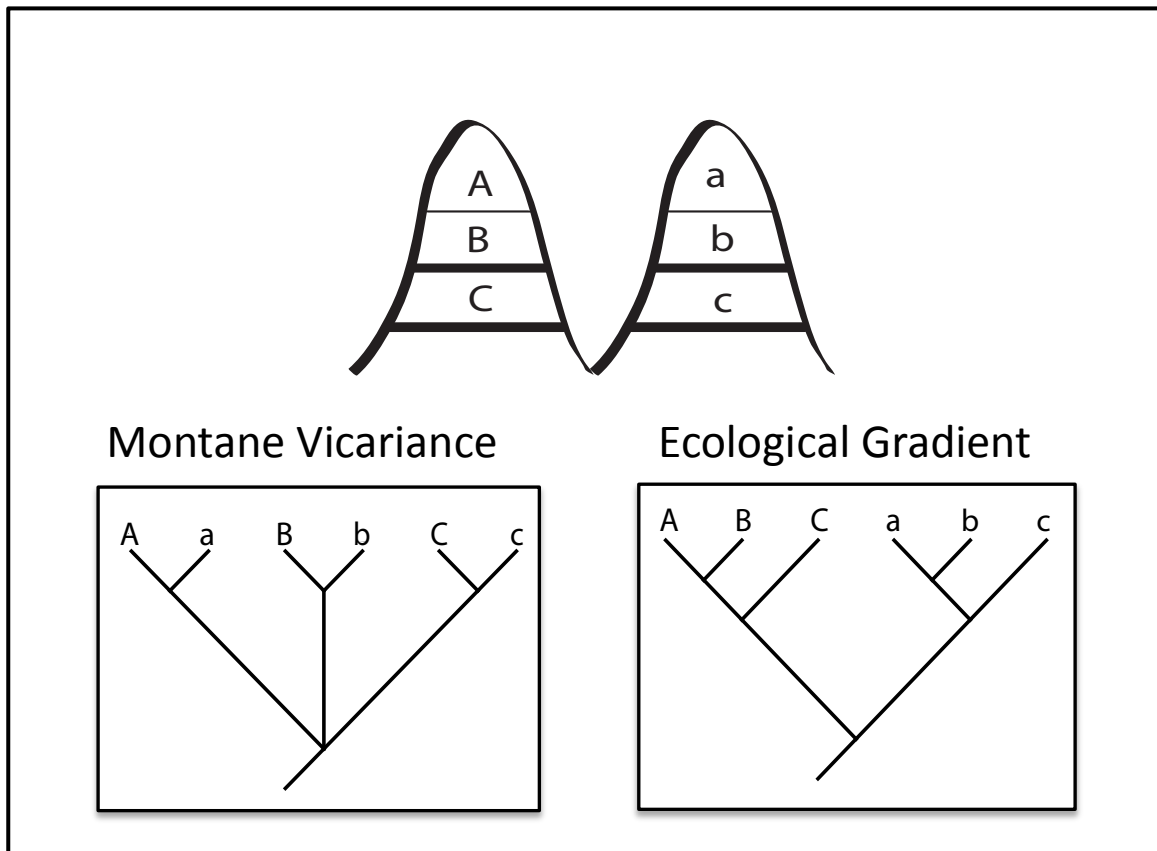
Figure 3.1. Phylogenetic relationships of populations distributed across elevational gradients and in different mountains. Under Montane Vicariance is expected that elevational belts form clades, while under Ecological Gradients is expected that mountains do form clades.
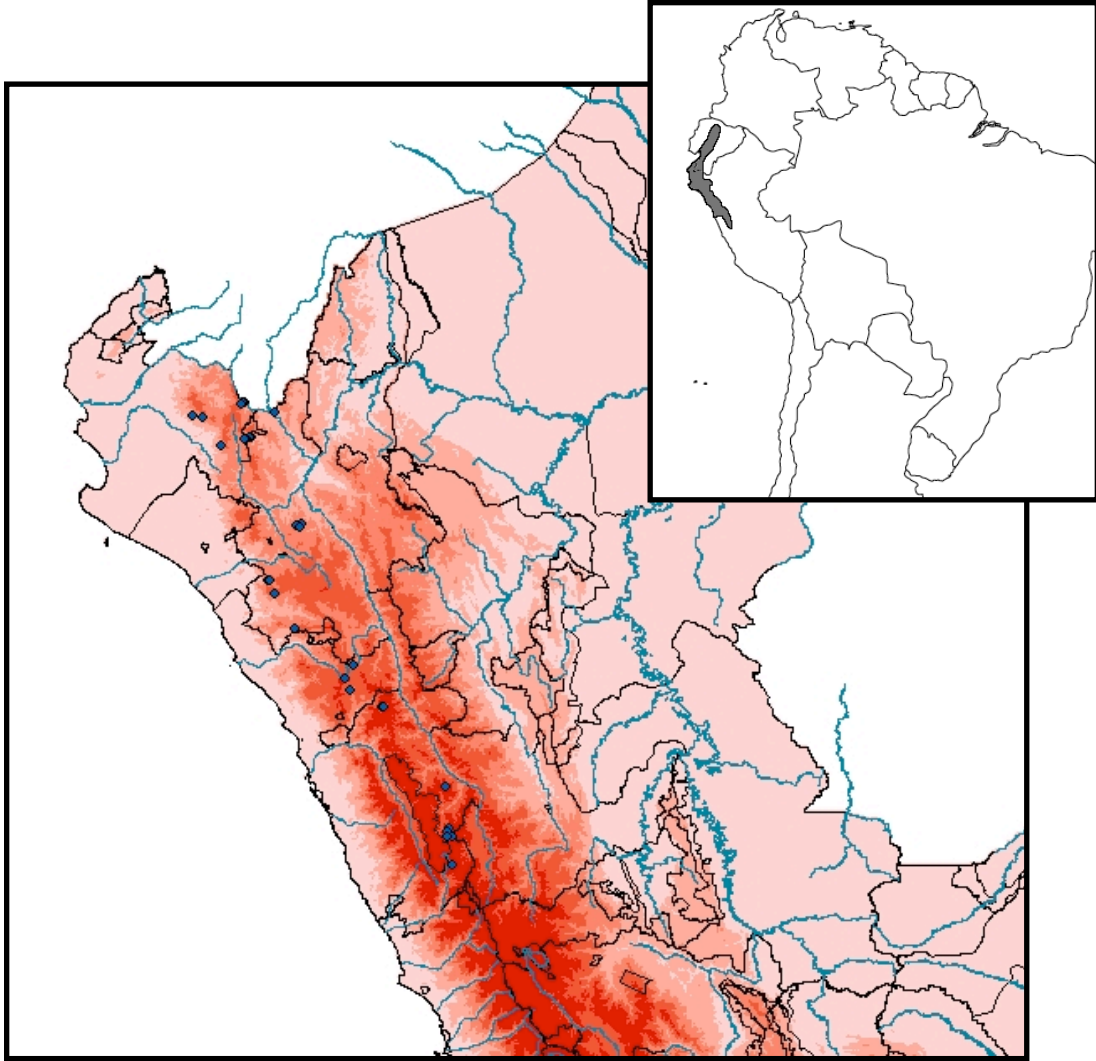
Figure 3.2. Sampled localities (blue dots) for the soft grass mouse *Akodon mollis* (Rodentia: Cricetidae: Sigmodontinae) along the Peruvian Andes. Inset shows the attributed distribution of *A. mollis* in Peru and Ecuador.
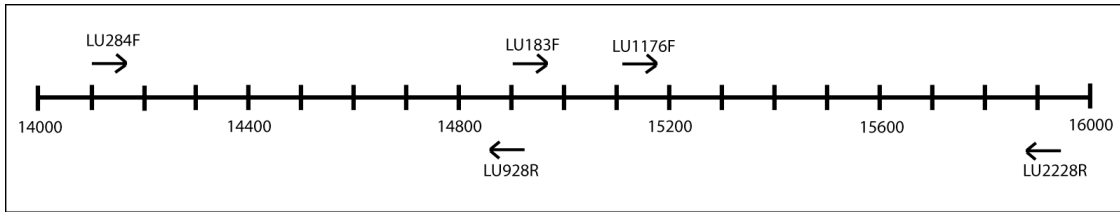
Figure 3.3. Primers used for amplification and sequencing of 1124 bp of the cytochrome b gene in *Akodon mollis.*
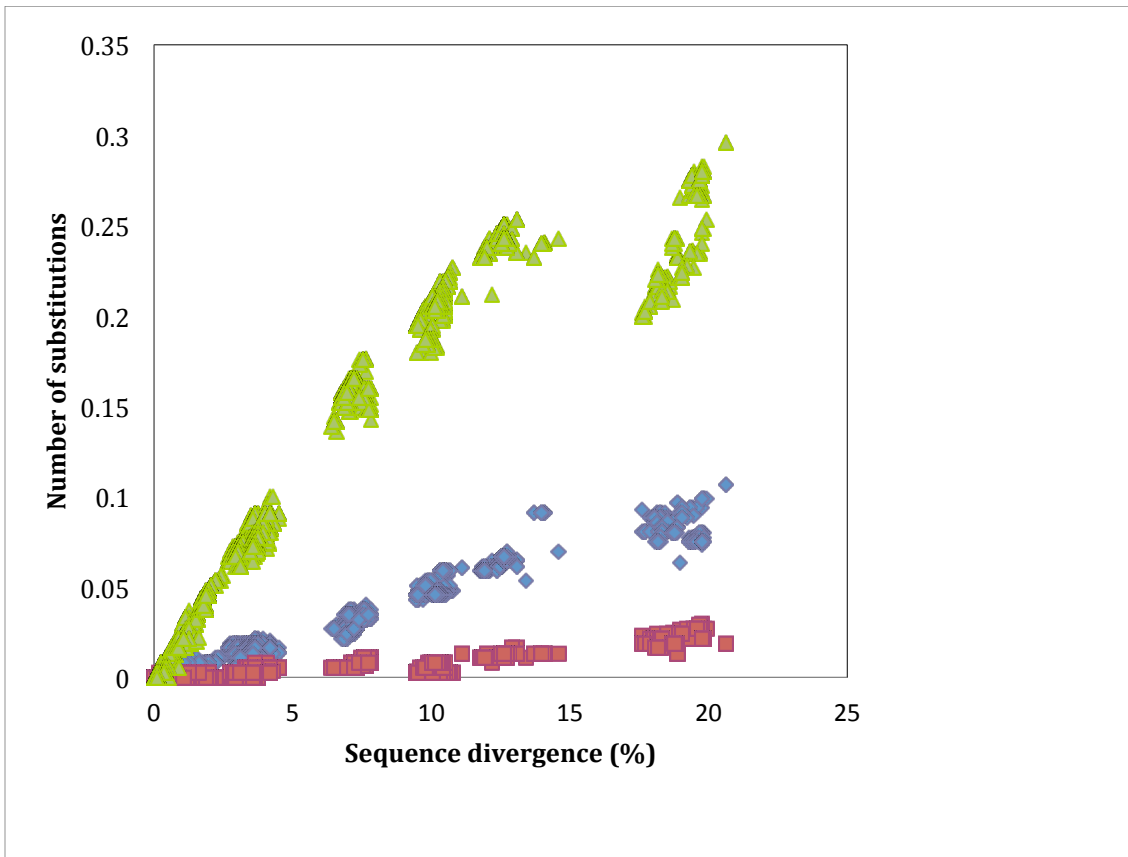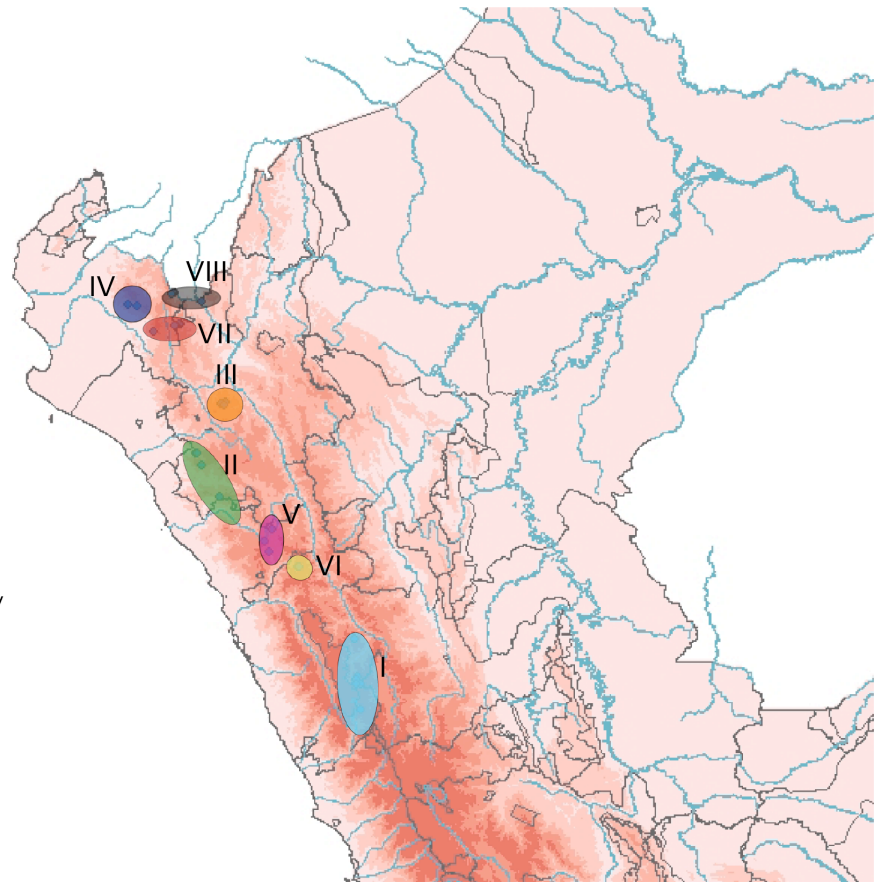

Figure 3.4. Saturation plots for the cytochrome b gene. 1st (rhomboid), 2nd (squares), and 3rd (triangle) codon position.

Figure 3.5. Best maximum–likelihood phylogram for *A. mollis* with corresponding color-coded areas in the Peruvian Andes. Support for nodes are shown as ML bootstrap/Bayesian posterior probability/MP bootstrap (values greater than 50% are presented).
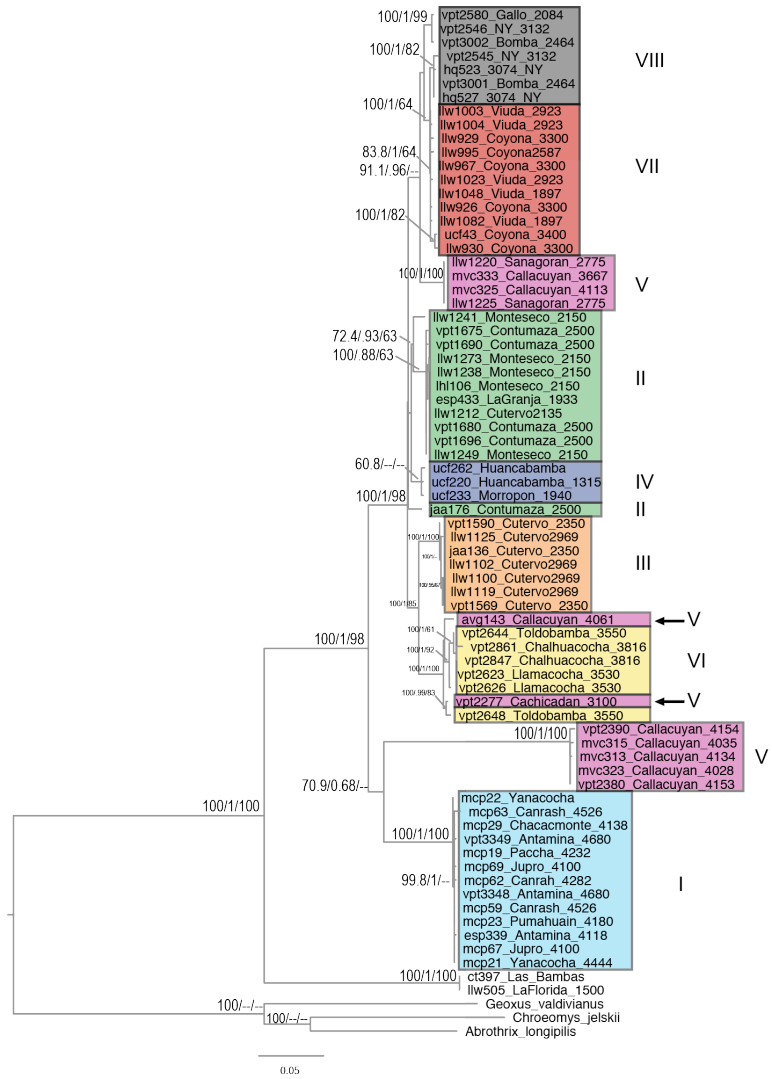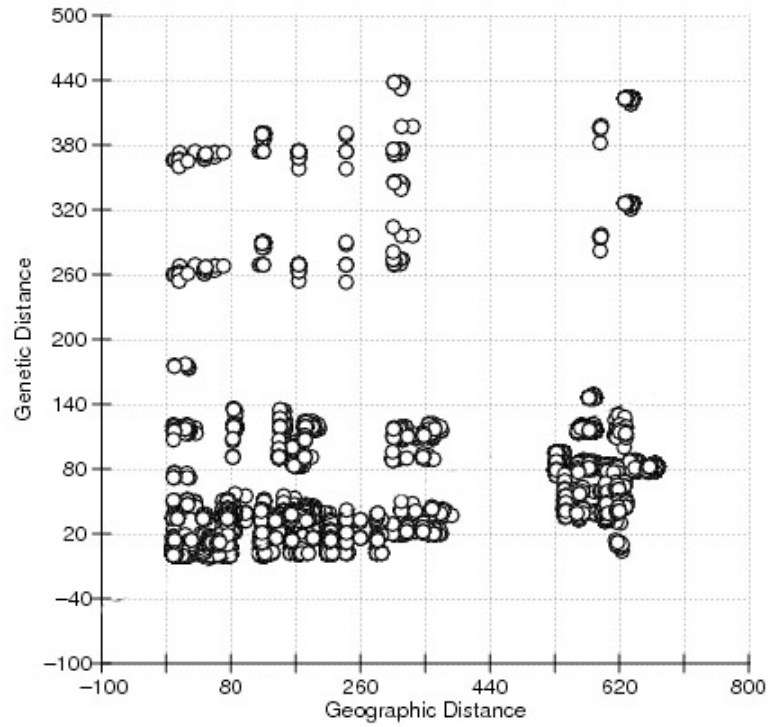
Figure 3.6. Isolation by distance diagram. Nucleotide pairwise differences as genetic distance in the *y* axis and pairwise geographic differences (in km) in the *x* axis. Mantel test: Z = 216791910.0223, r = 0.3626, p=1.
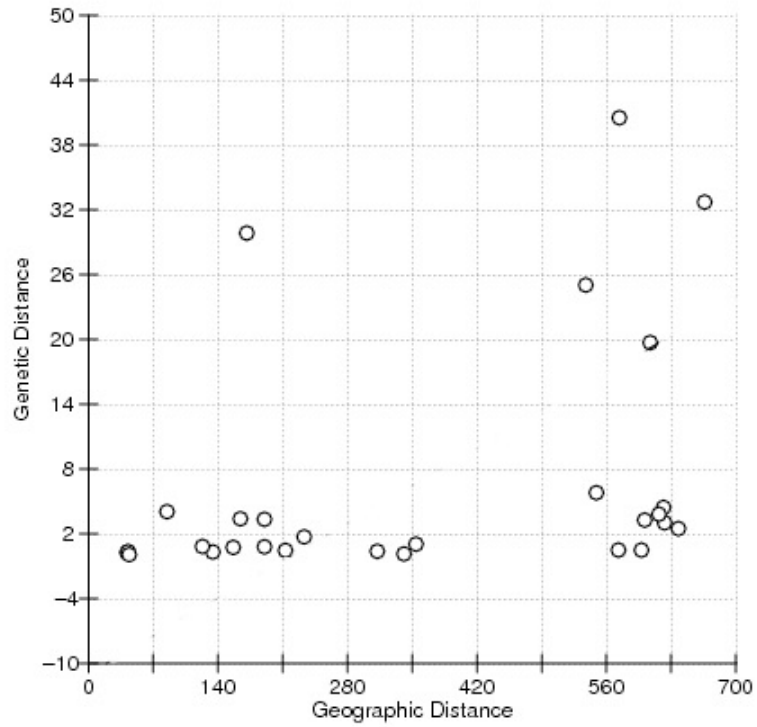
Figure 3.7. Isolation by distance diagram. Slatkin linearized F_{ST} as a function of the logarithmic geographic distance of each population. Mantel test: $Z = 499.1417$, $r = 0.3362$, $p = 0.868$
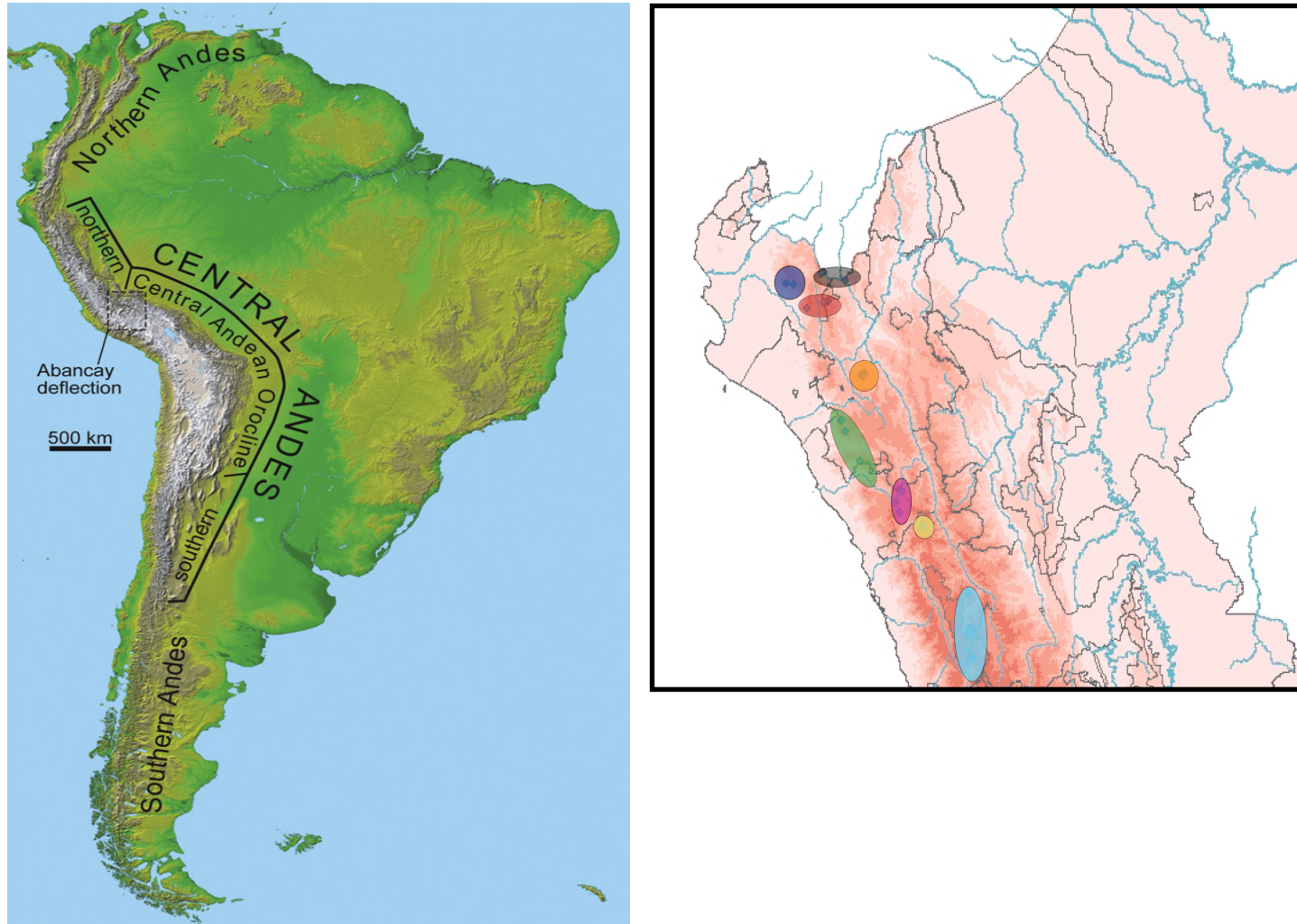
Fig. 3.8. Map of South America with the segmentation of the Andes (Modified from Picard et al., 2008). Note the distribution of *A. mollis* in the inset. This distribution corresponds to the southern Northern Andes, and northern Central Andes.

Table 3.1. Populations of *Akodon mollis.* Assignment based on geographical proximity and close elevational ranges.

Population	Average Northing	Average Easting	Elevational range	Number of specimens
Light blue	8935424.646	269379.4681	4087 -4517m	32
Green	9229010.692	719860.9493	1552 -2754m	26
Orange	9308642.018	748579.8472	2113 -2950m	20
Pink	9128556.211	809940.6842	2710 -4150m	19
Red	9416276.769	688527.2308	2142 -3254m	13
Yellow	9086812.333	189579	3618 -3747m	6
Blue	9429342.395	648984.0956	1300 -2403m	8
Gray	9458686.088	681755.7596	2084 -3132m	8

Table 3.2. Results of analysis of molecular variance (AMOVA) of *Akodon mollis,* by grouping the populations in two groups: **high** >3500m populations, and **low** <3500m populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	*p*-values
Among groups	1	1244.0022	14.7273 Va	36.10	0.01
Among populations within groups	7	1512.112	15.9823 Vb	39.17	0.00
Within populations	23	1240.882	10.0885 Vc	24.73	0.00

Table 3.3. Pairwise F_{ST} values for the populations depicted in Figure 3.5.

	Light blue	Green	Orange	Pink	Red	Yellow	Blue	Gray
Light blue								
Green	0.962							
Orange	0.952	0.802						
Pink	0.976	0.241	0.770					
Red	0.713	0.443	0.452	0.275				
Yellow	0.968	0.853	0.766	0.816	0.329			
Blue	0.751	0.317	0.425	0.121	0.214	0.331		
Gray	0.970	0.631	0.772	0.509	0.260	0.792	0.065	

Appendix 3.1. Specimens used in the analyses, 132 as listed below. Geographical coordinates and elevations were recovered from collector's notes. Abbreviations correspond to names of collectors as in field and catalog notes.

ANCASH: *Huari*, Canrash, 4517m, -9.676042; -77.05375 (mcp 59, mcp 62, mcp 63); *Huari*, Chacacmonte, 4087m, -9.684715; -77.105255 (mcp 29); *Huari*, Jupro, 4102m, -9.588604; -77.084414 (mcp 67, mcp 68, mcp 69, mcp 70, mcp 71, mcp 74); *Huari*, San Marcos, Juprog, 4109m, -9.56824; -77.07592 (vpt 3338, vpt 3341, vpt 3347, vpt 3348, vpt 3349, esp 339); *Huari*, Paccha, 4211m, -9.63179; -77.119506 (mcp 04, mcp 05, mcp 09, mcp 11, mcp 15, mcp 18, mcp 19, mcp 20); *Huari*, Pumahuain, 4180m, -9.663556; -77.130111 (mcp 23, mcp 24, mcp 25, mcp 26); *Huari*, Yanacocha, 4344m, -9.655458; -77.134007 (mcp 22, mcp 27, mcp 28); *Huari*, Yanacocha, 4400m, -9.652222; -77.135-77.134007 (mcp 21).

CAJAMARCA: *Contumaza*, Bosque Cachil, entre Cascas y Contumaza, 2754 m, -7.38925; -78.78188 (jaa 178, vpt 1673, vpt 1674, vpt 1675, vpt 1680, vpt 1690, vpt 1696); *Cutervo*, San Andrés de Cutervo, 2113m, -6.235533; -78.71885 (llw 1197, llw 1212); *Cutervo*, San Andrés de Cutervo, Cutervo National Park, 100m over El Tragadero, 2950m, -6.249972; -78.766528 (llw 1085, llw 1086, llw 1088, llw 1093, llw 1094, llw 1095, llw 1098, llw 1100, llw 1101, llw 1102, llw 1119, llw 1122, llw 1125, llw 1136); *Cutervo*, San Andrés de Cutervo, 4 km W San Andrés de Cutervo, 2424m, -6.25674; -78.7246 (jaa, 136, vpt 1569, vpt 1586, vpt 1590); *San Ignacio*, Tabaconas, Piedra Cueva in Cerro Coyona (Tabaconas-Namballe National Sanctuary), 3254m, -5.268429; -79.269934 (llw 926, llw 929, llw 930, llw 946, llw 967, llw976); *San Ignacio*, Tabaconas, Piedra Cueva in Cerro Coyona (Tabaconas-Namballe National Sanctuary), 2607m, -5.279798; -79.273998 (llw 995); *San Ignacio*, Tabaconas, Cerro La Viuda (Tabaconas-Namballe National Sanctuary' Buffer Zone), 2880m, -5.290794; -79.336961 (llw 1003, llw 1004, llw 1013, llw 1023); *San Ignacio*, Tabaconas, Cerro La Viuda (Tabaconas-Namballe National Sanctuary' Buffer Zone), 2142m, -5.284432; -79.321697 (llw 1048, llw 1082); *Santa Cruz*, 2 km E Monteseco, 1838m, -6.847342; -79.088503 (lhl 90, lhl 92, lhl 93, lhl 98, lhl 106, lhl 116, llw 1238, llw 1241, llw 1248, llw 1249, llw 1273, llw 1274, llw 1279, rco 10035,vpt 1636, vpt 1655, vpt 1656, vpt 1664).

LA LIBERTAD: *Sánchez Carrión*, Sanagorán, 2732m, -7.792068; -78.138445 (lhl 83, lhl 84, llw 1220, llw 1223, llw 1225, llw 1226, llw 1232); *Sánchez Carrión*, Sanagorán, 2500m, -7.783123; -78.146387 (vpt 2251, vpt 2263); *Santiago de Chuco*, Campamento Callacuyán, Quebrada Quishuara Sur, 3968m, -7.938683; -78.23468 (avg 143); *Santiago de Chuco*, Campamento Callacuyán, 3997m, -7.95126; -78.237412 (mvc 313, mvc 323); *Santiago de Chuco*, Campamento Callacuyán, 4061m, -7.925205; -78.240462 (mvc 315); *Santiago de Chuco*, Campamento Callacuyán, 4063m, -7.921906; -78.249845 (mvc 325, 326); *Santiago de Chuco*, Campamento Callacuyán, 3765m, -7.920278; -78.208885 (mvc 333); *Santiago de Chuco*, Campamento Callacuyán, 4150m, -7.952933; -78.252512 (vpt

2380, vpt 2390); *Santiago de Chuco,* Campamento Cachicadán, 2823m, -8.064632; -78.17321 (vpt 2277).

PIURA: *Huancabamba*, Canchaque, Agua Azul, 1300m, -5.35678; -79.60106 (ucf 220, ccn 65); *Huancabamba*, Huaricancha, 1987m, -5.315797; -79.411568 (uf 262); *Huancabamba*, El Carmen de la Frontera, Carmen de la Frontera, Alto Samaniego, 2403m, -5.11017; -79.35269 (ucf 43); *Morropón*, Chalaco, 2199m, -5.04272; -79.78925 (ucf 211, ccn 60); *Morropón*, Portachuelo, 1937m, -5.02561; -79.90633 (ucf 233); *Huancabamba*, Cuenca del Río Blanco, Campamento New York, 2923m, -4.902729; -79.373163 (hq 523, hq 527, vpt 2545, vpt 2546); *Huancabamba*, Minera Majaz, Campamento Bomba Quemada, 2288m, -4.891769; -79.354599 (vpt 3001, vpt 3002); *Huancabamba*, Cuenca del Río Blanco, Campamento Quebrada del Gallo, ~2200m, -4.884012; -79.342632 (vpt 2580, vpt 2583)

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