

**The evolutionary assembly of a species rich biota:
Avian speciation and differentiation in the Andean cloud forests**

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Introduction — Understanding the patterns and processes generating biodiversity is a central goal of systematics and evolutionary biology. One outstanding biodiversity hotspot is the cloud forest biome of the tropical Andes in northern South America¹. The long strip of humid forest that stretches from Venezuela to Bolivia sustain one of the richest assemblages of vascular plants and birds in the world². The few evolutionary studies of the rich Andean avifauna, in particular, have focused on characterizing major distribution patterns and phenotypic variation³⁻⁶. For example, it has been shown that the long and narrow geographic ranges of widespread birds along the humid Andes are fragmented by two types of barriers: intervening low valleys (e.g., Marañón valley in northern Peru, Táchira Depression in western Venezuela) and inhospitable highlands along ridgelines (e.g. páramos and snow peaks, see Fig. 1). Indeed, range discontinuity and the narrow elevational breadth of many Andean forest birds have promoted dramatic geographic variation in morphology. The diversification of Andean birds was also likely influenced by the altitudinal shifts of montane forests during the Pleistocene glacial cycles. Such cycles are postulated to have facilitated the separation, differentiation, and expansion of avian populations across barriers, but this hypothesis remains largely untested⁷.

Recent molecular-based phylogenetic studies have begun to validate the traditional view of the Andean avifauna as a collage of species stemming from different biogeographic origins. For instance, *Myadestes ralloides* (Turdidae)⁸, *Myioborus* spp. (Parulidae)⁹, and *Buarremon* (Emberizidae)¹⁰ originated from North and Central American ancestors, whereas many Andean hummingbird lineages (Trochilidae)¹¹ and *Thamnophilus* antshrikes (Thamnophilidae)¹² are derived lowland South American groups. Higher levels of phenotypic variation with increasing elevation has been interpreted to indicate that speciation is more rapid at higher elevations⁶. In a review of species-level molecular phylogenies, diversification rates in the mountains appeared to be relatively uniform in comparison to the declining rates of lowland bird lineages, suggesting that ongoing speciation is a key process in the building of montane avifaunas¹³. Taken together, the results suggest several patterns of population divergence, but we still lack a comprehensive phylogeographic framework and rigorous test of historical diversification processes in the tropical Andes^{7,13,14}.

How does speciation proceed in the Andean cloud forests? Is it geographically and chronologically coincident across species of similar biogeographic origin (South American vs. North American)? What is the role of barriers, elevation, and Pleistocene habitat shifts in population divergence? Here I propose a comparative approach to address these fundamental questions.

Hypotheses — First, I will test the hypothesis that multiple bird lineages share a similar geographic pattern of population structure, but they may exhibit contrasting timing of population splits according to their biogeographical origin. I predict that geographic correspondence of major phylogroups will be observed across species but that the topology (i.e., the geographic location of basal and terminal phylogroups) will consistently vary between species groups of Central American or South American origin. Second, I will test the hypothesis that the amount of genetic divergence between populations on either side of a barrier to gene flow will depend on the elevational range of the species. I predict that, overall, divergence time between

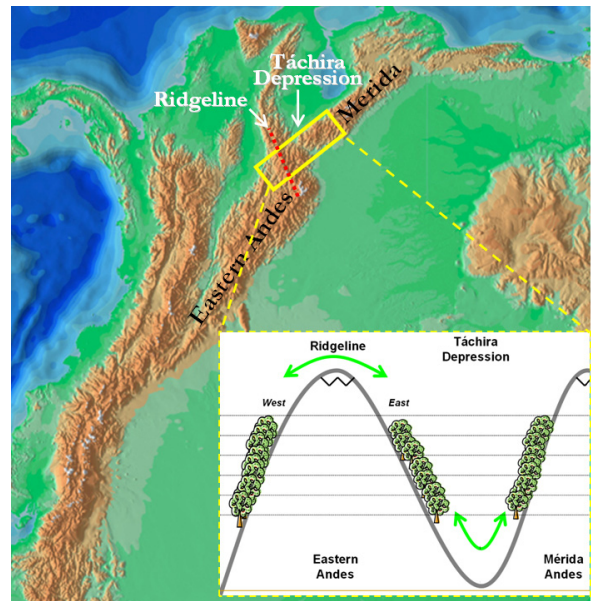


Fig.1. Northern South America showing the Andes in Ecuador, Colombia and Venezuela. The yellow box shows the location of the proposed transect crossing two barriers: the ridgeline of the Eastern Andes and (2) the Táchira Depression. The lower panel shows a schematic profile of the transect and the five altitudinal belts that will be sampled.

populations on either side of valley barriers will correlate positively with elevation, so that upper montane forest species will be more divergent than lower montane forest species.

Objectives — The objectives of this study are: 1) to build a comparative phylogeographic data set composed of mtDNA sequences for 25 species of widespread birds, encompassing a diverse array of taxonomic groups and elevational zones; and 2) to compare levels of population genetic structure, gene flow, and divergence times based on multilocus nuclear markers, between sister populations on either side of barriers.

Methods: 1. Comparative phylogeography — I have selected 25 widespread species with an attempt to balance the following biological criteria: taxonomic distribution (non-passerines, suboscine and oscine passerines), elevational range (lower montane, upper montane; narrow and broad elevational distributions), and biogeographic pattern (exclusively Andean, or also ranging into lowland South America, the Tepuis, or Central American mountains). Practical issues were considered such as the geographic coverage currently represented in tissue collections.

As a starting point, the LSU Museum of Natural Science holds the largest collection of vouchered tissue samples of Neotropical birds in the world, largely collected in the mountains of Bolivia, Peru, Ecuador, Panama, and other regions of the Andes and Central America. I will complete sampling by requesting tissue grants from other institutions and by conducting fieldwork to fill existing collecting gaps. These are most prominent in the Colombian and Venezuelan Andes, where patterns are expected to be complex due to the further subdivision of the cordillera into independent parallel ranges, and the location of the two barrier types within very close proximity. I will concentrate my collecting effort along a transect crossing the Táchira Depression and the ridgeline of the Eastern Andes (Fig. 1).

DNA will be extracted from pectoral muscle using standard protocols. For the comparative phylogeography, I will sequence the mitochondrial markers ND2 and *Cyt-b* (~2050 bp mtDNA) using primers that have reliably worked across diverse avian taxa¹⁵⁻¹⁸. For individual phylogeographies, Maximum Likelihood and Bayesian analyses will be carried out using PAUP 4.0¹⁹ and MrBayes 3.1²⁰, respectively, after selecting the model of molecular evolution that fit best the data using the BIC criterion implemented in ModelTest²¹. To compare phylogeographic patterns across species in relation to the alternative biogeographical scenarios, I will follow the statistical rationale described by Richards et. al.²², in which external information is used to generate realistic phylogeographic hypothesis *a priori*. I will test for molecular clock assumptions to estimate the timing of historical events (the sequence of population differentiations) using likelihood ratio tests. PAUP will be used to obtain branch lengths and apply a rate of 1.6-2.0% divergence per million years to date nodes²³; however, a relaxed molecular clock calibration will be explored to account for potential rate variation across taxonomic groups¹⁴.

2. Role of barriers and glacial cycles in population divergence — The phylogeographies produced will ultimately inform the selection of species for the coalescent analyses across barriers. Five target species will be selected, which should ideally have sister populations across the Táchira Depression or the ridgeline of the Eastern Andes, and occupy different elevational zones. The collecting transect across these two barriers will encompass five elevational points per slope, for a total of 15 localities. To estimate population genetic parameters across barriers and along elevation, I will sequence 10 independently segregating nuclear loci (to be selected) for ten individuals per elevational zone and slope. However, I will explore the results from simulation analyses in Mesquite²⁴ using time data from the geological literature²⁵ to get an idea of the statistical power and the behavior of the coalescent process between populations with different levels of connection across a barrier at a given time (as expected for populations of different elevational zones during the last glacial maximum). To estimate time since separation and the extent of post-separation gene flow between populations across barriers, I will analyze the multilocus sequence data to fit a population genetic model of divergence with gene flow. To do so, I will employ the Metropolis-coupled Markov Chain Monte Carlo simulations implemented in the coalescent-based IMA program²⁶. Regression models will be run to evaluate the relationship between elevation and genetic divergence and gene flow in Andean birds.

Significance — Elucidating how diversity arises and how biotas have been historically assembled is a fundamental goal in evolutionary biology. Understanding the patterns and processes of population

divergence acting upon a wide array of lineages would provide an improved conceptual framework that informs different research areas of biology, including ecology, behavior, taxonomy, and conservation. This empirical comparative study, therefore, also has the potential to take the lead in the study of the mechanisms governing population differentiation in montane systems. This type of research is particularly important in the Neotropics, where alpha diversity of birds remains weakly documented. Northern South America has a rich and complex biota composed of lineages with multiple biogeographic affinities³ as well as a rich geologic and climatic history^{27,28}. However, only a handful of phylogenetic and phylogeographic studies have included samples from Colombia and Venezuela^{9,10}; therefore, dense geographic sampling in these two countries will likely produce new insights.

The proposed research will have a broader impact in terms of my development as a systematist and evolutionary biologist, and with the establishment of collaborations with other scientists and students. First, fieldwork in South America will involve the participation of undergraduate students, who will receive training in field techniques and participate in general discussions of the concepts and methods of this project. Second, my interactions with established scientists in Colombia and Venezuela will strengthen my long-term research program in the region while contributing to ornithological skin and tissue collections.

Schedule — The proposed research represents the core of my dissertation research on avian speciation in the Andes. This is my second year of my doctoral studies at Louisiana State University, but during the last year I have acquired ND2 sequence data for ca. 600 individuals of 15 of the study species. The process for research permits is in its final stages of approval. I am planning a single, intensive fieldwork season during the fall 2009, that will consist of 10-day collecting trips to 15 localities in Colombia and Venezuela (along the transect described above). Sequencing for the comparative phylogeography will be completed by the summer 2009, whereas for the barrier analysis will be completed in early 2010. Papers will be submitted for publication between 2009 and early 2010.

Literature cited

1. Hawkins, B.A., et al. 2007. *Am. Nat.* 170:S16-S27.
2. Stotz, D.F., et al. 1996. *Neotropical birds: Ecology and conservation*, Chicago University Press.
3. Chapman, F.M. 1917. *Bull. Amer. Mus. Nat. His.* 36:1-728.
4. Terborgh, J. 1977. *Ecology* 58:1007-1019.
5. Remsen, J.V. 1984. *Science* 224:171-173.
6. Graves, G.R. 1985. *Auk* 102:556-579.
7. Price, T. 2007. *Speciation in Birds*, Roberts and Company.
8. Miller, M.J., et al. 2007. *Auk* 124:868-885.
9. Pérez-Eman, J.L. 2005. *Mol. Phy. Evol.* 37:511-528.
10. Cadena, C.D., et al. 2007. *Mol. Phy. Evol.* 44:993-1016.
11. McGuire, J.A., et al. 2007. *Syst. Bio.* 56:837-856.
12. Brumfield, R.T., & S.V. Edwards. 2007. *Evolution* 61:346-367.
13. Weir, J.T. 2006. *Evolution* 60:842-855.
14. Ribas, C.C., et al. 2007. *Proc. Roy. Soc. B* 274:2399-2408.
15. Hackett, S.J. 1996. *Mol. Phy. Evol.* 5:368-382.
16. Johnson, K.P., & M.D. Sorenson. 1998. *Mol. Phy. Evol.* 10:82-94.
17. Kocher, T.D., et al. 1989. *PNAS* 86:6196-6200.
18. Helm-Bychowski, K., & J. Cracraft. 1993. *Mol. Biol. Evol.* 10:1196-1214.
19. Swofford, D.L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony*, Sinauer Associates.
20. Huelsenbeck, J.P., & F. Ronquist. 2001. *Bioinformatics* 17:754-755.
21. Posada, D., & K.A. Crandall. 1998. *Bioinformatics* 14:817-818.
22. Richards, C.L., et al. 2007. *J. Biogeog.* 34:1833-1845.
23. Lovette, I.J. 2004. *Auk* 121:1-6.
24. Maddison, W.P., & D.R. Maddison. 2007. <http://mesquiteproject.org/>
25. Hooghiemstra, H., & T. Van der Hammen. 2004. *Phil. Trans. R. Soc. B* 359:173-181.
26. Hey, J., & R. Nielsen. 2007. *PNAS* 104:2785-2790.
27. Coates, A.G., et al. 2004. *Geo. Soc. Amer. Bull.* 116:1327-1344.
28. Nores, M. 2004. *Glob. Ecol. Biogeog.* 13:149-161.

Budget and Justification

<i>BUDGET</i>	<i>\$ amount</i>
Ground transportation	650
Subsistence and lodging	1,500
Total	2,150
Total funding requested	\$2,000

This proposal requests a total of \$2,000 to cover the costs of subsistence for me and two field assistants (undergraduate students), and to help defray the costs of ground transportation. At an estimated rate of \$100 per locality, \$1,500 is solicited for field subsistence and lodging. Funding for ground transportation has been largely secured, except for \$650 that is the estimated cost of transportation by public trucks in Venezuela, of which \$500 is solicited here.

Lab materials, reagents, sequencing, and computing facilities used so far have been supplied by the LSU Museum of Natural Science and the Brumfield Lab.