

ABSTRACT: Taking advantage of the large-scale genetic library generated by the Barcode of Life project, we studied COI adaptation to high altitude in American birds. Almost 22,804 COI sequences from around 2,000 avian species from the American continent were downloaded from BOLD. Using a complete phylogeny of the birds of the World we classified over 150 pairs of sister species into highland-lowland, highland-highland and lowland-lowland species pairs to compare their COI sequences. We found a tendency towards more amino acid substitutions when at least one of the species from the pair was a highland species and verified that the change in the amino acid/s occur more frequently in the highland species of the pair. We also analyzed the amino acids that did differ between the sister species pairs to assess their position and whether their properties differed and could therefore affect the protein structure and function. This is the first large-scale analysis of mitochondrial adaptation to high altitude in any taxonomic group. Results indicate that there is not a generalized adaptation of COI to hypoxic highlands in birds, but suggest that mutations in COI tend to be fixed more frequently in highland than in lowland species. This study constitutes an example of how DNA Barcode libraries allow diverse large-scale analyses beyond species identification and discovery.

INTRODUCTION

Several bird species live -and fly- in hypoxic sites (over 2,000 m above sea level, masl), which requires morphological and physiological adaptations. Previous studies have focused almost exclusively on haemoglobin adaptation, revealing that highland birds have a higher concentration of the protein and an increased affinity to oxygen due to amino acid substitutions (1), and that there is convergent evolution among highland species in these adaptations to altitude (2, 3). Although mitochondrial genes participate in the cellular respiratory process, their adaptive role to high altitude has been much less studied.

Cytochrome c oxidase subunit I (COI) is part of the protein complex that participates in the electron transport chain and could potentially present adaptive modifications in highland species. Previous studies showed that non-synonymous mutations in COI were found in highland insects (4) and mammals (5), but mutations that improve enzymatic kinetics of the cytochrome c oxidase complex in birds (geese) are not in the subunit I of the protein complex (6). In this context the objective of this study is to use the large-scale COI library available as a result of the Barcode of Life project to study its adaptation to high altitude in the birds of the Americas.

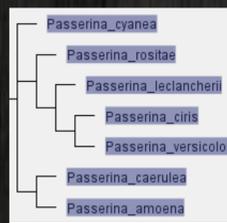
MATERIALS AND METHODS



- Over 22,000 COI sequences of at least 600 bp from 2,000 American birds were downloaded from BOLD SYSTEMS
- The maximum and minimum altitudes in which it can be found were determined for each of the 2,000 bird species (7)
- Sister species pairs were established using the phylogeny of the birds of the World by Jetz et al. (8) and Hackett's phylogeny backbone
- We considered as highland bird species those with a maximum altitude exceeding 2,500 masl and as lowland those with a maximum altitude below 1,500 masl
- Sister species pairs were therefore classified into three groups: highland-highland species, highland-lowland species and lowland-lowland species (Fig. 1)



Highland-Lowland



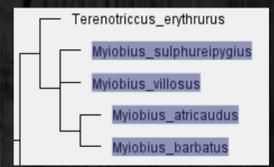
P. caerulea 0-1100 masl *P. amoena* 2000-3000 masl

Highland-Highland



M. frontalis 1800-4300 masl *M. capistratus* 500-4700 masl

Lowland-Lowland



M. atricaudus 0-1400 masl *M. barbatus* 600-1300 masl

FIGURE 1: Establishing sister species pairs and their categories (highland-lowland, highland-highland, and lowland-lowland species pairs).



- COI sequences from the three groups were aligned and analyzed in MEGA
- Uncorrected (p) and K2P genetic distances were estimated for each sister species pair and then used to relativize the number of amino acid substitutions between species



- The position of the amino acid substitutions on the COI secondary structure was determined following Kerr's consensus (9)
- The impact of each amino acid substitution was determined in relation to the presence of a change in its polarity, charge or water interaction.

RESULTS

Highland-Lowland

45 Sister pairs 547 Sequences

Highland-Highland

36 Sister pairs 538 Sequences

Lowland-Lowland

74 Sister pairs 1215 Sequences

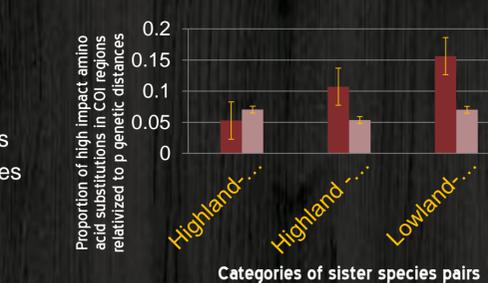
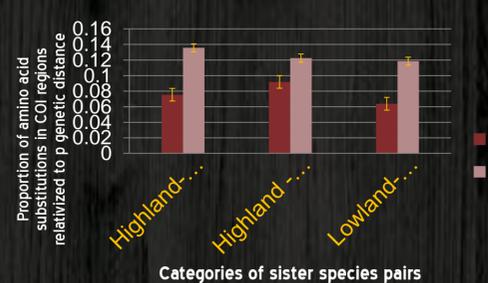
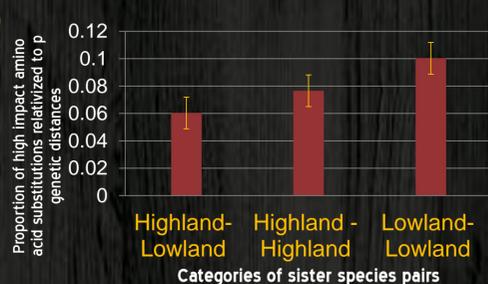
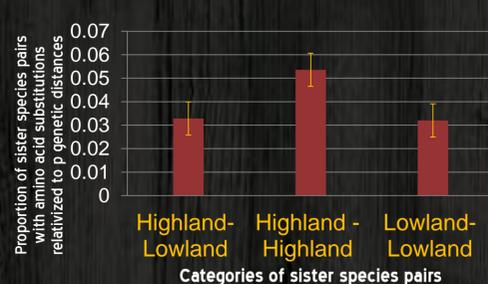
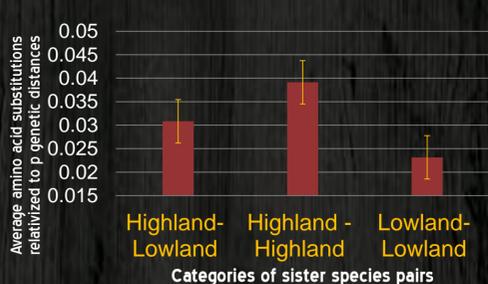


FIGURE 2: Amino acid substitutions among the three categories of species pairs: a) average amino acid differences between sister species; b) proportion of sister species pairs with amino acid substitutions; c) proportion of high impact amino acid substitutions between sister species pairs; d) proportion of amino acid substitutions in COI loops and helices; e) proportion of high impact amino acid substitutions in COI loops and helices.



When Highland-Lowland sister species pairs had differences in their amino acids, substitutions occurred usually in the Highland species



Amino acid differences occurred in the same proportion in all the orders in which they were present

CONCLUSIONS AND FUTURE PROSPECTS

- There is no evidence of a generalized adaptation of avian COI to highlands, but there is a tendency towards more amino acid substitutions in COI when at least one of the species from the sister pair is from the highlands and the change occurred in most cases in the highland species.
- High impact amino acid substitutions, however, did not appear more frequently in highland species. We are currently studying amino acid substitutions and their impact in more detail.
- We will also analyze convergence among high altitude species in COI structure as a consequence of amino acid substitutions and assess intraspecific variation between highland and lowland populations.

REFERENCES

- (1) Scott, G. R. (2011). Elevated performance: the unique physiology of birds that fly at high altitudes. *J. Exp. Biol.* 24, 2455-2452. (2) McCracken, K. G., Barger, C. P., Bulgarella, M., Johnson, K. P., Kuhner, M. K., Moore, A. V., Peters, J. L., Trucco, J., Valqui, T. H., Winker, K. and Wilson, R. E. (2009). Signatures of high-altitude adaptation hemoglobin of five species of Andean dabbling ducks. *Am. Nat.* 174, 631-650. (3) McCracken, K. G., Barger, C. P., Bulgarella, M., Sonsthagen, S. A., Trucco, J., Valqui, T. H., Wilson, R. E., Winker, K. and Sorenson, M. D. (2009). Parallel evolution in the major haemoglobin genes of eight species of Andean waterfowl. *Mol. Ecol.* 18, 3992-4005. (4) Zhang, Z.-Y., Chen, B., Zhao, D.-J. and Kang, L. (2013). Functional modulation of mitochondrial cytochrome c oxidase underlies adaptation to high-altitude hypoxia in a Tibetan migratory locust. *Proc. R. Soc. B.* 280, 20122758. (5) Luo, Y., Gao, W., Gao, Y., Tang, S., Huang, Q., Tan, X., Chen, J. and Huang, T. (2008). Mitochondrial genome analysis of *Ochotona curzoniae* and implication of cytochrome c oxidase in hypoxic adaptation. *MDN*. 352-357. (6) Scott, G. R., Schulte, P. M., Egginton, S., Scott, A. L. M., Richards, J. G. and Milsom, W. K. (2011). Molecular evolution of cytochrome c oxidase underlies high-altitude adaptation in bar-headed goose. *Mol. Biol. Evol.* 28, 351-363. (7) Del Hoyo, J., Elliott, A., Sargatal, J. and Christie, D. A. (1992-2013). Handbook of the birds of the world. *Lynx edicions*. (8) Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. and Moers, A. O. (2012). The global diversity of birds in space and time. *Nat.* 491, 444-448. (9) Kerr, K. C. R. (2011). Searching for evidence of selection in avian DNA barcodes. *Mol. Ecol.* 11, 1045-1055. *Images:* Handbook of the birds of the world *Alive*.