

Human and Hummingbird Hemoglobin Concentrations and Metabolic Rhythms at Altitude Determined with Statistical Modeling

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Abstract

Hummingbirds show remarkable adaptation to high altitude hypoxia whereas humans are imperfectly adapted to high altitude living.

Here we compare hemoglobin levels and metabolism derived from growth rhythms in hummingbirds and humans. To compare growth rhythms, we analyzed growth intervals in hummingbird tail feathers and human growing tissues such as hair.

We find that hemoglobin levels were higher in hummingbirds ($P < 0.001$) than in humans, but the influence of altitude on hemoglobin was more pronounced in humans (slope, steeper with increasing altitude, $P < 0.001$), and levels for both taxa converge at extreme elevations.

The power spectra from growth intervals in growing tissues which reflect metabolism in both species, were not different (low frequency/high frequency ratios (LF/HF) in the two species) $P > 0.22$ NS.

In a comparison among hummingbird species, we found no evidence that metabolic demands (based on power spectra derived from growth intervals) changed with increasing altitude, even while body mass increased significantly ($P > 0.02$). Our index of hummingbird metabolism (spectral LF/HF ratio) was consistent with estimates based on allometric conversion of mass for humans.

These results support the notion that hummingbird hemoglobin levels and metabolism are useful models for biologically adaptive strategies to life at high altitude. Humans and hummingbirds exhibit convergent phenotypes for hemoglobin concentration at extreme altitudes. However, whereas human health suffers above 2500 m, hummingbirds are evolutionarily successful and physiologically robust at very high altitudes. Such different outcomes may be in part due to ancient versus recent high altitude colonization, but may also reflect greater altitude-specialization of hummingbird species, fundamental differences between avian and mammalian respiratory systems, or the very different demands of thermoregulation in hummingbirds versus humans.

Keywords: Hemoglobin; Human; Hummingbirds; Evolution; Thermoregulation; Statistical modeling; High altitude

Introduction

Hummingbirds show remarkable adaptations to mountainous environments in the Americas. Although the hummingbird clade originated in the lowlands, some species are resident up to ~5000 m McGuire et al. [1], similar in elevation to the highest permanent human dwelling [2].

Acute adaptation to altitude requires autonomic nervous system (ANS) adjustments to cope with short term environmental influences such as ambient hypoxia and low temperatures [3]. However, long-term survival at altitude depends on additional adaptive strategies that modify metabolism and hemoglobin levels [4].

More than 2 million people are born, live, work and adapt to altitude in the Andes, East African high altitude plateau, and Himalayas but they cope with the ambient stress of altitude life in different ways and with varying success Claydon, et al. [5]; one way to adapt to altitude is by increasing the hemoglobin content of the blood to improve oxygen uptake and delivery to tissue [6]. Additional short-term adaptive mechanisms depend on changes in (ANS) function (ANSf) such as thermoregulation and respiratory function [3]. Many genetic and physiologic mechanisms have been discovered that also contribute to the success or failure of human adaptation to altitude Claydon, et al.

[5] however, no evidence of maladaptation in hummingbirds has been reported to date.

We hypothesized that altitude adapted hummingbirds might exhibit differences in hemoglobin content, metabolism, and ANSf when compared to human altitude dwellers. Therefore, we used field measurements of hemoglobin concentration and museum measurements of growth rhythms (scale bars for metabolism)) in hummingbird tail feathers to model altitudinal patterns of variation in human and hummingbirds metabolism.

Materials and Methods

We used hummingbird specimens from the Museum of

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Southwestern Biology of the University of New Mexico to measure growth bar-intervals which reflect the growth of the tail feathers on rectrices. We measured hemoglobin concentration of hummingbirds using a Hemocue, with a 1 g/dl correction for avian blood [7]. We obtained measurements from 1024 individual hummingbirds, representing 72 species, as authorized by the following permits: 76-2006-INRENA-IFFS-DCB, 087-2007-INRENA-IFFS-DCB, 135-2009-AG-DGFFS-DGEFFS, 0377-2010-AG-DGFFS-DGEFFS, 0199-2012-AG-DGFFS-DGEFFS, and 006-2013-MINAGRI-DGFFS/DGEFFS. All sample and data collection from hummingbirds was authorized by the Institutional Animal Care and Use Committee of the University of New Mexico (Protocol number 08UNM033-TR-100117; Animal Welfare Assurance number A4023-01). We used hemoglobin concentration estimates from a total of 6561 individual humans from altitude populations residing in the Andes, Himalayas and Ethiopia previously published (Figure 1) [8].

We gauged ANS function derived from the ratios of low/high frequencies (LF/HF) from spectral analyses of growth intervals of hummingbird feathers measured in 1173 feathers (53 individual birds), 94 non-avian theropod rock imprints of feathers and 23 non-avian theropod rock imprints [9]. Additionally we measured 46 rachis growth-intervals. We also included in the analyses oxygen hydrogen ratios measured along the length of the hairs (a measure of growth and metabolism) from 8 humans; 1 horse tail hair and hair from 1 Siberian mammoth to generate the power spectra.

Statistical analyses

In this section, we develop statistics related to ratio (LF/HF) of low frequency to high frequency spectral content (areas under the curve of the spectrum, AUC). The basic distributional theory is given in Qualls and Appenzeller [10]. There, we have from equations 6, 8 and 9 that the ratio LF/HF is a random variable with a distribution proportional to an F-distribution. The assumptions were normality or large samples, and disjoint frequency bands for the calculations of AUC1 and AUC3,

which nearly give independence of these two AUCs. The subscripts 1, 3 are arbitrary, though they refer to three bands of frequencies, low, mid, and high, where the band of mid frequencies, labeled 2, are left out of the computation of the ratio. In particular,

$$1) \quad LFHF = \frac{AUC_1}{AUC_3} \sim \frac{c_1 R_1}{c_3 R_3} * F_{R_1, R_3},$$

where F_{R_1, R_3} is a F statistic with degrees of freedom R_1, R_3 and

$$R_i = 2 \frac{E^2(AUC_i)}{Var(AUC_i)} \text{ and } c_i = \frac{1}{2} \frac{Var(AUC_i)}{E(AUC_i)} \text{ for } i=1,2.$$

Here E is expectation and Var is variance of the random variable AUC_i .

Note $c_i R_i = E(AUC_i)$; thus, a point estimate of $c_i R_i$ and $E(AUC_i)$ is AUC_i and a likelihood estimate of:

$$\frac{c_1 R_1}{c_3 R_3} \text{ is } \frac{AUC_1}{AUC_3} = LFHF.$$

The computation of the first and second moments $E(AUC_i)$ and $Var(AUC_i)$ are adapted from Priestley [11] and are given in above equations, Also note that the F-distribution used here is generalized in that the degrees of freedom R_i are not necessarily integers.

The F-distribution can be used to give a 95% confidence interval for LFHF:

$$P\left[\frac{c_1 R_1}{c_3 R_3} * F_{R_1, R_3}^{-1}(.025) \leq \frac{c_1 R_1}{c_3 R_3} * F_{R_1, R_3} \leq \frac{c_1 R_1}{c_3 R_3} * F_{R_1, R_3}^{-1}(.975)\right] = 0.95 \text{ or}$$

2) $(LFHF * F_{R_1, R_3}^{-1}(.025), LFHF * F_{R_1, R_3}^{-1}(.975))$ is a 95% confidence interval of the expected value of LFHF.

Here $F_{R_1, R_3}^{-1}(.025)$ and $F_{R_1, R_3}^{-1}(.975)$ are the 2.5 and 97.5 percentiles of this F-distribution to be computed from an F-table or by computer program.

Next we compute the error bars of LF/HF for graphical purposes. For symmetric error bars and based on the variance of the F-distribution. We have:

$$\text{Standard error } SE = \sqrt{Var\left[\frac{c_1 R_1}{c_3 R_3} * F_{R_1, R_3}\right]} = \frac{c_1 R_1}{c_3 R_3} \sqrt{Var[F_{R_1, R_3}]}, \text{ thus}$$

$$3) \quad SE = LFHF * \sqrt{2 \frac{R_3^2 (R_1 + R_3 - 2)}{R_1 (R_3 - 2)^2 (R_3 - 4)}}, \text{ provided } R_3 > 4.$$

If one allows asymmetric error bars, then one could use the 68% content interval (mean \pm SE is a 68% interval for the normal distribution) as follows.

$$3') \quad LFHF * (F_{R_1, R_3}^{-1}(0.16), F_{R_1, R_3}^{-1}(0.84)).$$

Example: Our collection of hummingbirds provided measurement of alternating light and dark bands in feathers. The widths of dark bands reflect metabolism during daylight and form a time series (529 observations) suitable for spectral analysis. Since these hummingbirds live and were collected at known altitudes, we are able to compute LF/HF ratios as a function of altitude. Using SAS 9.4, PROC SPECTRAL, and data step programming, we compute the parameters mentioned above from the spectra of the dark band series for hummingbird feathers at specific altitudes detailed in Table 1.

For the differences in LF/HF at different altitudes for hummingbirds (Figure 2), we use t-tests. There were no significant differences

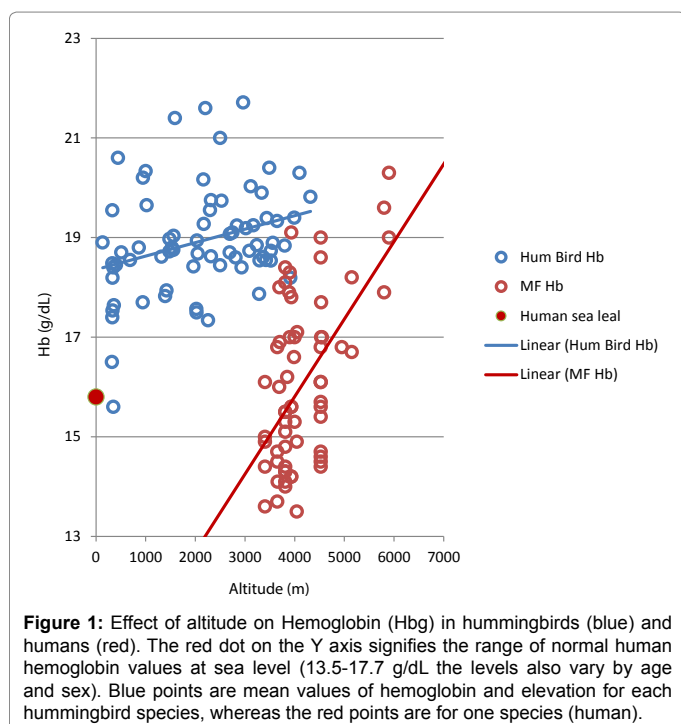
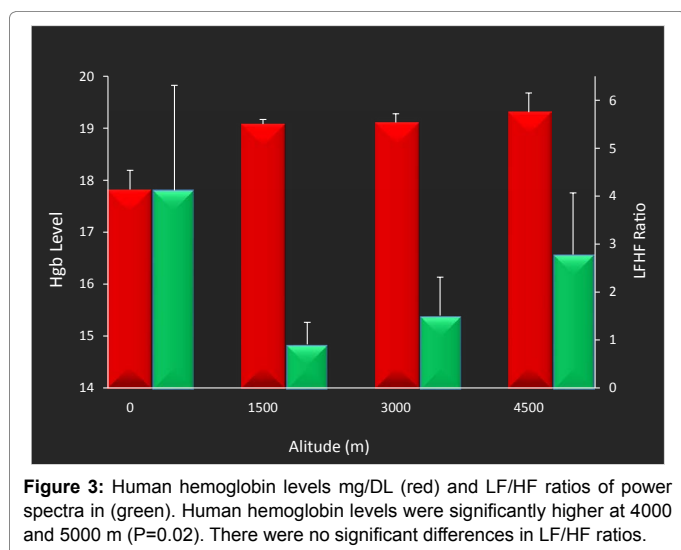
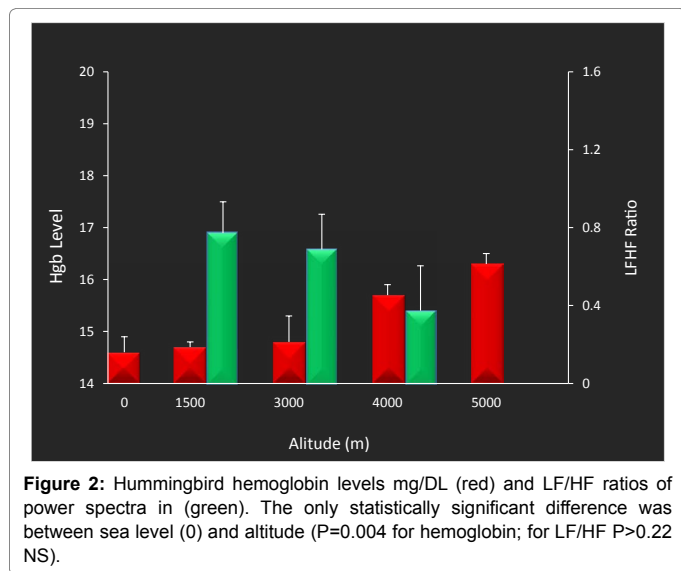


Figure 1: Effect of altitude on Hemoglobin (Hbg) in hummingbirds (blue) and humans (red). The red dot on the Y axis signifies the range of normal human hemoglobin values at sea level (13.5-17.7 g/dL the levels also vary by age and sex). Blue points are mean values of hemoglobin and elevation for each hummingbird species, whereas the red points are for one species (human).

Alt_1500	Total123	Sum1	Sum2	Sum3	LFHF12	LFHF13	Alt_1500	Sig1	Sig2	Sig3	R1	R3	SE_F	SE_LFHF
0	0.99	0.65	0.19	0.16	3.43	4.12	0	0.28	0.054	0.035	10.7	39.5	0.53	2.19
1500	1.02	0.36	0.27	0.39	1.35	0.91	1500	0.14	0.082	0.094	12.7	35.4	0.51	0.46
3000	0.99	0.46	0.23	0.31	2.01	1.51	3000	0.19	0.073	0.074	11.9	33.6	0.53	0.8
4500	0.99	0.6	0.18	0.21	3.23	2.78	4500	0.22	0.06	0.048	15	40.5	0.46	1.29

Table 1: Altitudes were pooled into representative categories of 0, 1500, 3000 and 4500 meters).



(all $P>0.15$). Similarly, for humans (Figure 3), there were no significant differences in LF/HF (all $P>0.22$).

Results

We used the measured width of the combined dark and light banding on hummingbird feathers as a proxy for 24-hour growth and metabolism [12].

Hemoglobin levels were higher in hummingbirds ($P<0.001$) than in humans, but the influence of altitude on hemoglobin was more pronounced in humans (slope, $P<0.001$) (Figure 1).

We confirmed an increased mass of hummingbird at altitude (Figures 4 and 5). The width of the sum of the dark and light bands of

hummingbird tail feathers; a proxy for metabolic rhythms, does not change with altitude (Figure 6).

We analyzed Hummingbird mass versus altitude and altitude temperature (Figure 7) and show the dark and light bands in a hummingbird tail feather (Figure 8).

Discussion

In growing tissues such as feathers, the growth bars are easily discernible by their repetitive patterns, visible in the dark and light bands shown in the feather (Figure 8). We focused our attention on the dark bands because they reflect metabolism during the day, whereas the light band grows during the night, a time when most hummingbirds at altitude go into torpor (a state of physical inactivity).

Humans living at altitude exhibit genetically based physiological adaptations to allow prolonged survival above 2500 m, at least some of which relate to blood hemoglobin concentration [2,8]. Such adjustments are not always capable of ensuring a healthy existence. For example in the high Andes the natives often suffer from chronic mountain sickness (CMS) or Monge's disease. Such patients have very high hematocrits (hemoglobin), pulmonary hypertension, and eventually, right heart failure that causes their early demise [4,6]. By contrast, this disease is rare in the Himalayas and has not been found in natives of the East African high altitude plateau [5] suggesting genetic underpinning for human long-term altitude survival. Consistent with this suggestion, genetic adaptations to the highlands have recently been reported from African highlanders [13].

The dispersal of modern humans across the globe from the jungles to the mountainous peaks of the Himalayas was aided by genetic adaptations to their environment. Examples of such adaptations are found in Africa (resistance to malaria) [14] and Scandinavia (light skin color) [15]. Such environmental adaptations were and continue to be beneficial for survival; they have been achieved by environmental pressures on genetic loci under selection [15]. Hummingbirds have great potential as model organisms for altitude adaptation because of their successful radiation to high altitude. Therefore, they invite a comparison of the adaptive strategies used by the birds with human altitude dwellers especially those that have been found less successful such as the Andeans of Peru who develop CMS and early death [4].

Evolution often conserves successful adaptive strategies and uses these repeatedly and in different species [12,15]. Our study implies that unlike variation among human individuals and populations, variation in hemoglobin among hummingbird species increases only subtly with increasing altitude. However, at extreme altitudes, human hemoglobin approaches that of hummingbirds (~ 19 g/dl; Figure 1). This phenotypic convergence is remarkable considering the contrasting evolutionary histories of these two taxa at altitude. Humans colonized high altitude thousands of years ago and maintained gene flow between high and low altitude populations [15] in contrast, several lineages within the hummingbird clade colonized altitude millions of years ago and gave rise to numerous high-altitude specialist species that long ago stopped exchanging genes with lowland relatives [16].

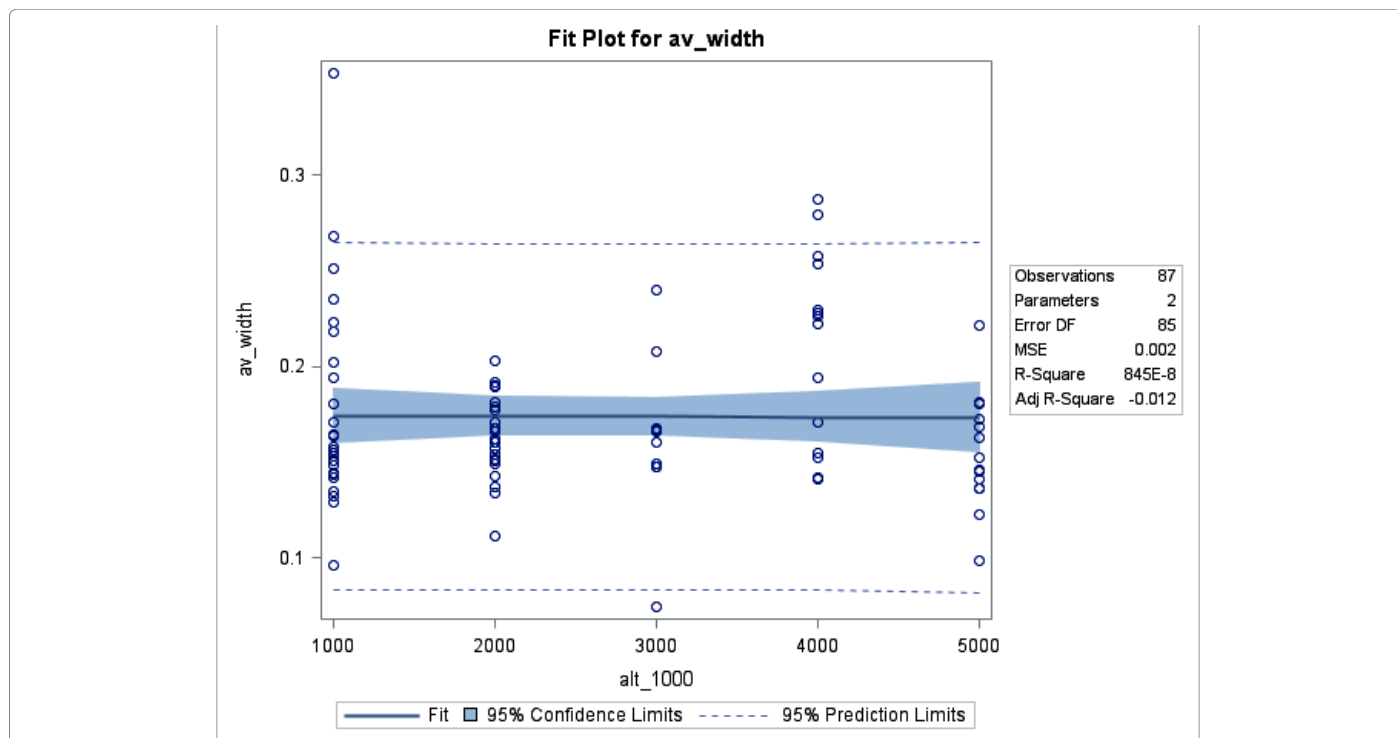


Figure 4: The width of the sum of the dark and light bands of hummingbird tail feathers; a proxy for metabolic rhythms, does not change with altitude, consistent with a circadian metabolism unaffected by altitude.

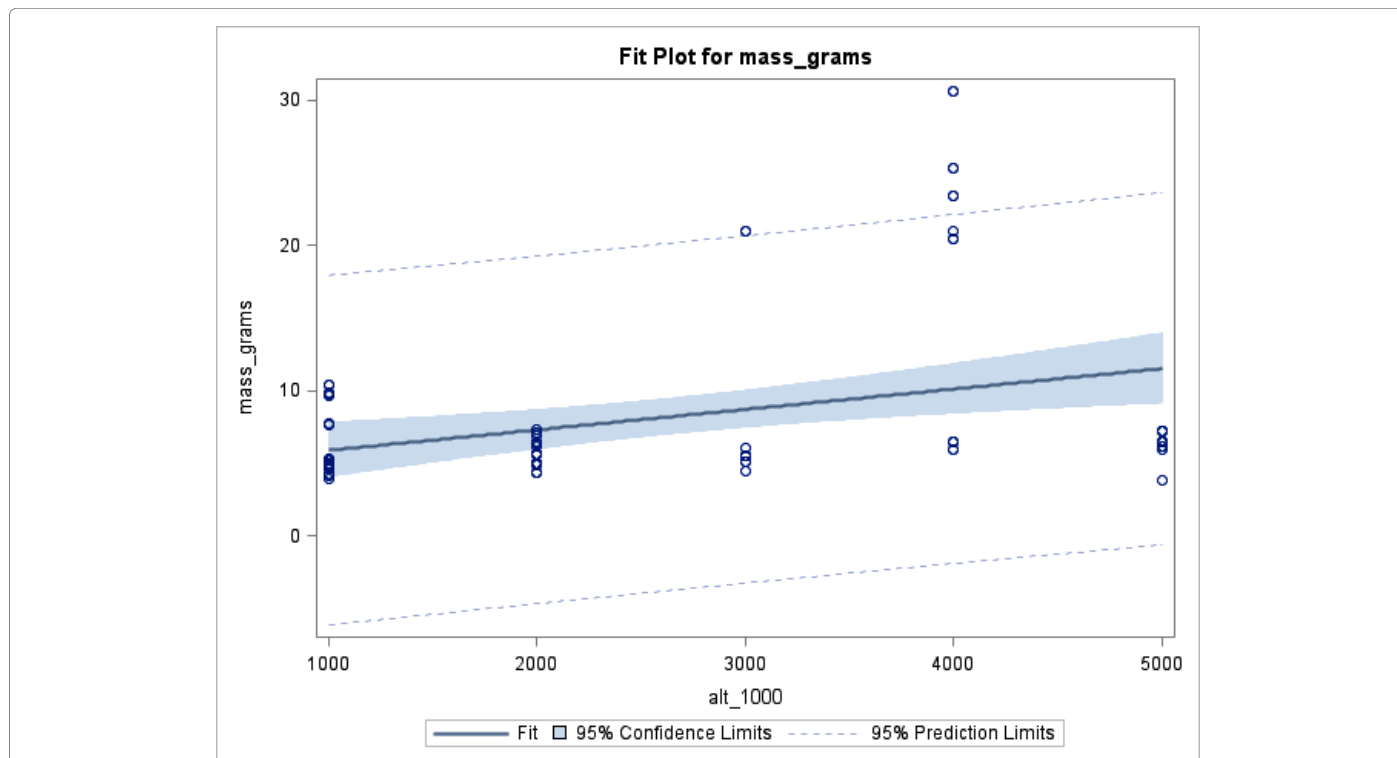


Figure 5: Body mass of the species increases with increasing altitude, implying that food intake and metabolism increases concomitant with altitude ($P=0.02$). This analysis includes individual birds on which we measured feather growth bars. A phylogenetic comparative analysis of Peruvian hummingbirds shows the same trend (Altshuler and Dudley [18]).

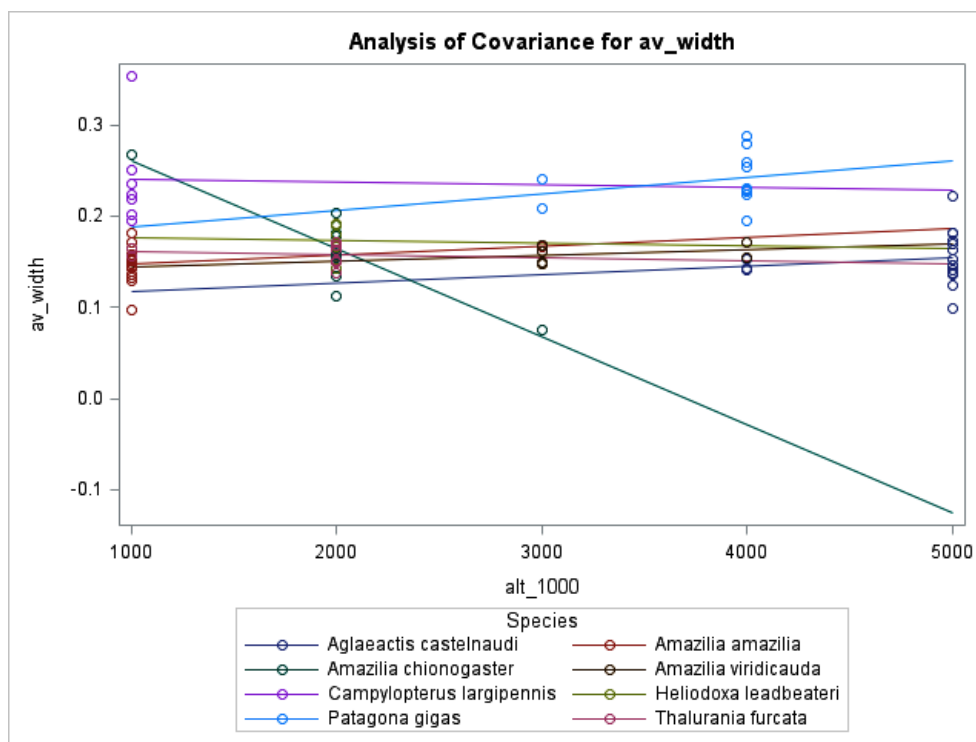


Figure 6: The average width of the sum of light and dark bands in hummingbird feathers, a measure of daily feather growth and metabolism, was not associated with altitude within species, except in *Amazilia chionogaster*, a species with an unusually broad elevation range (sea level to >3000 m). The hemoglobin of *Amazilia chionogaster* at ~3000 m does not differ statistically from that of other hummingbird species at the same elevation.

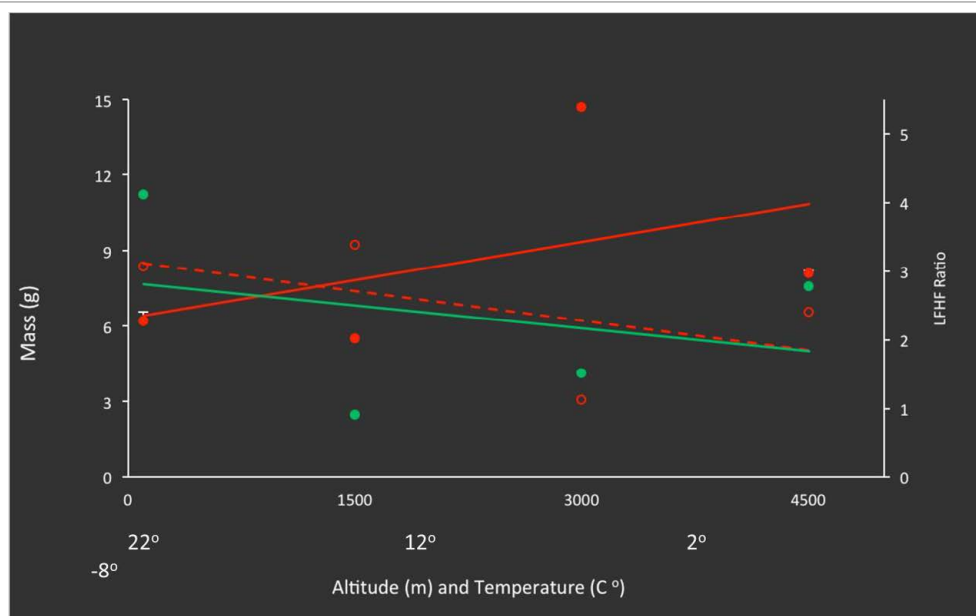


Figure 7: Hummingbird mass (solid red, trend line: solid red dashes) versus altitude (m) or equivalent temperature (Co) on X-axis to the left. Spectral low frequency/high frequency (LF/HF) ratio (solid green, trend line) for dark bands (accreted during daylight) in feather growth versus altitude on axis to the right. Hummingbird mass converted to metabolism per gram (open red circles, trend line: dashed red) by allometric relationship ($=0.78 \times X^{-0.33}$) versus altitude (m) on axis not shown. The temperatures shown on the X-axis are average temperatures based on a decrease rate of 9.8°C per 1,000 meters altitude gained.

All growing tissues, such as teeth, bone, and hair, reflect metabolism of the animals in which they are examined. Growth is costly in terms of energy used; therefore, to grow necessitates an increase in food consumption to meet the energy demands of increased metabolism

and body mass [3]. Here we derived metabolism from growth intervals of hummingbird feathers and, a variety of other growing tissues such as human and animal hairs, and dinosaur rock imprints of their feathers [9]. We show that altitude has no discernible effect on hummingbirds'



Figure 8: Distinctive light and dark banding in hummingbird tail feather. The dark band is a product of daylight metabolism; the light band accretes at night [8]. The feather is from a specimen of *Patagona gigas*. The scale bar visible in the left upper corner of the image indicates length in mm.

metabolic adjustments to ambient conditions as judged from their feather's growth. Although hummingbird species living at higher altitudes are generally heavier than their lowland relatives, there is extensive overlap among constituent species in highland and lowland communities. By contrast humans, lifelong exposure to altitude requires considerable metabolic adjustments [4,6,8], aided by steeper altitude related increases in blood hemoglobin content in Highlanders and generally smaller stature than lowlanders.

Our results support the notion that hummingbird hemoglobin levels and metabolism are useful models for the study of biologically adaptive strategies to life in ambient hypoxia.

Hummingbird thermoregulation has been examined [17,18]. Remarkably, because of their flying style, they generate enormous amounts of heat. To sustain metabolism for hovering in the hypodense air, hummingbirds must increase their food intake and enlarge their wing-span which, in turn, results in increased body mass at altitude as observed in this study. At low altitudes, feeding times in this taxon can be limited by high temperatures, necessitating frequent rest periods to allow for cooling [18]. At altitude, decreased ambient temperatures (Figure 7) might increase the available time for feeding and this could have been an additional factor in the evolutionary expansion of hummingbirds to high altitude and subsequent adaptation. Furthermore, the ability of hummingbirds to use the nightly torpor to conserve energy during inactive periods could be another pre-adaptation to altitude [18]. By contrast, although humans face less severe thermal constraints due to their larger body sizes, and microclimate provided by clothing, recent genetic studies have revealed the possibility that high-latitude, as well as cold-adapted alleles, may have pre-adapted humans to colonize high-altitude habitats [19,20].

In hummingbirds adaptation to altitude is also aided by amino-acid substitutions in hemoglobin that improve oxygen uptake and delivery at altitude by increasing O₂-binding affinity [21]. Functional amino acid substitutions in hemoglobin also occur in many other altitude vertebrates who live at high altitudes [22]. However, there is no good evidence to date that any of those variants enhance respiratory function at altitude or provide a fitness advantage under any circumstances [16].

Conclusion

Humans and hummingbirds exhibit convergent phenotypes for hemoglobin at extreme altitudes. Obviously, however, numerous aspects of high-altitude respiration and thermoregulation differ

between hummingbirds and humans, in part due to their different evolutionary histories at altitude. Study of daily metabolic rhythms in additional high altitude taxa using statistical modeling will be required to test whether these contrasting patterns are attributable to the different timing of colonization and different levels of altitude specialization. In this way, biorhythms may help to reveal why hummingbirds thrive while humans tend to suffer under hypobaric hypoxia.

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