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# The effects of atmospheric pressure on inter-specific competition and the morphological and hematological characteristics of elevational distributions of hummingbirds

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*Approved by the Thesis Committee:*

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**THE EFFECTS OF ATMOSPHERIC PRESSURE ON INTER-SPECIFIC  
COMPETITION AND THE MORPHOLOGICAL AND HEMATOLOGICAL  
CHARACTERISTICS OF ELEVATIONAL DISTRIBUTIONS OF  
HUMMINGBIRDS**

**BY**

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CHAMPAIGN, 2013**

**THESIS**

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**ABSTRACT**

Species diversity in mountainous regions is strongly influenced elevational range limits of species, but it is generally not known which abiotic or biotic factors maintain these limits. Using Black-chinned and Broad-tailed Hummingbirds, species with offset but overlapping elevational ranges in the southwestern USA, we investigate the role barometric pressure plays in influencing these limits. In chapter one, we used a hypobaric chamber to test the effects of variable air pressures on interspecific competitive dominance. We sequenced the genes that encode the two adult isoforms of haemoglobin and measured the O<sub>2</sub>-binding affinity of each isoform. In chapter two, we explored the morphological differences between species and the haematological response of species

exposed to variable air pressures. Results suggest that species variation in physiological and behavioral responses to variable air pressures show how biotic and abiotic forces might interact to maintain stable elevational distribution limits, and are potentially resistant to rising temperatures.

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## Chapter 1

# **Air pressure reverses competitive dominance between hummingbird species that differ in elevational range and hemoglobin-D O<sub>2</sub>-binding affinity**

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### INTRODUCTION

Well-defined elevational limits of species ranges were described long before the development of a theoretical framework capable of explaining such patterns in evolutionary terms (Humboldt, 1838). Understanding the ecological and evolutionary causes of these elevational limits will be essential to predicting how montane communities will respond to climate warming and threats from invasive species. Elevational gradients are natural laboratories in which to study mechanisms of range limitation because they encompass drastic climatic variation and community turnover across small spatial scales, eliminating the need to consider dispersal limitation or historical contingency as alternative explanations for the absence of a given species from any particular elevational zone. As a result, elevational gradients have been a focal point for spatial studies of species diversity and species interactions (Cabrera, Rada, & Cavieres, 1998; Caughley, Short, Grigg, & Nix, 1987; Feldman & McGill, 2013; Jankowski, Londoño, Robinson, & Chappell, 2013; Terborgh, 1977).

For most species, elevational limits are likely determined by some degree of interaction between abiotic and biotic forces (Jankowski et al., 2013; Jankowski, Robinson, & Levey, 2010). Abiotic forces such as temperature, air pressure, and UV light exposure vary monotonically with elevation (West, 1996) and impose physical challenges that can limit species occurrence on the basis of physiological tolerance (Bert, 1878; Darwin, 1959). Biotic factors such as competition, food availability, predation, and parasitism can also influence habitat suitability along elevational gradients, just as they do in other contexts (Darwin, 1959; MacArthur, 1972; Ricklefs, 2010). These forces can be at least partly disentangled using analyses of species distribution patterns combined with functional studies (e.g. (Brown, 1984; Case, Holt, McPeck, & Keitt, 2005; Gaston, 2009; Hewitt, 1999)).

A frequently observed pattern known as *elevational replacement* (Terborgh, 1971) occurs when ecologically similar, closely related species have distributions that abut along elevational contours (sometimes with overlap). This distributional pattern is thought to reflect the effects of interspecific competition and competitive exclusion (Terborgh & Weske, 1975). However, direct evidence of the influence of interspecific competition on species distribution limits is difficult to find (Bullock, Edwards, Carey, & Rose, 2000; Case et al., 2005; Jankowski et al., 2013). Previous studies of elevational replacement have found support for different mechanisms, usually some aspect of physiological tolerance or competitive ability, determining upper and lower range boundaries (Feinsinger,

Colwell, Terborgh, & Chaplin, 1979; Gifford & Kozak, 2012; Kozak & Wiens, 2006; Morin & Chuine, 2006). Jankowski et al. (Jankowski et al., 2010) showed that interspecific territoriality by elevational replacement species contributes to the maintenance of elevational range limits, and that competitive dominance by the lower-elevation replacement species limits the lower elevation limit of the high elevation replacement species in Central American thrushes (*Catharus* spp.). Previous work on hummingbirds also found that lower elevation species were dominant over high-elevation competitors where they overlap. Feinsinger et al. (Feinsinger et al., 1979) showed that Green Violetear hummingbirds (*Colibri thalassinus*) were dominant competitors that foraged as 'territorialists' at high elevations, but they became subordinate 'trap-line' foragers at lower elevations where they faced more intense competition. In this way, the dynamics of interspecific competition appear to change with elevation, potentially limiting species elevational distributions. A more recent observation supporting this hypothesis was made by Altshuler (Altshuler, 2006a) who found that interspecific competitive dominance at feeders shifted with elevation between migrant Rufous Hummingbirds (*Selasphorus rufus*), which are elevational generalists, and Broad-tailed Hummingbirds (*Selasphorus platycercus*), which are high-elevation specialists. The dominance of the more aggressive Rufous Hummingbirds appeared to be diminished at the highest elevations. This tendency for low elevation hummingbird species to be dominant over high-elevation competitors is thought to have a biomechanical basis, whereby smaller wings confer high maneuverability and superior competitive ability, but larger wings are necessary

to provide energetically efficient flight in the thin air at high elevations (Altshuler & Dudley, 2002; Altshuler, Dudley, & McGuire, 2004; Altshuler, Stiles, & Dudley, 2004; Feinsinger et al., 1979). These ecological mechanisms likely have a deep evolutionary basis because hummingbird elevational distributions tend to be narrow in amplitude (Parker, Stotz, & Fitzpatrick, 1996) and evolutionarily conserved over millions of years (Graham, Parra, Rahbek, & McGuire, 2009).

Under a rapidly warming climate, montane birds are predicted to expand on the upslope margin of their distributions and contract on the downslope margin. Upward range shifts of lowland species may result in declines for higher elevation species due to competition or other ecological pressures (Sekercioglu, Schneider, Fay, & Loarie, 2008). However, to the extent that elevational ranges may be limited by physiological tolerance to the partial pressure of oxygen ( $PO_2$ ), the predicted responses of montane species to climate warming may need to be reconsidered.

Under hypoxic conditions at high elevation, there are numerous modifications of the cardiorespiratory and cardiovascular systems that can influence tissue  $O_2$  delivery and, hence, whole-animal physiological performance (Scott, 2011; Storz, Scott, & Cheviron, 2010). For example, in hummingbirds and other avian taxa that are native to high altitudes, fine-tuned adjustments in hemoglobin (Hb)- $O_2$  affinity are known to make important contributions to hypoxia adaptation (Galen et al., 2015; Natarajan et al., 2015, 2016; Projecto-Garcia et al., 2013; Storz,

2016). Evolved changes in Hb function and other components of the O<sub>2</sub>-transport pathway may therefore contribute to species differences in the elevational limits of physiological tolerance. Where elevational replacement species come into contact, differences in the extent of physiological tolerance to abiotic stressors can lead to reciprocal competitive exclusion and elevational ranges that are narrower than they are in areas where only one species occurs (DuBay & Witt, 2014). On evolutionary timescales, the process of competition between relatives may be responsible for the origin of the elevational replacement pattern (Cadena, 2007; Freeman, 2015).

During the breeding season in the southern Rocky Mountains, the high elevation Broad-tailed Hummingbird (*Selasphorus platycercus*; 2000-3600 m) is replaced at low elevations by a similar species, the Black-chinned Hummingbird (*Archilochus alexandri*; 0-2500 m). The elevational ranges of the two abut one another on mountain slopes throughout the southwestern USA; in northern New Mexico, the species overlap between ~2000-2500 m elevation (Fig. 1a). These species specific elevational distributions are generally maintained in their winter distribution in Mexico, although the Broad-tailed Hummingbird also occurs at low elevations during migration. Black-chinned Hummingbirds tend to be competitively dominant over Broad-tailed Hummingbirds at feeders in the elevational zone of overlap where the two species co-occur (Feldman & McGill, 2013). The hypothesis that interference competition between these two species actively regulates their elevational limits is suggested by their morphological,

ecological, and phylogenetic similarity, the fact that both species are abundant, and the fact that they are known to overlap in their use of natural and human-provided nectar resources (Baltosser & Russell, n.d.; Camfield, Calder, & Calder, 2013). Feldman and McGill (Feldman & McGill, 2013) found no evidence that the degree of dominance of Black-chinned Hummingbirds changes at hummingbird feeders distributed across a ~400 m zone of elevational overlap in Colorado. However, the latter study was limited to observations of feeders under natural conditions, so it may not have been able to detect differential physiological tolerances that might subtly affect the relationship between energetic performance and elevation. Experimental tests are needed to determine whether the lower elevation species would continue to be dominant over its higher-elevation replacement under high-altitude conditions.

The objective of this study was to examine the effects of high-altitude hypobaria on flight activity and inter-specific competitive dominance between Black-chinned Hummingbirds and Broad-tailed Hummingbirds. We used a large hypobaric chamber to manipulate air pressure, which jointly alters air density and the partial pressure of oxygen ( $PO_2$ ), two parameters that are integral to the ability of hummingbirds to engage in metabolically expensive hovering flight (Altshuler & Dudley, 2002). We took advantage of the fact that wild-caught hummingbirds tend to quickly establish competitive dominance when pairs of individuals are introduced to a small cage with a feeder. If interspecies dominance changes predictably with reductions in pressure, it would suggest that differential tolerance

to hypobaria may be the ultimate cause of the consistent elevational position of the contact zone between these two species across southwestern USA. We also tested for species differences in the oxygenation properties of Hb, as genetically based changes in Hb-O<sub>2</sub> affinity are known to contribute to hypoxia adaptation in high elevation hummingbird species (Natarajan et al., 2016; Projecto-Garcia et al., 2013).

## MATERIALS AND METHODS

### *Study System*

We trapped adult male Broad-tailed Hummingbirds (*Selasphorus platycercus*) and Black-chinned Hummingbirds (*Archilochus alexandri*) during the summers of 2014 and 2015 in the foothills of the Sandia Mountains and Jemez Mountains, northern New Mexico. The majority of birds trapped from the zone of overlap, and occasionally birds were trapped from above and below the zone of overlap. All birds were captured using drop-door traps (Altshuler, Dudley, Heredia, & McGuire, 2010) and were trained to feed from a modified 20-ml syringe during the first two hours after capture. Individual birds were housed within a mesh cage (90 x 90 x 90 cm) and were provided with one modified syringe feeder, one high perch, and one low perch opposite the high perch (Fig. 1b). Cages were kept covered by a light colored cloth to minimize stress on the birds. Birds were fed daily on a sugar solution and a complete protein diet (Nektarplus). Hummingbird care was in accordance with the university animal care guidelines (IACUC Approval 16-200406-MC). The hypobaric chamber at the University of New Mexico was used to simulate elevational changes during experimental trials.

### *Activity Assay*

Adult males of each species were used to test whether there was a change in the level of activity of birds immediately following the reduction of pressure and how that change differed between high and low elevation species. Within approximately 48 hours of capture, cages were transported to the hypobaric chamber. Activity level was recorded at six pressures, starting at ambient pressure and transitioning to high-elevation equivalent pressure over the course of about 90 minutes: 633 mmHg (1600 m), 590 mmHg (2200 m), 550 mmHg (2800 m), 512 mmHg (3400 m), 475 mmHg (4000 m), and 441 mmHg (4600 m). Even though it would be above the elevational distribution of either species, we chose 4600 m as the peak simulated elevation because the intensity of aerobic exercise that the birds would experience within a 0.73 m<sup>3</sup> cage is below what they would experience during competitive bouts in the wild, and intense exercise exacerbates O<sub>2</sub> diffusion limitation in a way that is similar to extreme elevation (Piiper & Scheid, 1983). Accordingly, we judged that a 4600 m challenge would approximate the level of hypoxic challenge that would occur during competitive bouts under natural circumstances at pressures that are ~15-20% higher. In the wild, Broad-tailed Hummingbirds routinely occur above 3000 m elevation, but rarely exceed ~3600 m, and in fact only seven peaks within their geographic distribution exceed 4400 m (Camfield et al., 2013) . In contrast, Black-chinned Hummingbirds rarely ascend above ~2600 m (Baltosser & Russell, n.d.). Once a given pressure was reached in the hypobaric chamber, birds were observed for the subsequent 10 minutes, during which the number of seconds spent hovering



was recorded. Any signs of respiratory stress such as ruffling of feathers or panting were noted.

To analyze differences in activity between species, we compared the proportion of time that was spent hovering at each of the six pressures. Proportion data were logit-transformed and then the reduction in activity between 1600 m and 4600 m was compared between species using a t-test. We analyzed at the degree of reduction over the range of pressures between species using an information theoretic approach to test drivers of activity reduction (Anderson & Burnham, 2002). We compared seven models, comprising all possible combinations of these two variables and their interaction. Models were ranked based on Akaike's information criterion adjusted for small sample size (AICc). Models that had higher AICc scores than nested models were excluded from consideration (Arnold, 2010).

### *Competition Trial*

Adult males of each species were also used to test whether competitive dominance between high and low elevation species changes as a function of pressure. We simultaneously released one individual of each species into a new cage and then observed the interactions that ensued for the duration of the 45-minute trial. During the trial, we watched for aggressive behavior, which usually consisted of a hovering bird trying to displace the bird that was occupying the high perch. When birds exhibited aggressive behavior, we recorded failed and

successful attempts to displace the opposite species from the high perch. We recorded the proportion of time that each bird spent occupying the high perch. We noted any signs of respiratory stress such as fluffing and panting. We identified the dominant individual, or winner, based on asymmetric agonistic behavior during the trial, as follows: First, the winner had to exhibit at least some aggressive behavior; second, if both birds exhibited aggression, but one was more than 25% more successful at displacing the other bird from its perch, the bird with the higher rate of displacement was named the winner; third, when the rates of displacement were similar, the proportion of time spent on the high perch was used to determine the winner.

To test whether inter-specific competitive dominance was affected by pressure, we compared the number of trials won by each species at 1600 m and 4600 m using a one-tailed Fisher's exact test, reflecting our *a priori* hypothesis that the high species would be dominant at simulated high elevations.

#### *Cloning and sequencing of globin genes*

We cloned and sequenced the full complement of adult-expressed  $\alpha$ - and  $\beta$ -type Hb genes ( $\alpha^A$ -,  $\alpha^D$ -, and  $\beta^A$ -globin) from 12 individual specimens (four Broad-tailed Hummingbirds and eight Black-chinned Hummingbirds). We extracted RNA from whole blood using the RNeasy kit, and we amplified full-length cDNAs of the three genes using a OneStep RT-PCR kit (Qiagen, Valencia, CA, USA). We designed paralog-specific primers using 5' and 3' UTR sequences, as described

previously (Opazo et al., 2015; Projecto-Garcia et al., 2013). We cloned reverse transcription (RT)-PCR products into pCR4-TOPO vector using the TOPO<sup>®</sup> TA Cloning<sup>®</sup> Kit (Invitrogen, Carlsbad, CA, USA), and we sequenced at least five clones per gene in each individual in order to recover both alleles. This enabled us to determine full diploid genotypes for each of the three adult-expressed globin genes in each individual specimen. All sequences were deposited in GenBank under the accession numbers KX240778-KX240785, KX241081-KX241101, and KX241355-KX241362.

#### *Analysis of Hb function*

After cloning and sequencing the  $\alpha^A$ -,  $\alpha^D$ -, and  $\beta^A$ -globin genes of the 12 specimens mentioned above, we used isoelectric focusing (IEF) to separate each of two main Hb isoforms that are expressed in adult red blood cells. The tetrameric Hb protein is composed of two  $\alpha$ -chain subunits and two  $\beta$ -chain subunits. Whereas the major HbA isoform incorporates products of the  $\alpha^A$ - and  $\beta^A$ -globin genes ( $\alpha^A_2\beta^A_2$ ), the minor HbD isoform incorporates products of the  $\alpha^D$ - and  $\beta^A$ -globin genes ( $\alpha^D_2\beta^A_2$ ); the two isoforms therefore only differ with respect to the  $\alpha$ -type subunits (Grispo et al., 2012; Hoffmann & Storz, 2007). After separating the HbA and HbD isoforms by means of IEF using precast Phast gels (pH 3–9)(GE Healthcare Bio-Sciences, Pittsburgh, PA, USA; 17-0543-01), we excised the gel bands (each of which represented a structurally distinct Hb tetramer), subjected the samples to trypsin digestion, and performed a tandem mass-spectrometry (MS/MS) analysis to identify the resultant peptides, as

described previously (Opazo et al., 2015). Database searches of the resultant MS/MS spectra were performed using Mascot (Matrix Science, v1.9.0, London, UK); peptide mass fingerprints were queried against a custom database of globin sequences from the two species. We identified all significant protein hits that matched more than one peptide with  $P < 0.05$ . After confirming the identity of HbA and HbD, we then performed densitometric measurements on the IEF gel images to quantify the relative abundance of the two isoforms in the hemolysates of each individual specimen.

Using pooled samples from hummingbird specimens with identical genotypes for each of the adult-expressed globin genes, we separated the HbA and HbD isoforms and stripped the samples of red cell organic phosphates and other anions by means of ion-exchange fast protein liquid chromatography (FPLC) using a HiTrap QHP column (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). We measured O<sub>2</sub>-equilibrium curves using 3 µl thin-film samples of purified Hb solutions (0.3 mM heme concentration) at 37°C, 0.1 M HEPES buffer (pH 7.4). Using standard experimental conditions, we measured O<sub>2</sub>-binding properties of purified Hb solutions under four treatments: (i) in the absence of allosteric effectors ('stripped'), (ii) in the presence of Cl<sup>-</sup> (in the form of KCl (0.1 M)), (iii) in the presence of inositol hexaphosphate (IHP)(IHP/Hb tetramer ratio = 2.0), and (iv) in the simultaneous presence of both anionic effectors. We measured the O<sub>2</sub>-equilibrium curves using a modified O<sub>2</sub> diffusion chamber by monitoring absorption at 436 nm while subjecting thin-film samples to gas mixtures with

varying  $PO_2$  (prepared using Wösthoff gas-mixing pumps that perfuse the chamber). We estimated two parameters,  $P_{50}$  ( $O_2$  tension at half-saturation) and  $n_{50}$  (Hill's cooperativity coefficient at half-saturation), by fitting the sigmoidal Hill equation ( $Y = PO_2^{n_{50}} / (P_{50}^{n_{50}} + PO_2^{n_{50}})$ ) to the experimental  $O_2$  saturation data ( $Y$ ) using a nonlinear regression model. Free  $Cl^-$  concentrations were controlled with a model 926S Mark II chloride analyzer (Sherwood Scientific Ltd, Cambridge, UK).

## RESULTS

### *Activity Assay*

Activity data during simulated ascent were collected from seventeen Broad-tailed Hummingbirds and fifteen Black-chinned Hummingbirds. These data were used to test whether activity differed between high and low elevation species under reduced pressure. As pressure was reduced, the proportion of time spent hovering by Black-chinned Hummingbirds was reduced (Fig. 2). The magnitude of reduction in activity by Black-chinned Hummingbirds was greater than that of the reduction by broad-tailed Hummingbirds (t-test,  $P = 0.016$ ; Fig. 3). For three birds of each species, activity was only recorded at 1600 m and 4600 m. The difference between species remained consistent when we excluded data from birds that were collected outside of the elevational overlap. When we compared linear models of activity level as a function of pressure, species, and pressure-species interaction, we found that the best supported model included all three parameters; however, a model containing only pressure was only moderately

worse than the best model, ( $\Delta AICc=1.8$ ), suggesting that support for the inclusion of species identity in the model was not overwhelmingly strong (Table 1).

### *Competition Trials*

Competition data from 18 low and 16 high trials showed that competitive dominance shifted from the low elevation species to the high elevation species under reduced pressure (Fisher's Exact Test :  $P=0.0028$ ). Under normoxic conditions, the low elevation, Black-chinned Hummingbirds were dominant, winning 15 of the 18 trials. However, Broad-tailed Hummingbirds tended to be dominant under reduced pressure conditions, winning 11 of the 16 trials. All trials included in the analyses were between novel pairings of birds, but eight of the 34 pairs of birds used in the low trials included non-naïve individuals that had previously participated in a competition trial against a different opponent; the competitive performance of non-naïve individuals was consistent with expectations based on the overall result.

### *Species differences in the oxygenation properties of Hb*

If the hummingbird species exhibit differences in competitive performance under different barometric pressures, it is of interest to identify physiological factors that might contribute to such differences. We therefore tested for differences in Hb function that could potentially contribute to species differences in flight performance under hypobaric hypoxia.

Our mass spectrometry experiments confirmed that both hummingbird species express two structurally distinct Hb isoforms, HbA (pI = 8.8-9.0) and HbD (pI = 7.0-7.4). HbA accounted for >85% of total Hb in both species, which is consistent with data from other hummingbird taxa examined to date (Natarajan et al., 2016; Opazo et al., 2015). Analysis of O<sub>2</sub>-equilibrium curves revealed that the HbD isoform exhibited a uniformly higher O<sub>2</sub>-affinity than the HbA isoform in both species. This is indicated by the lower values of  $P_{50}$  (the  $PO_2$  at which heme is 50% saturated) for HbD relative to HbA (Table 2, Fig. 5). The isoform differences in O<sub>2</sub>-affinity were observed in the absence ('stripped') and presence of allosteric effectors (Table 2, Fig. 5). Both isoforms exhibited a high degree of cooperative O<sub>2</sub>-binding, as estimated Hill coefficients ( $n_{50}$ 's) were consistently >2 in the presence of IHP (Table 2).

The sequence data revealed no amino acid differences between the two species in the  $\alpha^A$ - or  $\beta^A$ -globin genes, and the MS/MS analysis confirmed that the HbA isoforms of the two species were structurally identical. Consistent with these results, the estimated  $P_{50}$  values for the two species were essentially identical (Table 2, Fig. 5). In contrast to the lack of structural and functional differences in HbA, the HbD isoforms of the two species were distinguished by two amino acid substitutions at  $\alpha^D$  sites 11 and 55. Comparison with orthologous sequences from other hummingbirds (Natarajan et al., 2016; Projecto-Garcia et al., 2013) indicated that the two differences are attributable to  $\alpha^D11\text{Thr}\rightarrow\text{Ser}$  and  $\alpha^D55\text{Val}\rightarrow\text{Ile}$  substitutions that occurred in the Broad-tailed Hummingbird

lineage. As a result of the independent or joint effects of these two  $\alpha^D$  substitutions, the HbD of Broad-tailed Hummingbird exhibited a significantly higher intrinsic O<sub>2</sub>-affinity than the lowland variant (the  $P_{50}$  of the 'stripped' Hb was slightly lower; Table 2, Fig. 5). This significant difference persisted in the simultaneous presence of both allosteric effectors ('KCl+IHP'; Table 2, Fig. 5), the experimental treatment that is most relevant to *in vivo* conditions in avian red blood cells.

## DISCUSSION

This study tested the effects of barometric pressure on competitive interactions and performance. In a single-variable manipulation, we found significant effects of barometric pressure on interspecific dominance and species-specific activity levels. This is consistent with the hypothesis that barometric pressure variation is an important determinant of species elevational range limits.

### *Differential effect of pressure on activity levels*

The drastic reductions in flight activity upon acute exposure to the air pressure of 4600 m elevation suggest that Black-chinned Hummingbirds were more strongly affected by the pressure reduction than were Broad-tailed Hummingbirds. During the experiment, we noticed a tendency of Black-chinned Hummingbirds, but not Broad-tailed Hummingbirds, to fluff their feathers and pant during pressure reduction to 4600m. We believe that these behaviors were indicative of respiratory stress following acute hypoxia exposure. The fact that both species reduced their activity levels overall during simulated ascent suggests that Broad-



tailed Hummingbird individuals also may have experienced hypoxia-induced stress; alternatively, the birds may have merely calmed over time since their introduction to the chamber.

### *Reversal of competitive dominance*

The reversal of competitive dominance under different abiotic conditions is thought to be an important mechanism that maintains species diversity across space (Dunson & Travis, 1991). There are other well documented cases in which a single abiotic parameter reverses interspecific competitive dominance; for example, water temperature variation can reverse competitive dominance between freshwater fish (Reese & Harvey, 2011). However, this is the first controlled experiment to our knowledge in which a single abiotic variable has been shown to reverse competitive dominance between bird species.

Our simple assay of behavioral dominance is uniquely possible in hummingbirds due their rapid assimilation to captivity and their willingness to utilize and defend new, artificial sources of nectar. Free-ranging Black-chinned Hummingbirds tend to be dominant over Broad-tailed Hummingbirds at feeders (Feldman & McGill, 2013), and this is consistent with our observations at flower patches in the elevational zone of species overlap; therefore, the fact that Black-chinned Hummingbirds were dominant under standard pressure conditions in our experimental cages suggest that our assay of dominance was providing an ecologically relevant assessment.

### *Lift versus O<sub>2</sub>-uptake as the underlying mechanism*

The apparent differences between species in their responses to hypobaria raise the question of which aspect of hypobaria was affecting flight activity and dominance. One possibility is that the low-density air made it disproportionately difficult for Black-chinned Hummingbirds to generate lift, perhaps due to slightly smaller relative wing sizes. To compensate for lower density air, hummingbird species with small wings (high wing-loading) tend to increase stroke amplitude to increase stroke amplitude to increase lift production with minimal additional metabolic energy expenditure (Altshuler, 2003). On the other hand, slight variations in wing morphology appear to be related to rates of O<sub>2</sub> consumption during forward-flight and hovering (Welch Jr. & Suarez, 2008); furthermore, hummingbird species that are native to high altitudes tend to have evolved larger wings to maintain lift-generation while minimizing additional O<sub>2</sub> demand or diminishment of maneuverability (Altshuler & Dudley, 2002). In contrast, a reduction in the  $PO_2$  of inspired air can compromise tissue O<sub>2</sub> delivery to working muscles, a problem that is especially acute for birds that need to fuel the high rates of aerobic metabolism associated with flapping flight (Bartholomew & Lighton, 1986; Butler, 1970; Scott, 2011; Scott & Milsom, 2006). It is therefore possible that the species difference in competitive dominance under hypoxia is primarily attributable to differences in aerobic performance capacities. For example, at low  $PO_2$  it may be that Broad-tailed Hummingbirds are simply better able to maintain O<sub>2</sub> flux to metabolizing tissues due to physiological differences affecting any number of convective or diffusive steps in the O<sub>2</sub> transport pathway

(e.g., convective O<sub>2</sub> transport by Hb). There is also the potential for a synergistic interaction, as intrinsic physiological differences between the two species could further accentuate purely biomechanical differences in flight performance at reduced air densities.

#### *Evidence for adaptation in the minor haemoglobin isoform*

Given that Broad-tailed Hummingbirds were competitively dominant over Black-chinned Hummingbirds only when  $PO_2$  was reduced, it is of interest to identify the physiological factors that might contribute to species differences in physiological performance capacities under hypoxia. Although the HbA isoforms of the two species were structurally and functionally identical, the increased HbD O<sub>2</sub>-affinity (reduced  $P_{50}$ ) of the Broad-tailed Hummingbird is consistent with well-documented elevational trends in the oxygenation properties of Hbs in hummingbirds and other avian taxa (Galen et al., 2015; Natarajan et al., 2015, 2016; Projecto-Garcia et al., 2013; Storz, 2016). In the case of the Broad-tailed Hummingbird and Black-chinned Hummingbird, we do not have any direct evidence for a causal connection between the increased HbD O<sub>2</sub>-affinity and increased aerobic capacity under hypoxia. However, under severe hypoxia, theoretical and experimental results demonstrate that even a slight increase in Hb-O<sub>2</sub> affinity can limit the inevitable reduction in tissue O<sub>2</sub> delivery by safeguarding arterial O<sub>2</sub> saturation, especially during exercise when O<sub>2</sub>-equilibration at the blood-gas interface is diffusion limited (Bencowitz, Wagner, & West, 1982; Mairbäurl, 1994; Storz, 2016; Willford, Hill, & Moores, 1982). Thus,

even slight differences in arterial  $O_2$  saturation can potentially translate into appreciable differences in whole-animal aerobic capacity, especially in conjunction with changes at other steps in the  $O_2$ -transport pathway (Scott, 2011; Scott & Milsom, 2006)

## Conclusions

Upward elevational shifts in response to warming are predicted, and have already been observed in tropical montane avifaunas (Forero-Medina et al., 2011; Freeman & Class Freeman, 2014). The results of our study imply that hummingbird species' elevational range limits are set by pressure due to interactions between  $PO_2$  and evolved genetic characteristics, and the effects of those interactions on inter-specific competitive dynamics. This suggests that hummingbirds might not make predictable upslope shifts in response to changing temperatures. Buermann et al. (Buermann et al., 2011) used conservative climate projections to predict that Andean hummingbird species would move upslope by 300-700 m during the course of the 21<sup>st</sup> century in order to track their climatic niches. One of the key results of Buermann et al. was that this degree of elevational increase would not be sufficient to create aerodynamic problems for hummingbirds because of their ability to flexibly adjust wing-beat amplitude in order to compensate for hypodense air. The effects of reduced  $PO_2$  are not as easy to model, but the present study suggests that genetic specialization on  $PO_2$  will have a stabilizing effect on elevational range limits under global warming.

# Chapter 1

## FIGURES

Figure 1. (a) Elevational distributions and equivalent air pressures for the lowland Black-chinned Hummingbird (*Archilochus alexandri*) and montane Broad-tailed Hummingbird (*Selasphorus platycercus*). Photos used by permission; courtesy of Dr. Raymond S. Matlack (bottom) and Carlos Caceres (top). (b) Cage design for competition trials within the hypobaric chamber, showing two perches and syringe-feeder.

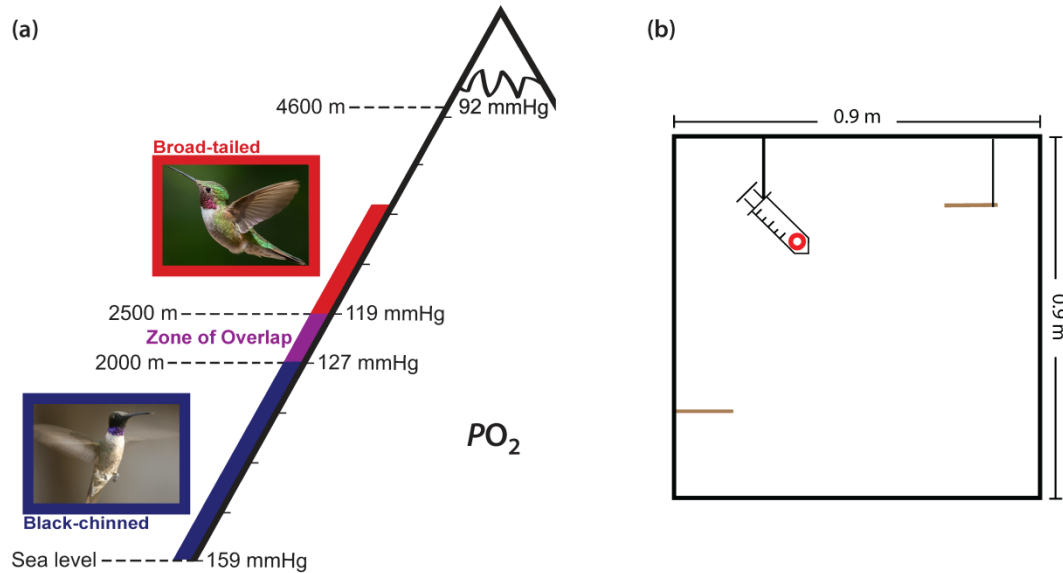


Figure 2. (a) The average proportion of time spent hovering at each simulated elevation for Black-chinned Hummingbirds (blue) and Broad-tailed Hummingbirds (red). Standard error bars surround each point. (b) The proportion of time individual hummingbirds spent hovering at various simulated elevations. Lines connect points corresponding to individual birds. Both species became less active with decreased pressure, but the reduction in activity was more dramatic for Black-chinned Hummingbirds.

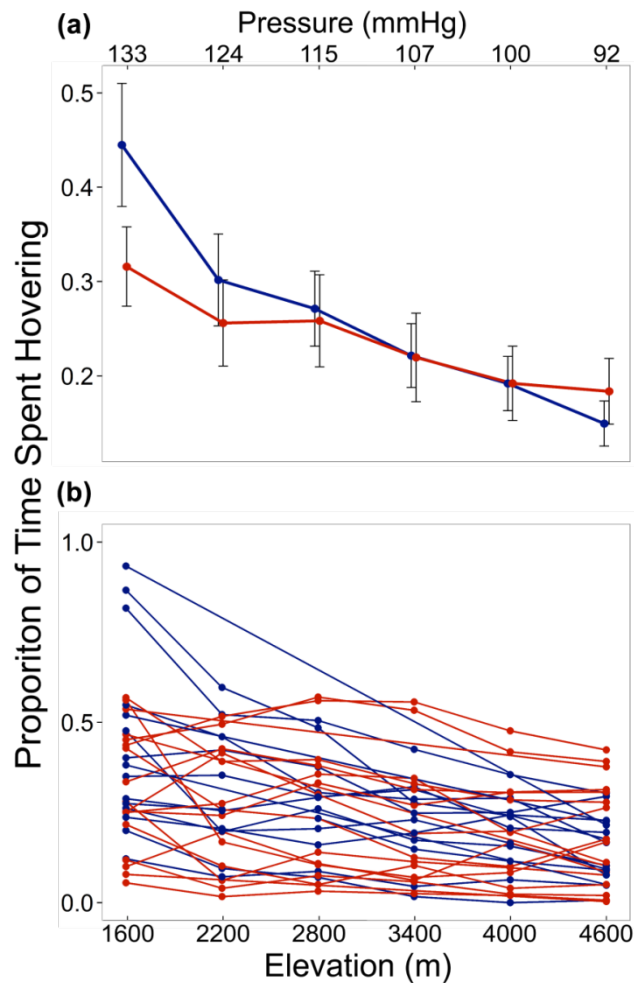


Figure 4: The dominance relationship changes in reduced pressure conditions (Fisher's Exact Test :  $P = 0.0028$ ). Under normobaric pressure (1600 m) Black-chinned Hummingbirds were dominant, winning 15 out of the 18 trials. While under reduced pressure (4600 m), Broad-tailed Hummingbirds were dominant, winning 11 of the 16 trials.

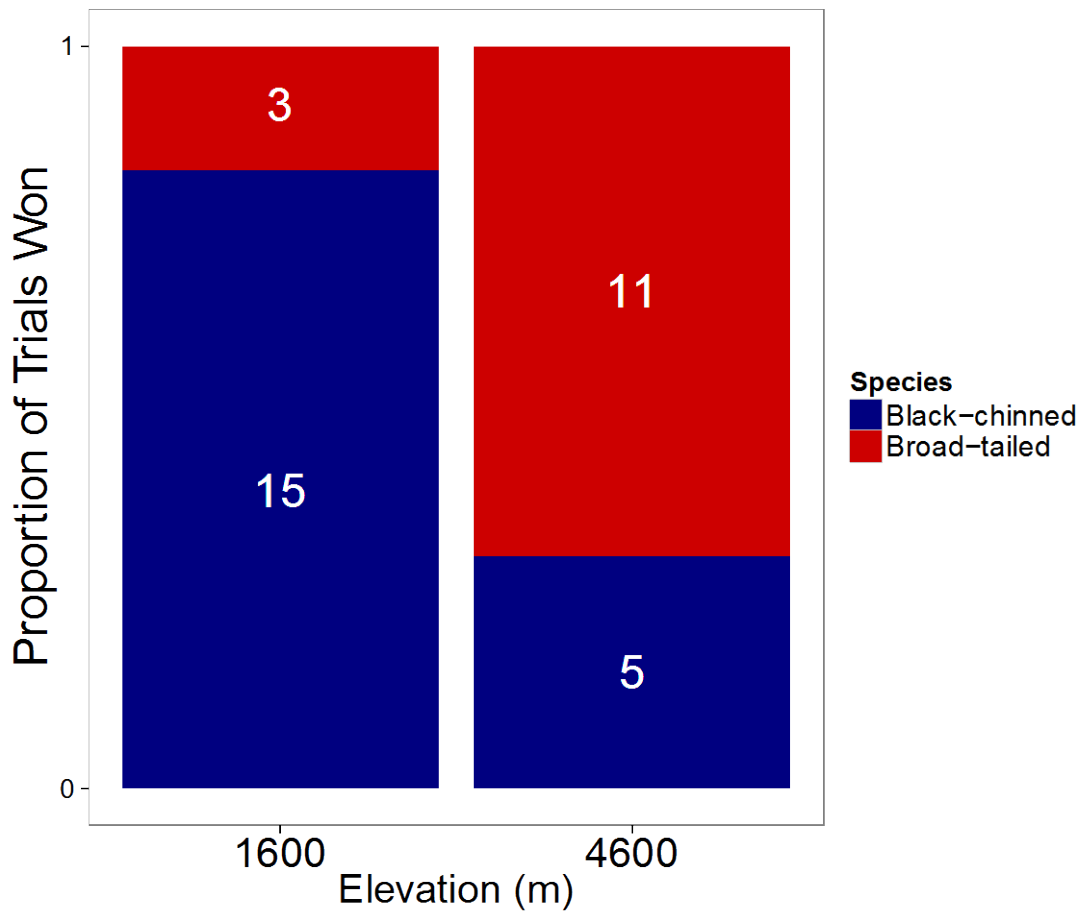
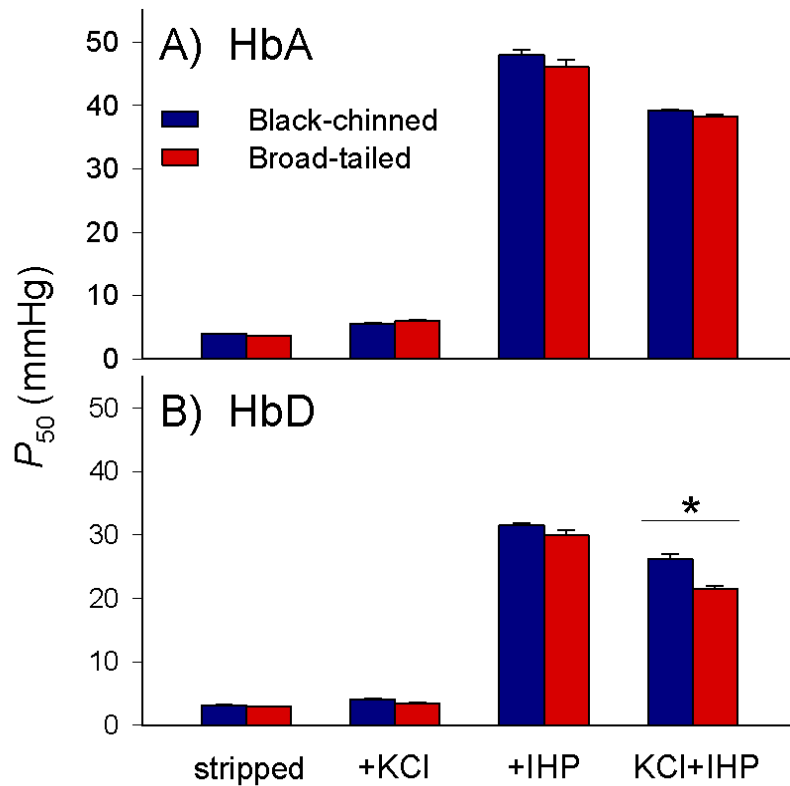


Figure 5: O<sub>2</sub>-affinities ( $P_{50}$ , mmHg;  $\pm 1$  SE) of purified HbA and HbD isoforms from Black-chinned and Broad-tailed Hummingbirds.  $P_{50}$  is the  $PO_2$  at which Hb is half-saturated, so the lower the  $P_{50}$ , the higher the Hb-O<sub>2</sub> affinity. The asterisk denotes a statistically significant difference between  $P_{50}$  values for the same treatment. O<sub>2</sub> equilibrium curves were measured in 0.1 mM HEPES buffer at pH 7.4 ( $\pm 0.01$ ) and 37°C in the absence (stripped) and presence of Cl<sup>-</sup> ions (0.1 M KCl) and IHP (at two-fold molar excess over tetrameric Hb). As explained in the text, the 'KCl+IHP' treatment is most relevant to *in vivo* conditions in avian red blood cells.





## Chapter 1

### TABLES

**Table 1.** Comparison of models explaining the proportion of time spent hovering at pressures equivalent to 1600-4600 m (based on data illustrated in Figure 2). All seven possible combinations of the three explanatory variables were compared, but two models that did not improve AICc value relative to a nested version of the same model were excluded from consideration. P-values are listed for each included parameter in each model; bold p-values indicate  $<0.05$ .

Model Parameters	AIC <sub>c</sub>	Δ AIC <sub>c</sub>	AIC <sub>c</sub> _wt	k	p-value		
					Pressure	Species	Species:Pressure
<b>Species +</b>							
<b>Species:Pressure</b>	442.0	0.00	0.48	5	-	<b>0.011</b>	<b>5.4e-8</b>
<b>Species + Pressure</b>	443.6	1.6	0.22	4	<b>9.7e-8</b>	0.211	-
<b>Pressure</b>	443.8	1.8	0.20	3	<b>1.0e-7</b>	-	-
<b>Species:Pressure</b>	445.2	3.2	0.10	4	-	-	<b>6.6e-7</b>
<b>Species</b>	468.9	26.9	0.00	3	-	0.251	-

**Table 2.** O<sub>2</sub> affinities ( $P_{50}$ , mmHg) and cooperativity coefficients ( $n_{50}$ )( $\pm 1$  SE) of purified HbA and HbD isoforms from Black-chinned Hummingbirds and Broad-tailed Hummingbirds (*Archilochus alexandri* and *Selasphorus platycercus*, respectively), measured under four different experimental treatments. O<sub>2</sub> equilibria were measured in 0.1 mM HEPES buffer at pH 7.4 ( $\pm 0.01$ ) and 37°C in the absence (stripped) and presence of Cl<sup>-</sup> ions (0.1 M KCl]) and IHP (at two-fold molar excess over tetrameric Hb).  $P_{50}$  and  $n_{50}$  values were derived from O<sub>2</sub> equilibrium curves, where each value was interpolated from linear Hill plots based on 4 or more equilibrium steps between 25 and 75% saturation.

Species	Hb isoform	Stripped		+ KCl		+ IHP		KCl + IHP	
		$P_{50}$	$n_{50}$	$P_{50}$	$n_{50}$	$P_{50}$	$n_{50}$	$P_{50}$	$n_{50}$
<i>Archilochus alexandri</i>	HbA	3.97 $\pm$ 0.05	2.03 $\pm$ 0.05	5.58 $\pm$ 0.10	2.23 $\pm$ 0.09	47.93 $\pm$ 0.82	2.63 $\pm$ 0.11	39.12 $\pm$ 0.23	2.81 $\pm$ 0.05
	HbD	3.10 $\pm$ 0.10	2.00 $\pm$ 0.13	4.07 $\pm$ 0.08	2.01 $\pm$ 0.08	31.55 $\pm$ 0.29	2.47 $\pm$ 0.07	26.19 $\pm$ 0.76	2.32 $\pm$ 0.15
<i>Selasphorus platycercus</i>	HbA	3.69 $\pm$ 0.03	1.91 $\pm$ 0.03	5.99 $\pm$ 0.16	2.09 $\pm$ 0.13	46.02 $\pm$ 1.19	2.37 $\pm$ 0.12	38.25 $\pm$ 0.27	2.61 $\pm$ 0.05
	HbD	2.88 $\pm$ 0.06	1.91 $\pm$ 0.08	3.48 $\pm$ 0.05	1.91 $\pm$ 0.05	29.93 $\pm$ 0.79	2.34 $\pm$ 0.12	21.45 $\pm$ 0.53	2.11 $\pm$ 0.12

## Chapter 2

# A comparison of the morphological and hematological characteristics of elevational replacement hummingbird species

### INTRODUCTION

Discovering the functional differences that underpin divergence in the fundamental niches of closely related species has been a major goal of evolutionary biology. As a result, there has been much research about adaptation to altitude, an aspect of the realized niche that often differs between close relatives (Altshuler, 2006b; DuBay & Witt, 2014; Projecto-Garcia et al., 2013; Ruiz, Rosenmann, & Veloso, 1989; Weber, 2002; Zhang, Wu, Chamba, & Ling, 2007). Previous research has focused on the limits of physiological tolerance and identifying the limiting factors that prohibit species from moving upslope or down slope (Jankowski et al., 2013, 2010). Bird species have been shown to have specialized on high-altitude conditions through adaptations such as changes to cardio-pulmonary systems (Weinstein et al., 1985) or genetic adaptations to oxygen-transport proteins (e.g. (Projecto-Garcia et al., 2013)). The preponderance of evidence supports a role for evolved physiological tolerance in determining an upper or lower distribution boundary (Feinsinger & Chaplin, 1975; Gifford & Kozak, 2012; Kozak & Wiens, 2010)

Hummingbirds exhibit the most metabolically intensive form of flight, stationary hovering, which demands high oxygen consumption despite limited energy reserves (Suarez, 1998). Energy expenditure is increased further under cold challenge and hypodense air at high altitudes (Lasiewski, 1963; Welch & Suarez, 2008). High-altitude hummingbird species exhibit physical and behavioral adaptations that affect their performance in low pressure conditions. Increased stroke amplitude and/or having larger wings while hovering compensates for decreased lift generation in thin air (Altshuler, 2003; Altshuler, Dudley, et al., 2004). These differences have been suggested to be responsible, at least in part, for competitive differences under high elevation conditions (Altshuler, 2001, 2006a; Gaffney et al., 2016(submitted)). Hummingbirds have a unique morphological characteristic that sets them apart from other bird species, in that they have extraordinarily large increase in wing area as body mass increases. The exponent of the allometric relationship ( $Y=aW^b$ ) of hummingbird wings was estimated at nearly 1.4X-2X that of all other birds (Rayner, 1988).

To investigate differences in the fundamental niche of closely related hummingbird species that differ in their elevational ranges, we examined and compared their hematological and morphological characteristics. The high elevation Broad-tailed Hummingbird, and low elevation Black-chinned Hummingbird are ideal candidates for exploring intrinsic differences that may contribute to observed performance and competitive differences with respect to elevation and hypobaric hypoxia (Altshuler, 2001; Gaffney et al.,

2016(submitted)). Hummingbirds maintain high blood oxygen carrying capacity but have the smallest erythrocytes and smallest genomes known among birds (Gregory, Andrews, McGuire, & Witt, 2009). Comparison of species hematological characteristics and the hematological response to hypobaric hypoxia is expected to be informative regarding the ways in which each species may be genetically specialized on different altitudes, with a blood-O<sub>2</sub> transport system that is optimized for a particular partial pressure of O<sub>2</sub>. Finally, wing size and wing loading may be morphological parameters that are adapted to a particular optimum pressure that may differ between species(Altshuler & Dudley, 2002).

We tested the following key questions with respect to the comparison between elevational replacement sister species of hummingbirds: **1)** Do high and low elevation species differ in the size of their wings? **2)** How do wings scale relative to body size within and between species? **3)** Are the parameters affecting blood-oxygen carrying capacity ([Hb], Hct, MCV, MCH, MCHC, [RBC]) the same or different between the species? **4)** Does the hematological response to experimentally reduced pressure differ between high and low elevation?

## **MATERIALS AND METHODS**

### *Study System*

We trapped adult male Broad-tailed Hummingbirds (*Selasphorus platycercus*) and Black-chinned Hummingbirds (*Archilochus alexandri*) during the summers of 2015 and 2016 in the foothills of the Sandia Mountains and Jemez Mountains,

northern New Mexico. The majority of birds were trapped in the elevational zone of overlap (2000–2500 m), and occasionally birds were trapped from above and below this zone of overlap. All birds were captured using drop-door traps (Altshuler *et al.* 2010) and were trained to feed from a modified 20-ml syringe during the first two hours after capture. Individual birds were housed within a mesh cage (90 x 90 x 90 cm) and were provided with one modified syringe feeder, one high perch, and one low perch opposite the high perch. Cages were kept covered by a light colored cloth to minimize stress on the birds. Birds were fed daily on a sugar solution and a complete protein diet (Nektarplus, NEKTON; Günnter Enderle, Pforzheim, Baden-Württemberg, Germany). Hummingbird care was in accordance with the university animal care guidelines (IACUC Approval 16-200406-MC). Acclimatization experiments took place in a custom built hypobaric chamber at the University of New Mexico, which is 6.1 m long and 2.4 m in diameter and ventilated with fresh ambient air within 72 hours of capture.

### *Morphological Comparison*

Adult males of each species were collected during the summers of 2015-2016. Hummingbird body mass was measured, during specimen preparation, within 0.01 g using a digital balance. Wings were photographed against ½ cm graph paper and traced using Adobe Illustrator. From these photos, we acquired the total wing area,  $S$ , and length,  $R$ , and the aspect ratio was calculated as

$AR=4R^2/S$  and wing disc loading  $P_w=\frac{w}{\pi\left(\frac{2R}{2}\right)^2}$  (Feinsinger & Chaplin, 1975).

Measurements of the supracoracoideus, pectoralis major, wet lung masses and

heart masses were taken and measured within 0.001 g were recorded using a digital balance. Measurements of skull length, scapula length, femur length, femur width, humerus length and humerus width were measured, from skeletal specimens in the Museum of Southwestern Biology, in millimeters using digital calipers (Pittsburgh 6", Item #68304). Bone widths were measured at the center most part of the bone.

#### *Acclimatization Experiment*

Adult males of each species were used to test whether there was a change in the total hemoglobin concentration ([Hb]), hematocrit (Hct) and mean cell volume (MCV) under reduced pressure and if that change differed between high and low elevation species. Within 48 hours of capture, cages were transported to the hypobaric chamber. Birds were acclimatized at either low pressure, 441 mmHg (4600 m), or were kept at 633 mmHg (1600 m), or for four hours. Immediately post-acclimatization or for birds kept at 633 mmHg, pre-acclimatization, whole blood samples were obtained by venipuncture on the underside of the wing with a heparinized microcapillary tube and Hemocue 201+ cuvettes. An additional 10  $\mu$ l of blood was collected using a 10  $\mu$ l pipet (ErgoOne Model 10, 0.5-10  $\mu$ l) and put into 1990  $\mu$ l of 0.85% saline solution. Red blood cell counts (million/mL) were measured using a hemocytometer. The 3x3mm hemocytometer contains nine 1x1 mm squares, and subsequently made up of twenty-five 0.04 mm<sup>2</sup> squares. The 200 fold dilution was loaded onto the hemocytometer which was covered with a cover slip, depth between the bottom of the chamber and the cover was

0.1 mm. Viewed under a microscope at 40x magnification the total number of red blood cells were counted in seven of the 25 center squares. If cells touched the upper and left limits of the cell they were counted, cells touching the right and lower limits were not taken into account. Packed cell volume (PCV) or Hct (%) was measured using digital calipers after centrifuging. When possible, two samples were taken to measure Hct and values were averaged. Approx. 5 µl of blood was taken to measure [Hb] (g/dL) using a HemoCue Hb 201<sup>+</sup> haemoglobin photometer. Due to the HemoCue's photometric method, when measuring avian blood samples it produces values that are ~1 g/dL greater than those measured using a cyanomethaemoglobin spectrophotometer (Simmons & Lill, 2006) and thus we corrected our [Hb] values by 1 g/dL. After drawing blood samples, birds were euthanized by thoracic compression and prepared as a study skin with a spread wing and tissues deposited at the Museum of Southwestern Biology Department for Genomic Resources (Appendix A-L).

### *Analyses*

#### *Morphological Comparison Analysis*

To test for species differences in morphology, we compared species averages using unpaired t-tests (Table 1). The allometric relationship ( $y=aW^b$ ) was calculated for intraspecific and interspecific differences in the scaling of wing area with body mass. We performed linear regressions on log-transformed data to calculate scaling coefficients for both Black-chinned Hummingbirds and Broad-tailed Hummingbirds separately (intraspecific) and together (interspecific) (Table 2). By combining our data with data from Gregory et al., 2009, we tested



interspecific patterns of wing-area scaling with body mass across 38 species of hummingbird, weighted based on the sample size of each species.

#### *Acclimatization Experiment Analyses*

Packed cell volume (PCV) is the proportion of total blood volume comprised of erythrocytes; we estimated PCV based by measuring the column of erythrocytes in a microcapillary tube of whole blood after centrifuging (approx. 18,000 x g; Eppendorf Centrifuge 5415D) for five minutes. We use 'hematocrit' or 'Hct' interchangeably with PCV in this thesis. Mean corpuscular volume (MCV), the average volume of a single erythrocyte, was calculated as:

$$\mathbf{MCV} \text{ femto liters(fl)} = \frac{\text{PCV} * 10}{\text{RBC}}$$

Mean corpuscular hemoglobin (MCH), the average hemoglobin content of an individual erythrocyte, was calculated as:

$$\mathbf{MCH} \text{ picogram(pg)} = \frac{\text{Hb} * 10}{\text{RBC}}$$

Mean corpuscular hemoglobin concentration (MCHC) was calculated as:

$$\mathbf{MCHC} \text{ (g/L)} = \frac{\text{Hb} * 100}{\text{PCV}}$$

Red blood cell concentration (RBC), the number of red blood cells in a given volume of blood, was calculated using the following equation:

$$\mathbf{RBC}(\text{cells/mm}^3) = \frac{\text{Sum of 7 hemocytometer cells} * \frac{1}{0.28} * 10,000}{1e9}$$

We were unable to obtain measurements for Hct and RBC for 10 and 5 individuals, respectively. We estimated missing values of Hct based on [Hb],

using linear model parameterized with data from 53 hummingbird species (523 individuals;  $R^2 = 0.65$ ):

$$\text{Hct} = 0.2 + 0.019(\text{Hb})$$

Species averages were compared without missing data for RBC comparisons. Using the six hematological parameters, we performed a principal components analysis to test whether species could be differentiated by these six parameters.

## RESULTS

### *Morphological Comparison*

Black-chinned Hummingbirds have higher wing loading than Broad-tailed hummingbirds (t-test:  $p=5.04e-6$ , Figure 1). Broad-tailed Hummingbird wings are, on average,  $149.6 \text{ mm}^2$  larger than those of Black-chinned Hummingbirds. Relative to Black-chinned Hummingbirds, Broad-tailed Hummingbirds also had larger body masses, larger wing-areas, longer wings, longer total wingspan, higher aspect ratios, heavier pectoralis major muscles and lungs, and longer scapula and humerus bones (Table 1). Broad-taileds had strikingly larger wing-areas, wing lengths and humerus lengths (Figure 4).

There does not appear to be an intraspecific scaling relationship between wing area and body mass in Black-chinned or Broad-tailed Hummingbirds, scaling coefficients of 0.10 and 0.04, respectively (Table 2, Figure 2A). Among these two species there does appear to be an interspecific scaling relationship between wing area and body mass, with a scaling coefficient of 0.48 (Table 2, Figure 2B). With the addition of 36 species from Gregory et al 2009, the interspecific scaling

relationship strengthened (Scaling coefficient = 1.2, R<sup>2</sup> = 0.886, Figure 3).

Correlation coefficients for all pair wise comparisons of morphological measurements indicate that wing area, wing length and hummers length easily separate out Black-chinned from Broad-tailed individuals, with all other variables having much overlap between species (Figure 4).

#### *Acclimatization Experiment*

Black-chinned Hummingbirds had higher [Hb] than Broad-tailed hummingbirds, 19.64 g/dL and 18.83g/dL respectively. (Figure 5, t-test:  $p=0.009$ ). Black-chinned hummingbirds had, on average, 12.93 fl lower MCV in post-acclimatization treatment compared to pre-acclimatization (Figure 5; t-test:  $p=0.02$ ). Black-chinneds did not vary in any other parameter between pre- and post-acclimatization. Broad-tailed Hummingbirds hematological parameters did not vary between pre- and post-acclimatization (Table 3).

The principal components analysis revealed that species could not be distinguished using hematological measurements alone (Figure 6). Although the first and second principal component, which included explained 48.2 and 33.2 percent of the variation in the hematological measurements, respectively, the loadings for all six variables did not vary equally or in the same direction.

### **DISCUSSION**

We examined morphological differences and investigated the interspecific and intraspecific scaling relationship of wing area with body size. We also examined

the effect barometric pressure on species hematological response and found no consistent effect of short-term exposure to low pressure within or between species.

### *Morphological Comparison*

Broad-tailed hummingbirds have larger wing areas and lower wing loading values than do Black-chinned Hummingbirds. This may allow Broad-tailed hummingbirds to outcompete Black-chinned Hummingbirds in low density air, due to the beneficial nature of having more wing surface area in less dense air. The higher wing loading in Black-chinned hummingbirds may allow them to gain competitive advantages in higher density air, where larger wings and the ability to move more air with fewer movements, becomes less necessary and maneuverability becomes extremely beneficial.

Intraspecific and Interspecific wing area to body size scaling varied quite drastically. Within these two particular hummingbird species we can see no evidence of an intraspecific scaling pattern. Examining these two species of hummingbird together begins to reveal an interspecific pattern of increased wing area with body size. When compared across many hummingbird species and clades, scaling was consistently around 1.2, which falls in to the range of expected scaling of hummingbirds between 1-1.3 (Rayner, 1988). Alone, Broad-taileds and Black-chinneds wing area scales extremely sublinearly, with an increase in the scaling coefficient as additional species observations were added.

### *Acclimatization Experiment*

Individuals post-acclimatization generally did not differ from those pre-acclimatization in the blood parameters we examined. In particular, they did not show evidence of the predicted erythropoietic response to hypoxia that would increase [Hb] and [Hct]. The one difference that we observed between pre- and post acclimatization was a slight decrease in MCV of Black-chinned Hummingbirds following acclimatization to hypobaric hypoxia; however, this particular result was subject to small sample sizes. Black-chinned Hummingbirds had strikingly higher [Hb] than Broad-tailed Hummingbirds. This may be reflective of the difference in HbD O<sub>2</sub>-affinity between species. Broad-tailed Hummingbird has two genetic changes to hemoglobin,  $\alpha$ D11 (Thr → Ser) and  $\alpha$ D55 (Val → Ile), that confer higher affinity to its HbD isoform (Gaffney et al., 2016 (submitted)). This intrinsic difference may result in the lower [Hb] we see in Broad-taileds when compared to Black-chinneds, while other parameters remained similar. At elevations 2000-2500 m, Black-chinned Hummingbirds may elevate their blood-O<sub>2</sub> carrying capacity to compensate for a reduced in S<sub>a</sub>O<sub>2</sub>. By contrast, the higher O<sub>2</sub>-affinity of HbD in Broad-tailed Hummingbirds may facilitate their maintenance of lower [Hb] under hypobaric hypoxia, a condition that might be advantageous as it is known to be in Tibetan humans (Beall et al., 2010).

In humans, increased [Hb] is associated with increased blood viscosity which in turn produces higher vascular resistance, reducing V<sub>O<sub>2</sub> max</sub> (Connes, 2006; Guyton & Richardson, 1961) . Thus a blunted erythropoietic (EPO) response in

these hummingbirds, similar to those in many other high altitude species, may be adaptive (e.g. Storz et al., 2009; Weber, 2002). Highland mice have been shown to have lower DPG/Hb ratios compared to lowland mice (Snyder, 1982). This has been suggested, in conjunction with suppressed sensitivity to anions, to be an adaptive mechanism to maintain increased blood-O<sub>2</sub> affinity in reduced oxygen environments (Storz, 2010; Storz et al., 2009, 2010). Alternatively, the amount of time exposed to low pressure may not have been substantial enough to produce visible changes to red blood cell number or hemoglobin concentrations.

### *Conclusions*

Broad-tailed Hummingbirds are only slightly larger than Black-chinned Hummingbirds in body mass, with extensive body mass overlap between the two species. However, the wings of Broad-tailed Hummingbirds are substantially longer, with larger area, longer humerus bones, and heavier flight muscles. Its lower wing loading likely gives this high-elevation species an advantage producing lift in low-density air. Conversely, the smaller wings of Black-chinned Hummingbirds may confer maneuverability, a possible basis for its competitive advantage at mid and low elevations. Hummingbird wing area scales superlinearly with body size across hummingbird species, but there is no evidence of this scaling relationship within species. At similar elevations, Broad-tailed Hummingbirds maintain lower [Hb] than Black-chinned Hummingbirds, suggesting that the latter species needs to compensate for its low affinity Hb. Both species appear to have blunted erythropoietin responses to hypobaric hypoxia treatment.

## Chapter 2

### FIGURES

Figure 1. Black-chinned Hummingbirds(n=23) have higher wing disc loading than Broad-tailed Hummingbirds(n=29) (t-test:  $p=5.04e-6$ ).

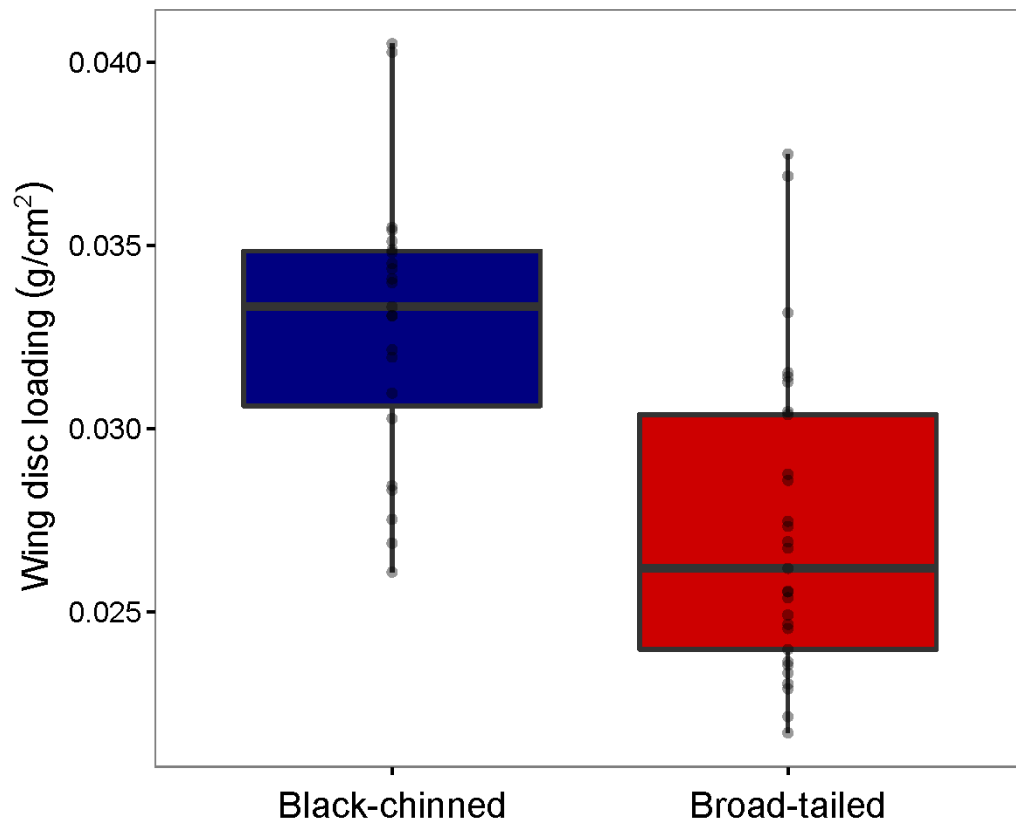


Figure 2. Mean wing area as a function of body mass for Black-chinned and Broad-tailed Hummingbirds. A) Intraspecific scaling: There is no association of wing area and body mass in either Black-chinned (Slope =  $0.2 \pm 0.13$  standard error; blue) and Broad-tailed hummingbirds (Slope =  $0.1 \pm 0.10$  standard error; red). B) Interspecific scaling: There is a positive association of wing area to body mass when both hummingbird species are included (Scaling coefficient is  $0.48 \pm 0.13$  standard error and is different from zero,  $p < 0.01$ ).

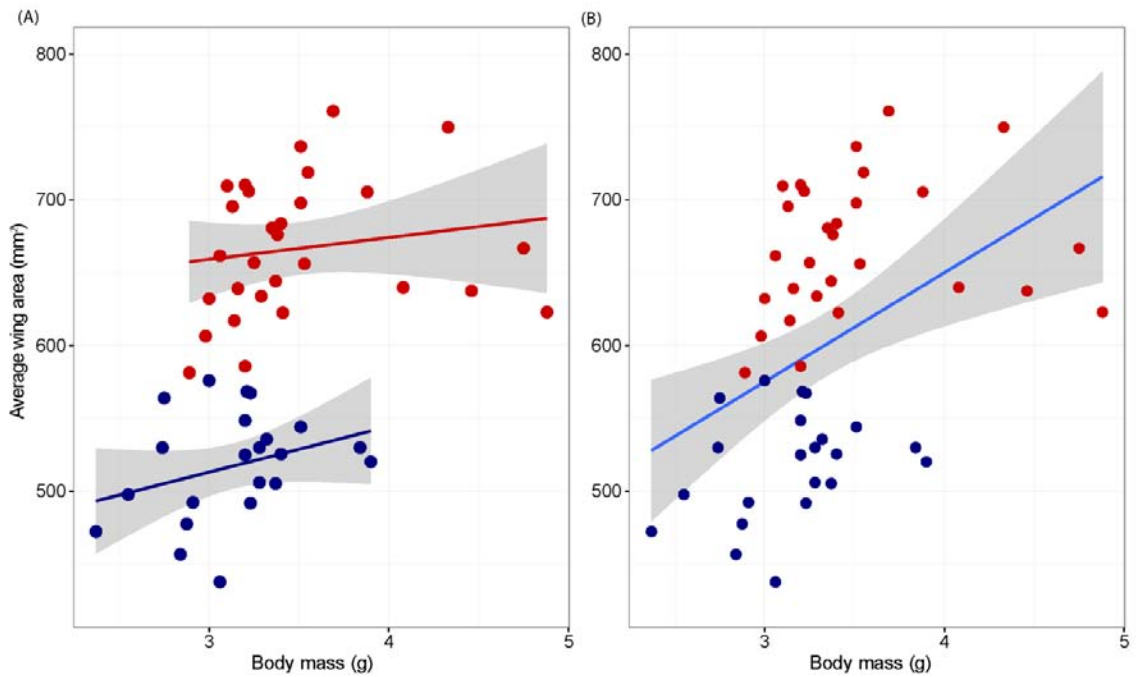




Figure 3. Mean wing area and mean body mass are positively associated among 38 hummingbird species. Scaling coefficient of 1.2 and standard error of  $\pm 0.07$ .

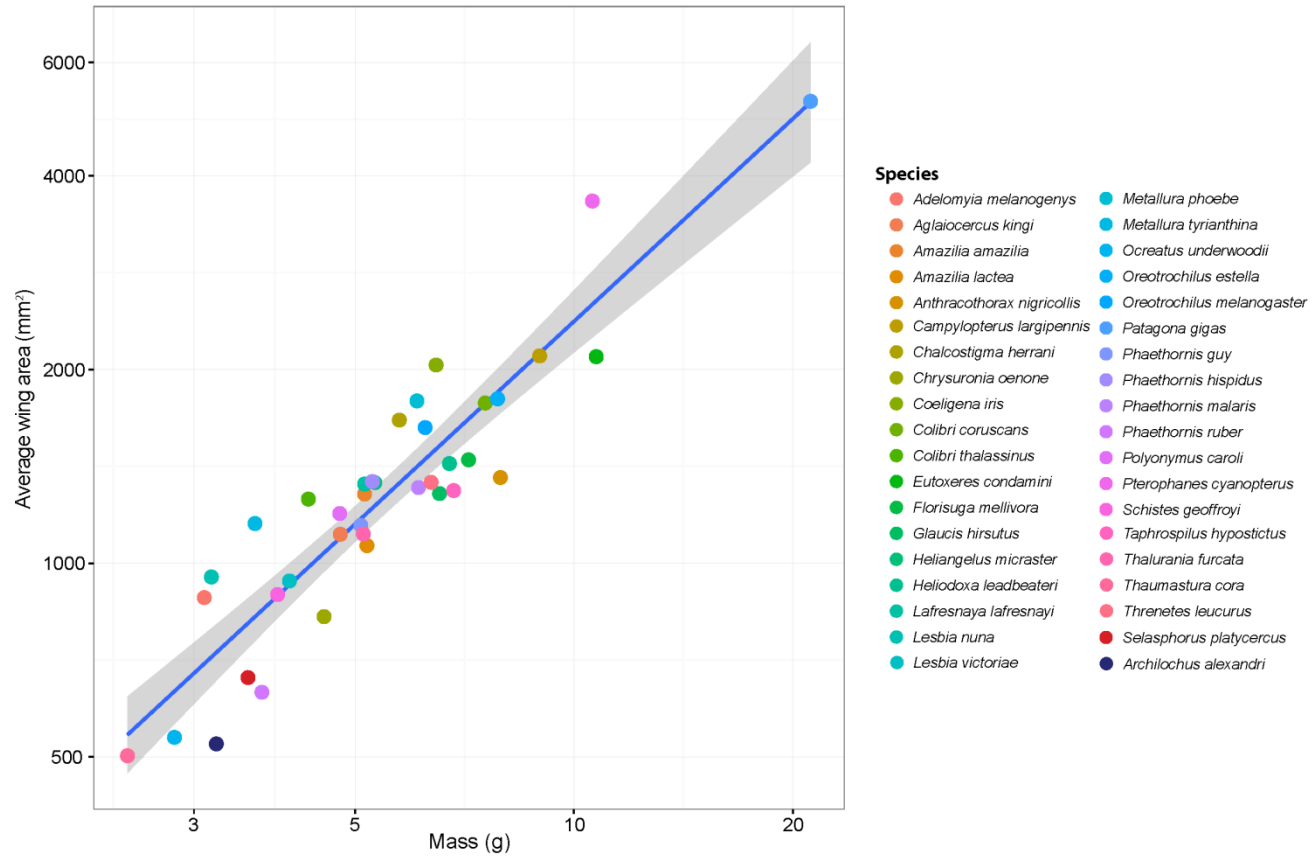


Figure 4. Correlation matrix of body mass (g), supracoracoideus mass (g), pectoralis major mass (g), heart mass (g), average lung mass (g), average wing area (mm<sup>2</sup>), average wing length (mm), skull length (mm), scapula length (mm), femur width (mm), femur length (mm), humerus width (mm) and humerus length (mm)., Interspecific correlation coefficient (black), Black-chinned intraspecific correlation coefficient (blue) and Broad-tailed intraspecific correlation coefficients (red) with significant differences noted ( $p < 0.1$ .,  $p < 0.05$  \*,  $p < 0.01$  \*\*,  $p < 0.001$  \*\*\*).

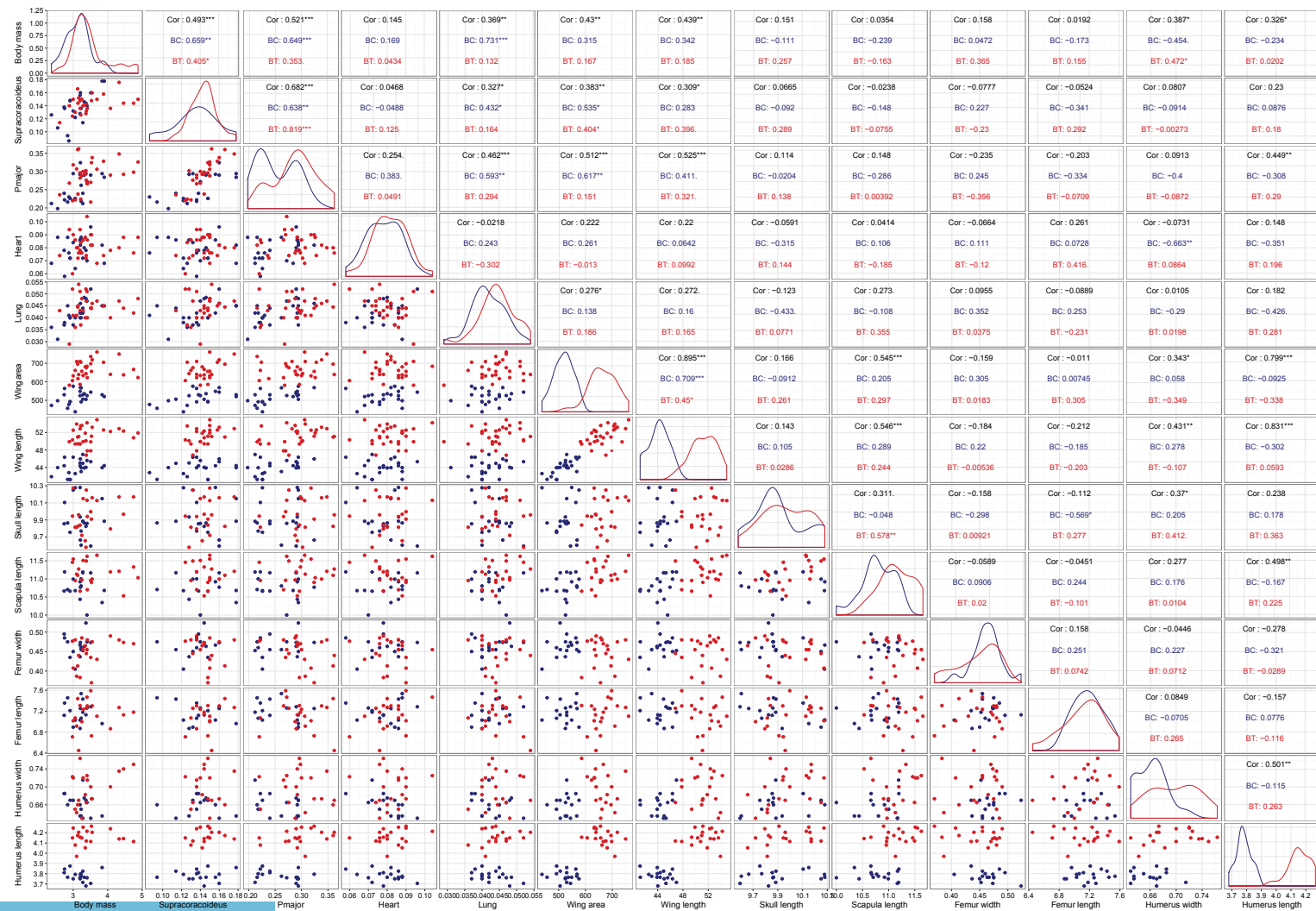


Figure 5. Hemoglobin concentrations (g/dL) (Hb), total red blood cell counts (cells/mm<sup>3</sup>) (RBC), mean cell volume (fl) (MCV), mean cellular hemoglobin (pg) (MCH), mean cellular hemoglobin concentration (g Hb/ml RBC) (MCHC) and Haematocrit (%) (Hct), for black-chinneds (blue) and broad-taileds (red) in either the normal pressure (1600 m elevation) or reduced pressure (4600 m simulated elevation) treatment. Black-chinneds differed between treatments for MCV (t-test;  $p=0.01$ ) and differed from broad-taileds in [Hb] (t-test;  $p=0.009$ ).

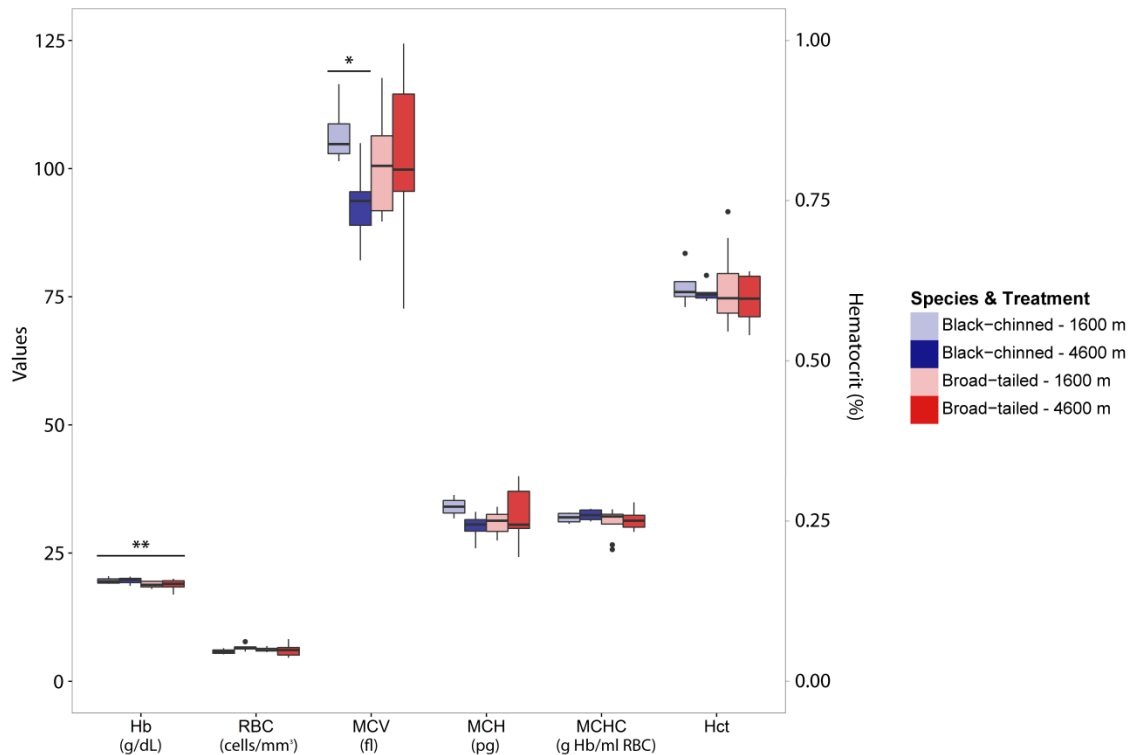
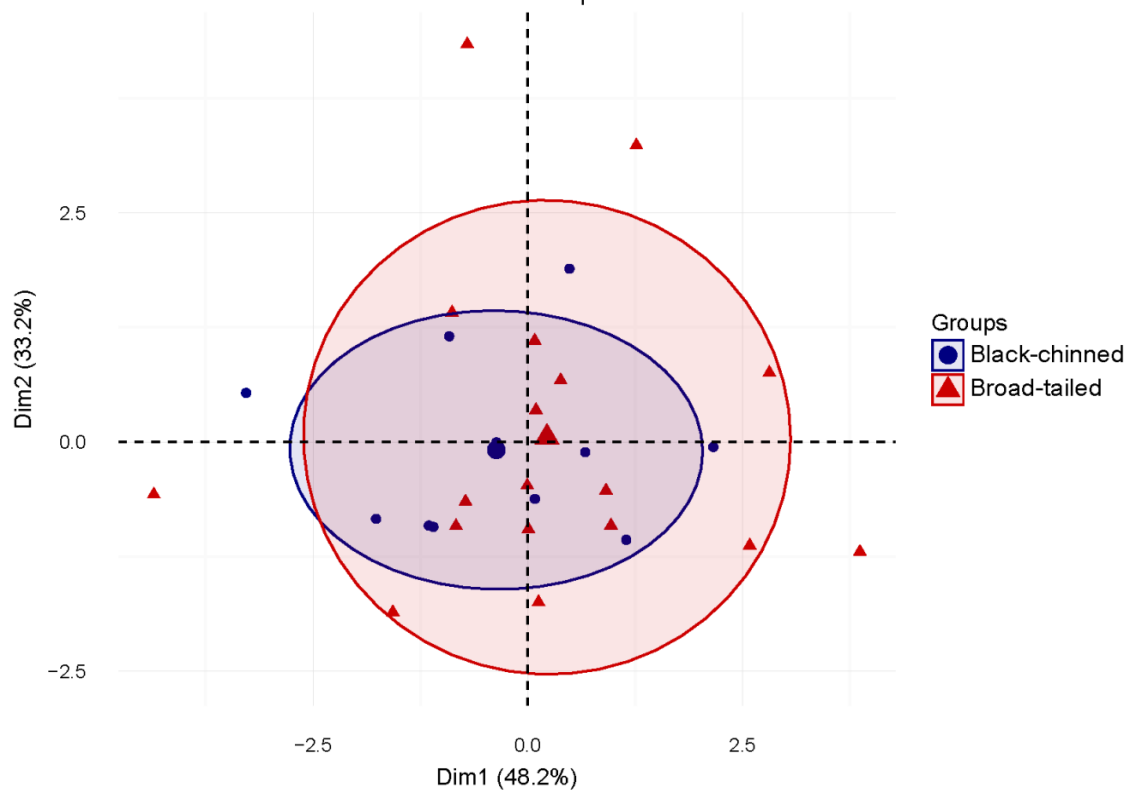


Figure 6. Principal components analyses of six blood parameters show that we cannot differentiate Black-chinned Hummingbirds from Broad-tailed Hummingbirds.



## Chapter 2

### TABLES

**Table 1.** Species morphological measurements  $\pm$  SD, all bolded variables were statically different between species at an  $\alpha=0.05$  level.

	<i>Black-chinned Hummingbirds</i>		<i>Broad-tailed Hummingbirds</i>	
	Value	N	Value	N
<b>Bodymass (g)</b>	<b>3.13 <math>\pm</math> 0.37</b>	<b>23</b>	<b>3.48 <math>\pm</math> 0.54</b>	<b>30</b>
Supracoracoideus (g)	0.14 $\pm$ 0.02	22	0.14 $\pm$ 0.01	26
<b>Pectoralis major (g)</b>	<b>0.25 <math>\pm</math> 0.04</b>	<b>24</b>	<b>0.29 <math>\pm</math> 0.04</b>	<b>30</b>
Heart (g)	0.078 $\pm$ 0.01	24	0.08 $\pm$ 0.01	30
Average lung (g)	0.042 $\pm$ 0.005	24	0.044 $\pm$ 0.005	30
Skull length (mm)	9.91 $\pm$ 0.21	19	9.96 $\pm$ 0.2	22
<b>Scapula length (mm)</b>	<b>10.79 <math>\pm</math> 0.33</b>	<b>17</b>	<b>11.15 <math>\pm</math> 0.32</b>	<b>21</b>
<b>Humerus length (mm)</b>	<b>3.78 <math>\pm</math> 0.05</b>	<b>18</b>	<b>4.16 <math>\pm</math> 0.08</b>	<b>19</b>
<b>Humerus width(mm)</b>	<b>0.66 <math>\pm</math> 0.02</b>	<b>16</b>	<b>0.7 <math>\pm</math> 0.04</b>	<b>20</b>
Femur length (mm)	7.18 $\pm$ 0.19	17	7.12 $\pm$ 0.28	20
Femur width (mm)	0.46 $\pm$ 0.03	17	0.45 $\pm$ 0.04	21
<b>Average wing area (mm<sup>2</sup>)</b>	<b>517.18 <math>\pm</math> 36.65</b>	<b>23</b>	<b>666.81 <math>\pm</math> 46.94</b>	<b>29</b>
<b>Avgerage wing length (mm)</b>	<b>44.06 <math>\pm</math> 1.68</b>	<b>23</b>	<b>51.29 <math>\pm</math> 2.05</b>	<b>29</b>
<b>Wingspan (mm)</b>	<b>104.81 <math>\pm</math> 3.75</b>	<b>23</b>	<b>119.97 <math>\pm</math> 3.58</b>	<b>30</b>
<b>Aspect Ratio</b>	<b>7.53 <math>\pm</math> 0.42</b>	<b>23</b>	<b>7.92 <math>\pm</math> 0.61</b>	<b>29</b>
<b>Wing disc loading</b>	<b>0.033 <math>\pm</math> 0.004</b>	<b>23</b>	<b>0.027 <math>\pm</math> 0.004</b>	<b>29</b>

**Table 2.** Scaling coefficients for average wing area and body mass with sample sizes. Scaling coefficients that differ from zero are bolded ( $p < 0.001$ ).

	N	Scaling Coefficient	Data
Intraspecific			
Black-chinned	28	0.20	This study
Broad-tailed	24	0.10	This study
Interspecific			
<b>Black-chinned &amp; Broad-tailed</b>	<b>52</b>	<b>0.48</b>	<b>This study</b>
<b>38 hummingbird species</b>	<b>136</b>	<b>1.20</b>	<b>Gregory et al 2009; this study</b>

**Table 3.** Hemoglobin concentrations (g/dL) (Hb), total red blood cell counts (cells/mm<sup>3</sup>) (RBC), mean cell volume (fl) (MCV), mean cellular hemoglobin (pg) (MCH), mean cellular hemoglobin concentration (g Hb/ml RBC) (MCHC) and Haematocrit (%) (Hct), for black-chinneds and broad-taileds. Values are reported for the reduced pressure (4600 m) and normal pressure (1600 m) treatments and overall species averages  $\pm$  SD. Black-chinneds differed between treatments for MCV (t-test;  $p=0.01$ ) and differed from broad-taileds for [Hb] (t-test;  $p=0.009$ )

Measurement	<i>Black-chinned Hummingbirds</i>			<i>Broad-tailed Hummingbirds</i>		
	1600 m (n=4)	4600 m (n=6)	Species Average	1600 m (n=9)	4600 m (n=9)	Species Average
Hb (g/dL)	19.63 $\pm$ 0.68	19.65 $\pm$ 0.68	19.64 $\pm$ 0.64	18.89 $\pm$ 0.59	18.83 $\pm$ 0.98	18.86 $\pm$ 0.79
RBC (cells/mm <sup>3</sup> )	5.79 $\pm$ 0.54	6.56 $\pm$ 0.65	6.25 $\pm$ 0.70	6.15 $\pm$ 0.38	6.00 $\pm$ 1.12	6.07 $\pm$ 0.81
MCV (fl)	106.86 $\pm$ 6.68	92.98 $\pm$ 7.77	98.53 $\pm$ 9.99	100.27 $\pm$ 9.72	102.17 $\pm$ 16.94	101.21 $\pm$ 13.43
MCH (pg)	34.04 $\pm$ 2.00	30.13 $\pm$ 2.48	31.69 $\pm$ 2.97	30.83 $\pm$ 2.14	32.17 $\pm$ 5.07	31.5 $\pm$ 3.84
MCHC (g Hb/ml RBC)	31.87 $\pm$ 1.08	32.42 $\pm$ 1.12	32.20 $\pm$ 1.08	30.93 $\pm$ 2.85	31.59 $\pm$ 1.83	31.26 $\pm$ 2.35
Hct (%)	0.62 $\pm$ 0.04	0.61 $\pm$ 0.01	0.61 $\pm$ 0.02	0.62 $\pm$ 0.06	0.60 $\pm$ 0.04	0.61 $\pm$ 0.05

## LIST OF APPENDICES

Appendix A. Individuals experimental identifier names, species names, hold times and notation of whether an individual participated in a competition trial.

Appendix B. Experimental IDs, date of activity assay experiment, name of the observer of who recorded the experiment data, the number of seconds a bird spent hovering at 633 mmHg (1600 m) and 590 mm Hg (2200 m).

Appendix C. Experimental IDs, the number of seconds a bird spent hovering at 550 mmHg (2800 m), 512 mm Hg (3400 m), 475 mm Hg (4000 m) 441 mmHg (4600m), and 633 mmHg after the conclusion of the activity assay.

Appendix D. Experimental IDs, Museum of Southwestern Biology Bird Division NK number, and URLs for each specimen that was collected.

Appendix E. Competition experiment number, date and observer of competition experiment, experimental ID of birds that participated and the simulated elevation.

Appendix F. Competition experiment number, notation of whether the Black-chinned or Broad-tailed Hummingbird showed aggressive behavior, number of successful and failed displacement attempts by the Black-chinned Hummingbird.



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Appendix H. Competition experiment number, percentage of time spent on the high perch by the Black-chinned and Broad-tailed Hummingbird, and the determined winner of the competition trial.

Appendix I. Species names, Latitude (degrees, minutes), Longitude (degrees, minutes), the elevation (meters), date and time at which the individual was caught. The date and time of the experiment an individual participated in, individual experimental IDs, and Museum of Southwestern Biology Bird Division NK numbers.

Appendix J. Museum of Southwestern Biology Bird Division NK numbers, measurements of an individual's body mass (g), supracoracoideus (g), pectoralis major (g) , heart (g), average lung (g), total wingspan (mm), body width (mm), and average wing area measurement ( $\text{mm}^2$ )

Appendix K. Museum of Southwestern Biology Bird Division NK numbers, measurements of an individual's average wing length ( $\text{mm}^2$ ), aspect ratio, skull length(mm), scapula length (mm), femur width (mm), femur length (mm), humerus with (mm), and humerus length (mm).

Appendix L. URLs for each specimen collected.

Appendix A. Individuals experimental ID names, species names, hold times and notation of whether an individual participated in a competition trial.

Experiment ID	Species	Hold Time Until Experiment (hours)	Competition participation
BTHU_1	<i>Selasphorus platycercus</i>	70.0	Yes
BTHU_2	<i>Selasphorus platycercus</i>	70.0	Yes
BTHU_3	<i>Selasphorus platycercus</i>	70.0	Yes
BCHU_1	<i>Archilochus alexandri</i>	70.0	Yes
BCHU_2	<i>Archilochus alexandri</i>	70.0	Yes
BCHU_3	<i>Archilochus alexandri</i>	70.0	Yes
2015BT01	<i>Selasphorus platycercus</i>	50.6	Yes
2015BC05	<i>Archilochus alexandri</i>	27.1	Yes
2015BC07	<i>Archilochus alexandri</i>	27.6	Yes
2015BC08	<i>Archilochus alexandri</i>	18.8	Yes
2015BT07	<i>Selasphorus platycercus</i>	49.9	Yes
2015BT08	<i>Selasphorus platycercus</i>	48.3	Yes
2015BC09	<i>Archilochus alexandri</i>	47.5	Yes
2015BC10	<i>Archilochus alexandri</i>	46.0	Yes
2015BC11	<i>Archilochus alexandri</i>	25.5	No
2015BC12	<i>Archilochus alexandri</i>	14.7	Yes
2015BT09	<i>Selasphorus platycercus</i>	70.0	Yes
2015BT19	<i>Selasphorus platycercus</i>	40.4	Yes
2015BT20	<i>Selasphorus platycercus</i>	40.5	No
2015BT22	<i>Selasphorus platycercus</i>	18.7	No
2015BC18	<i>Archilochus alexandri</i>	16.4	Yes
2015BC02	<i>Archilochus alexandri</i>	50.6	Yes
2015BC03	<i>Archilochus alexandri</i>	48.6	Yes
2015BC04	<i>Archilochus alexandri</i>	49.2	Yes
2015BC06	<i>Archilochus alexandri</i>	70.0	Yes
2015BC16	<i>Archilochus alexandri</i>	38.5	Yes
2015BC17	<i>Archilochus alexandri</i>	26.2	Yes
BCHU_106	<i>Archilochus alexandri</i>	70.0	Yes
2015BT02	<i>Selasphorus platycercus</i>	49.5	Yes
2015BT03	<i>Selasphorus platycercus</i>	50.5	Yes
2015BT04	<i>Selasphorus platycercus</i>	27.6	Yes
2015BT06	<i>Selasphorus platycercus</i>	70.0	Yes
2015BT10	<i>Selasphorus platycercus</i>	13.3	Yes
2015BT11	<i>Selasphorus platycercus</i>	13.1	Yes
2015BT16	<i>Selasphorus platycercus</i>	15.4	Yes
2015BT15	<i>Selasphorus platycercus</i>	38.9	Yes
2015BT05	<i>Selasphorus platycercus</i>	74.7	Yes

Appendix B. Experimental IDs, date of activity assay experiment, name of the), observer of who recorded the experiment data, the number of seconds a bird spent hovering at 633 mmHg (1600 m) and 590 mm Hg (2200 m).

Experiment ID	Activity Experiment Date	Activity Observer	Seconds hovering at 1600 m	Seconds hovering at 2200 m
BTHU_1	7-Aug	Ariel Gaffney	322	
BTHU_2	7-Aug	Sebastian Restrepo-Cruz	47	
BTHU_3	7-Aug	Sebastian Restrepo-Cruz	280	
BCHU_1	7-Aug	Sebastian Restrepo-Cruz	229	
BCHU_2	7-Aug	Ariel Gaffney	560	
BCHU_3	7-Aug	Ariel Gaffney	312	
2015BT01	28-May	Ariel Gaffney	0	3
2015BC05	1-Jun	Kobie Boslough	241	254
2015BC07	5-Jun	Kobie Boslough	329	276
2015BC08	11-Jun	Ariel Gaffney	4.5	31
2015BT07	11-Jun	Kobie Boslough	60	118
2015BT08	11-Jun	Kobie Boslough	151	249
2015BC09	16-Jun	Ariel Gaffney	157	119
2015BC10	16-Jun	Kobie Boslough	120	57
2015BC11	19-Jun	Kobie Boslough	165	155
2015BC12	19-Jun	Kobie Boslough	0	17
2015BT09	19-Jun	Ariel Gaffney	150	165
2015BT19	24-Jul	Ariel Gaffney	201	256
2015BT20	24-Jul	Deborah Boro	341	235
2015BT22	24-Jul	Ariel Gaffney	33	10
2015BC18	24-Jul	Ariel Gaffney	142	123
2015BC02	28-May	Ariel Gaffney	490	313
2015BC03	28-May	Ariel Gaffney	520	358
2015BC04	28-May	Ariel Gaffney	286	118
2015BC06	5-Jun	Ariel Gaffney	73	43
2015BC16	16-Jul	Kobie Boslough	210	212
2015BC17	16-Jul	Kobie Boslough	173	153
BCHU_106				
2015BT02	28-May	Mariana Villegas	152	145
2015BT03	28-May	Kobie Boslough	168	37
2015BT04	1-Jun	Ariel Gaffney	257	155
2015BT06	5-Jun	Ariel Gaffney	337	101
2015BT10	16-Jun	Kobie Boslough	130	61
2015BT11	16-Jun	Ariel Gaffney	262	310
2015BT16	16-Jul	Ariel Gaffney	71	24
2015BT15	16-Jul	Ariel Gaffney	271	297
2015BT05				

Appendix C. Experimental IDs, the number of seconds a bird spent hovering at 550 mmHg (2800 m), 512 mm Hg (3400 m), 475 mm Hg (4000 m) 441 mmHg (4600m), and 633 mmHg after the conclusion of the activity assay.

Experiment ID	Seconds hovering at 2800 m	Seconds hovering at 3400 m	Seconds hovering at 4000 m	Seconds hovering at 4600 m	Seconds hovering at 1600 m again
BTHU_1				226	
BTHU_2				2	
BTHU_3				61	
BCHU_1				30	
BCHU_2				129	
BCHU_3				135	
2015BT01	0	5	0	0	
2015BC05	226	149	150	177	
2015BC07	183	172	174	100	
2015BC08	60	7	1	3	
2015BT07	65	37	100	51	
2015BT08	180	115	119	67	
2015BC09	123	138	99	56	159
2015BC10	42	10	0	4	0
2015BC11	140	89	69	54	117
2015BC12	66	46	34	82	0
2015BT09	214	207	117	158	104
2015BT19	228	188	183	184	293
2015BT20	238	198	171	167	168
2015BT22	19	15	12	4	17
2015BC18	96	116	146	137	217
2015BC02	303	255	213	182	
2015BC03	291	166	145	106	
2015BC04	156	104	94	62	
2015BC06	52	27	38	29	
2015BC16	176	188	143	46	112
2015BC17	175	193	124	117	195
BCHU_106					
2015BT02	199	162	184	188	
2015BT03	84	68	58	46	
2015BT04	140	75	60	106	
2015BT06	63	42	50	101	
2015BT10	30	62	24	30	22
2015BT11	336	334	286	254	288
2015BT16	45	35	14	12	46
2015BT15	342	320	251	235	291
2015BT05					

Appendix D. Experimental IDs, Museum of Southwestern Biology Bird Division NK number, and URLs for each specimen that was collected.

Experiment ID	NK Number	Arctos URL
BTHU_1	222332	<a href="http://arctos.database.museum/guid/MSB:Bird:45350">http://arctos.database.museum/guid/MSB:Bird:45350</a>
BTHU_2	222333	<a href="http://arctos.database.museum/guid/MSB:Bird:45351">http://arctos.database.museum/guid/MSB:Bird:45351</a>
BTHU_3	222334	<a href="http://arctos.database.museum/guid/MSB:Bird:45352">http://arctos.database.museum/guid/MSB:Bird:45352</a>
BCHU_1	222335	<a href="http://arctos.database.museum/guid/MSB:Bird:45353">http://arctos.database.museum/guid/MSB:Bird:45353</a>
BCHU_2	222336	<a href="http://arctos.database.museum/guid/MSB:Bird:45354">http://arctos.database.museum/guid/MSB:Bird:45354</a>
BCHU_3	222337	<a href="http://arctos.database.museum/guid/MSB:Bird:45355">http://arctos.database.museum/guid/MSB:Bird:45355</a>
2015BT01	250954	<a href="http://arctos.database.museum/guid/MSB:Bird:45356">http://arctos.database.museum/guid/MSB:Bird:45356</a>
2015BC05	250955	<a href="http://arctos.database.museum/guid/MSB:Bird:45357">http://arctos.database.museum/guid/MSB:Bird:45357</a>
2015BC07	250956	<a href="http://arctos.database.museum/guid/MSB:Bird:45358">http://arctos.database.museum/guid/MSB:Bird:45358</a>
2015BC08	250957	<a href="http://arctos.database.museum/guid/MSB:Bird:45359">http://arctos.database.museum/guid/MSB:Bird:45359</a>
2015BT07	250958	<a href="http://arctos.database.museum/guid/MSB:Bird:45360">http://arctos.database.museum/guid/MSB:Bird:45360</a>
2015BT08	250959	<a href="http://arctos.database.museum/guid/MSB:Bird:45361">http://arctos.database.museum/guid/MSB:Bird:45361</a>
2015BC09	250960	<a href="http://arctos.database.museum/guid/MSB:Bird:45362">http://arctos.database.museum/guid/MSB:Bird:45362</a>
2015BC10	250961	<a href="http://arctos.database.museum/guid/MSB:Bird:45363">http://arctos.database.museum/guid/MSB:Bird:45363</a>
2015BC11	250962	<a href="http://arctos.database.museum/guid/MSB:Bird:45364">http://arctos.database.museum/guid/MSB:Bird:45364</a>
2015BC12	250963	<a href="http://arctos.database.museum/guid/MSB:Bird:45365">http://arctos.database.museum/guid/MSB:Bird:45365</a>
2015BT09	250964	<a href="http://arctos.database.museum/guid/MSB:Bird:45366">http://arctos.database.museum/guid/MSB:Bird:45366</a>
2015BT19	250974	<a href="http://arctos.database.museum/guid/MSB:Bird:45373">http://arctos.database.museum/guid/MSB:Bird:45373</a>
2015BT20	250975	<a href="http://arctos.database.museum/guid/MSB:Bird:45374">http://arctos.database.museum/guid/MSB:Bird:45374</a>
2015BT22	250976	<a href="http://arctos.database.museum/guid/MSB:Bird:45375">http://arctos.database.museum/guid/MSB:Bird:45375</a>
2015BC18	250977	<a href="http://arctos.database.museum/guid/MSB:Bird:45376">http://arctos.database.museum/guid/MSB:Bird:45376</a>
2015BC02		
2015BC03		
2015BC04		
2015BC06		
2015BC16		
2015BC17		
BCHU_106		
2015BT02		
2015BT03		
2015BT04		
2015BT06		
2015BT10		
2015BT11		
2015BT16		
2015BT15		
2015BT05		

Appendix E. Competition experiment number, date and observer of competition experiment, experimental ID of birds that participated and the simulated elevation.

Experiment Number	Date of Experiment	Observer	Individual 1	Individual 2	Simulated Elevation (m)
1	22-Jul	Ariel Gaffney	BCHU_1	BTHU_1	4600
2	22-Jul	Ariel Gaffney	BCHU_1	BTHU_3	4600
3	23-Jul	Ariel Gaffney	BCHU_1	BTHU_1	1600
4	23-Jul	Ariel Gaffney	BCHU_1	BTHU_3	1600
5	23-Jul	Ariel Gaffney	BCHU_1	BTHU_2	1600
6	7-Aug	Sebastian	BCHU_2	BTHU_1	4600
7	7-Aug	Ariel Gaffney	BCHU_106	BTHU_3	4600
8	7-Aug	Ariel Gaffney	BCHU_3	BTHU_2	4600
9	7-Aug	Ariel Gaffney	BCHU_2	BTHU_2	4600
10	7-Aug	Ariel Gaffney	BCHU_3	BTHU_3	4600
11	7-Aug	Sebastian	BCHU_106	BTHU_1	4600
12	7-Aug	Ariel Gaffney	BCHU_106	BTHU_2	4600
13	11-Aug	Ariel Gaffney	BCHU_106	BTHU_3	1600
14	11-Aug	Ariel Gaffney	BCHU_106	BTHU_2	1600
15	11-Aug	Ariel Gaffney	BCHU_2	BTHU_1	1600
16	11-Aug	Ariel Gaffney	BCHU_2	BTHU_3	1600
17	11-Aug	Ariel Gaffney	BCHU_2	BTHU_2	1600
18	11-Aug	Ariel Gaffney	BCHU_3	BTHU_2	1600
19	11-Aug	Ariel Gaffney	BCHU_3	BTHU_1	1600
20	11-Aug	Ariel Gaffney	BCHU_106	BTHU_1	1600
21	28-May	Ariel Gaffney	2015BC03	2015BT02	4600
22	28-May	Mariana Villegas	2015BC02	2015BT01	4600
23	28-May	Ariel Gaffney	2015BC04	2015BT03	4600
24	1-Jun	Ariel Gaffney	2015BC05	2015BT04	4600
25	5-Jun	Kobie Boslough	2015BC07	2015BT06	4600
26	5-Jun	Ariel Gaffney	2015BC06	2015BT05	4600
27	11-Jun	Kobie Boslough	2015BC08	2015BT08	1600
28	11-Jun	Ariel Gaffney	2015BC07	2015BT07	1600
29	16-Jun	Kobie Boslough	2015BC09	2015BT10	1600
30	16-Jun	Ariel Gaffney	2015BC10	2015BT11	1600
31	19-Jun	Kobie Boslough	2015BC12	2015BT09	1600
32	16-Jul	Kobie Boslough	2015BC17	2015BT16	1600
33	16-Jul	Ariel Gaffney	2015BC16	2015BT15	4600
34	24-Jul	Ariel Gaffney	2015BC18	2015BT19	1600

Appendix F. Competition experiment number, notation of whether the Black-chinned or Broad-tailed Hummingbird showed aggressive behavior, number of successful and failed displacement attempts by the Black-chinned Hummingbird.

Experiment No.	Aggression by BCHU	Aggression by BTHU	BCHU Successful displacements	BCHU Failed displacements
1	No	Yes	0	0
2	No	Yes	0	0
3	Yes	Yes	Switches places with BTHU many times, multiple attempts to displace BTHU	
4	Yes	Yes	1	0
5	Yes	Yes	Switches places with BTHU a few times, number not noted	
6		Yes	Switches places with BTHU many times, multiple attempts to displace BTHU	
7	No	Yes	0	0
8	Yes	Yes	Switches places with BTHU many times, multiple attempts to displace BTHU	
9	Yes	Yes	Switches places with BTHU many times, multiple attempts to displace BTHU	
10	No	Yes	No aggressive behavior	No aggressive behavior
11	Yes	Yes	3	2
12	No	Yes	0	0
13	Yes	Yes	1	1
14	Yes	Yes	0	1
15	Yes	Yes	Very few interactions between birds, exact number not recorded	
16	Yes	Yes	5	0
17	Yes	Yes	4	1
18	Yes	Yes	1	0
19	Yes	Yes	1	0
20	Yes	Yes	3	0
21	Yes	Yes	22	10
22	Yes	Yes	6	24
23	Yes	Yes	16	18
24	Yes	Yes	18	50
25	Yes	Yes	15	9
26	Yes	Yes	3	45
27	Yes	Yes	0	0
28	Yes	Yes	3	0
29	Yes	Yes	13	21
30	Yes	Yes	7	0
31	Yes	Yes	0	1
32	Yes	Yes	24	5
33	Yes	Yes	13	22
34	Yes	Yes	19	25



Appendix G. Competition experiment number, number of successful and failed displacement attempts by the Broad-tailed Hummingbird, displacement rates.

Experiment No.	BTHU Successful displacements	BTHU Failed displacements	BCHU displacement rate of BTHU	BTHU displacement rate of BCHU
1	2	7	0.00	0.22
2	0	greater than 2	No aggression by BCHU	BTHU could not displace BCHU but attempted multiple times
3	Switches places with BCHU many times, more than one attempts to displace BCHU		Approximately even	
4	0	1	1.00	0.00
5	Switches places with BCHU a few times, number not noted		Approximately even	
6	Switches places with BCHU many times, more than one attempts to displace BCHU		Approximately even	
7	1	3	0.00	0.33
8	Switches places with BCHU many times, more than one attempts to displace BCHU		Approximately even	
9	Switches places with BCHU many times, more than one attempts to displace BCHU		Approximately equal until 15 minutes into the trial all aggression by BCHU stopped and BCHU stopped hovering and clung to high perch	Approximately equal until 15 minutes into the trial all aggression by BCHU stopped and BCHU stopped hovering and clung to high perch
10	>2	not recorded	No aggression	BTHU would displace BCHU and then leave perch to hover and feed
11	5	0	0.60	1.00
12	1	0	0.00	1.00
13	0	1	0.50	0.00
14	2	0	0.00	1.00
15	Very few interactions between birds, exact number not recorded		Approximately even	
16	0	1	1.00	0.00
17	0	3	0.80	0.00
18	0	3	1.00	0.00
19	1	0	1.00	1.00
20	1	2	1.00	0.33
21	7	6	0.69	0.54
22	9	2	0.20	0.82
23	15	15	0.47	0.50
24	19	58	0.26	0.25
25	12	133	0.63	0.08
26	11	1	0.06	0.92
27	5	17	0.00	0.23
28	5	0	1.00	1.00
29	26	40	0.38	0.39
30	4	30	1.00	0.12
31	4	16	0.00	0.20
32	2	3	0.83	0.40
33	7	21	0.37	0.25
34	11	30	0.43	0.27

Appendix H. Competition experiment number, percentage of time spent on the high perch by the Black-chinned and Broad-tailed Hummingbird, and the determined winner of the competition trial.

Experiment Number	% of Time on High perch BCHU	% of Time on High perch BTHU	Winner
1	90	10	BTHU
2	100	0	BTHU
3	25	75	BTHU
4	1	0	BCHU
5	80	20	BCHU
6	70	30	BCHU
7	90	10	BTHU
8	25	75	BTHU
9	95	5	BTHU
10	75	25	BTHU
11	5	90	BTHU
12	25	75	BTHU
13	30	70	BTHU
14	5	95	BTHU
15	90	10	BCHU
16	99	1	BCHU
17	100	0	BCHU
18	95	5	BCHU
19	95	5	BCHU
20	90	10	BCHU
21	90	10	BCHU
22	5	95	BTHU
23	95	5	BCHU
24	10	90	BTHU
25	85	15	BCHU
26	2	98	BTHU
27	100	0	BCHU
28	95	5	BCHU
29	60	40	BCHU
30	95	5	BCHU
31	95	5	BCHU
32	90	10	BCHU
33	65	35	BCHU
34	65	45	BCHU

Appendix I. Species names, Latitude (degrees, minutes), Longitude (degrees, minutes), the elevation, date and time at which the individual was caught. The date and time of the experiment an individual participated in, individuals experimental ID names, and Museum of Southwestern Biology Bird Division NK numbers.

Species	LAT Degrees	LAT Minutes	LONG Degrees	LONG Minutes	Elevation Caught	Date Captured	Time Captured	Experiment date	Experiment time	Experiment ID	NK Number
<i>Selasphorus platycercus</i>	35	59.814	106	48.45	2537	10-Jul-14				BTHU_1	222332
<i>Selasphorus platycercus</i>	35	59.814	106	48.45	2537	10-Jul-14				BTHU_2	222333
<i>Selasphorus platycercus</i>	35	59.814	106	48.45	2537	10-Jul-14				BTHU_3	222334
<i>Archilochus alexandri</i>	35	4.674	106	36.97	1585	28-Jul-14				BCHU_1	222335
<i>Archilochus alexandri</i>	35	5.43	106	35.54	1600	31-Jul-14				BCHU_2	222336
<i>Archilochus alexandri</i>	35	4.446	106	37.21	1575	3-Aug-14				BCHU_3	222337
<i>Selasphorus platycercus</i>	35	6.61	106	22.69	2050	26-May-15	9:16	28-May-15	11:50	2015BT01	250954
<i>Archilochus alexandri</i>	35	6.61	106	22.69	2050	31-May-15	9:46	1-Jun-15	12:55	2015BC05	250955
<i>Archilochus alexandri</i>	35	4.45	106	37.21	1575	4-Jun-15	7:45	5-Jun-15	11:21	2015BC07	250956
<i>Archilochus alexandri</i>	35	4.45	106	37.21	1575	10-Jun-15	15:45	11-Jun-15	10:36	2015BC08	250957
<i>Selasphorus platycercus</i>	35	6.61	106	22.69	2050	9-Jun-15	8:45	11-Jun-15	10:41	2015BT07	250958
<i>Selasphorus platycercus</i>	35	6.61	106	22.69	2050	9-Jun-15	10:15	11-Jun-15	10:36	2015BT08	250959
<i>Archilochus alexandri</i>	35	4.45	106	37.21	1575	14-Jun-15	10:15	16-Jun-15	9:46	2015BC09	250960
<i>Archilochus alexandri</i>	35	4.45	106	37.21	1575	14-Jun-15	11:50	16-Jun-15	9:51	2015BC10	250961
<i>Archilochus alexandri</i>	35	4.45	106	37.21	1575	18-Jun-15	8:45	19-Jun-15	10:15	2015BC11	250962

## Appendix I. (cont.)

Species	LAT Degrees	LAT Minutes	LONG Degrees	LONG Minutes	Elevation Caught	Date Captured	Time Captured	Experiment date	Experiment time	Experiment ID	NK Number
Archilochus alexandri	35	4.45	106	37.21	1575	18-Jun-15	18:45	19-Jun-15	9:28	2015BC12	250963
Selasphorus platycercus	35	59.814	106	48.45	2537	15-Jun-15	11:45	19-Jun-15	9:28	2015BT09	250964
Selasphorus platycercus	35	59.814	106	48.45	2537	21-Jun-15	10:35	25-Jun-15	14:00	2015BT12	250965
Selasphorus platycercus	35	6.61	106	22.69	2050	30-Jun-15	19:55	2-Jul-15	1:10	2015BT13	250966
Selasphorus platycercus	35	6.61	106	22.69	22.69	2050	7-Jul-15	8:03	8-Jul-15	12:05	250967
Archilochus alexandri	35	4.45	106	37.21	37.21	1575	24-Jun-15	9:20	25-Jun-15	12:45	250968
Archilochus alexandri	35	6.61	106	22.69	22.69	2050	30-Jun-15	19:30	2-Jul-15	1:10	250969
Archilochus alexandri	35	6.61	106	22.69	22.69	2050	30-Jun-15	19:35	7-Jul-15	12:05	250970
Selasphorus platycercus	35	9.25	106	21.81	21.81	2166	22-Jul-15	18:37	24-Jul-15	10:59	250974
Selasphorus platycercus	35	9.25	106	21.81	21.81	2166	22-Jul-15	19:38	24-Jul-15	12:06	250975
Selasphorus platycercus	35	9.25	106	21.81	21.81	2166	23-Jul-15	17:23	24-Jul-15	12:06	250976
Archilochus alexandri	35	6.61	106	22.69	22.69	2050	23-Jul-15	18:33	24-Jul-15	10:59	250977
Archilochus alexandri	35	6.61	106	22.69	22.69	2050	23-Jul-15	19:34	24-Jul-15	12:06	250978
Archilochus alexandri	35	6.61	106	22.69	22.69	2050	23-Jul-15	18:57	24-Jul-15	12:06	250979
Selasphorus platycercus	35	9.25	106	21.81	21.81	2166	23-Jul-15	16:56	24-Jul-15	9:00	250980
Selasphorus platycercus	35	9.25	106	21.81	21.81	2166	20-Jul-15	19:06	24-Jul-15	12:06	250990
Selasphorus platycercus	35	6.61	106	22.69	22.69	2050	6-Jul-16	8:06	7-Jul-16	9:40	275861
Selasphorus platycercus	35	6.61	106	22.69	22.69	2050	6-Jul-16	8:59	7-Jul-16	9:40	275862
Selasphorus platycercus	35	6.61	106	22.69	22.69	2050	13-Jul-16	7:38	14-Jul-16	9:44	275863

## Appendix I. (cont.)

Species	LAT Degrees	LAT Minutes	LONG Degrees	LONG Minutes	Elevation Caught	Date Captured	Time Captured	Experiment date	Experiment time	Experiment ID	NK Number
Archilochus alexandri	35	6.61	106	22.69	2050	13-Jul-16	8:27	14-Jul-16	10:39	08BC2016	275864
Archilochus alexandri	35	6.61	106	22.69	2050	13-Jul-16	8:44	14-Jul-16	9:29	09BC2016	275865
Selasphorus platycercus	35	6.61	106	22.69	2050	19-Jul-16	11:31	21-Jul-16	9:45	07BT2016	275866
Selasphorus platycercus	35	6.61	106	22.69	2050	7-Jul-15	8:03	8-Jul-15	12:05	10BC2016	250967
Archilochus alexandri	35	7.86	106	29.05	1800	20-Jul-16	11:03	22-Jul-16	10:30	08BT2016	275868
Selasphorus platycercus	35	6.61	106	22.69	2050	21-Jul-16	9:35	22-Jul-16	10:30	09BT2016	275869
Selasphorus platycercus	35	6.61	106	22.69	2050	25-Jul-16	11:14	27-Jul-16	3:25	01BC2016	275870
Archilochus alexandri	35	4.45	106	37.21	1575	8-Jun-16	14:35	9-Jun-16	9:45	02BC2016	275891
Archilochus alexandri	35	4.45	106	37.21	1575	8-Jun-16	18:37	9-Jun-16	9:45	03BC2016	275892
Archilochus alexandri	35	4.45	106	37.21	1575	13-Jun-16	10:40	14-Jun-16	6:10	04BC2016	275893
Archilochus alexandri	35	4.45	106	37.21	1575	13-Jun-16	14:08	14-Jun-16	5:45	05BC2016	275894
Archilochus alexandri	35	6.61	106	22.69	2050	15-Jun-16	8:16	16-Jun-16	8:40	01BT2016	275895
Selasphorus platycercus	34	59.88	106	19.18	2340	14-Jun-16	16:57	16-Jun-16	8:40	02BT2016	275896
Selasphorus platycercus	34	59.88	106	19.18	2340	14-Jun-16	17:50	16-Jun-16	8:40	06BC2016	275897
Archilochus alexandri	35	10.42	106	22.63	2238	21-Jun-16	9:11	23-Jun-16	8:30	03BT2016	275898
Selasphorus platycercus	35	10.42	106	22.63	2238	21-Jun-16	10:28	23-Jun-16	8:30	07BC2016	275899
Archilochus alexandri	35	6.61	106	22.69	2050	6-Jul-16	7:51	7-Jul-16	9:40	10BT2016	275900
Selasphorus platycercus	35	6.61	106	22.69	2050	26-Jul-16	12:03	28-Jul-16	8:00	11BT2016	276051
Selasphorus platycercus	35	7.86	106	29.05	1800	29-Jul-16	8:16	29-Jul-16	12:10	08BC2016	276052

## Appendix I. (cont.)

Species	LAT Degrees	LAT Minutes	LONG Degrees	LONG Minutes	Elevation Caught	Date Captured	Time Captured	Experiment date	Experiment time	Experiment ID	NK Number
Selasphorus platycercus	35	6.61	106	22.69	2050	1-Aug-16	7:48	2-Aug-16	2:30	12BT2016	276053
Selasphorus platycercus	35	6.61	106	22.69	2050	1-Aug-16	8:07	2-Aug-16	2:30	13BT2016	276054
Selasphorus platycercus	35	6.61	106	22.69	2050	1-Aug-16	9:15	2-Aug-16	2:30	14BT2016	276055
Selasphorus platycercus	35	6.61	106	22.69	2050	3-Aug-16	7:34	4-Aug-16	2:30	15BT2016	276057
Selasphorus platycercus	35	6.61	106	22.69	2050	3-Aug-16	8:09	4-Aug-16	2:30	16BT2016	276058
Selasphorus platycercus	35	6.61	106	22.69	2050	3-Aug-16	8:58	5-Aug-16	10:28	17BT2016	276059
Archilochus alexandri	35	6.61	106	22.69	2050	3-Aug-16	9:34	5-Aug-16	10:28	11BC2016	276060
Selasphorus platycercus	35	6.61	106	22.69	2050	3-Aug-16	9:14	5-Aug-16	11:28	18BT2016	276111

Appendix J. Museum of Southwestern Biology Bird Division NK numbers, measurements of an individual's body mass (g), supracoracoideus (g), pectoralis major (g) , heart (g), average lung (g), total wingspan (mm), body width (mm), and average wing area measurement (mm<sup>2</sup>)

NK Number	Body Mass (g)	Supra (g)	P.major (g)	Heart (g)	Average Lung (g)	Total Wing Span (mm)	Body Width (mm)	Average Wing Area (mm <sup>2</sup> )
222332	2.98	0.14	0.30	0.072	0.04			
222333	3.55	0.13	0.29	0.084	0.046			
222334	3.74	0.14	0.31	0.089	0.038			
222335	2.91	0.15	0.29	0.074	0.046			
222336	3.02	0.15	0.26	0.072	0.036			
222337	2.93	0.13	0.27	0.072	0.042			
250954	3.00	0.15	0.32	0.090	0.041	125.23	20.17	632.26
250955	2.37	0.13	0.21	0.068	0.036	99.66	15.90	472.27
250956	2.84	0.09	0.22	0.088	0.045	97.47	14.95	456.79
250957	2.88	0.09	0.23	0.076	0.041	102.17	16.54	477.50
250958	3.40		0.27	0.104	0.051	125.58	17.11	683.75
250959	3.20	0.14	0.28	0.094	0.051	121.21	15.50	710.34
250960	3.28		0.21	0.072	0.042	111.47	17.52	529.95
250961	3.23	0.14	0.22	0.076	0.042	103.02	15.15	491.91
250962	3.28	0.11	0.24	0.086	0.044	107.70	17.82	506.00
250963		0.12	0.22	0.072	0.038			
250964	3.22	0.13	0.22	0.084	0.037	122.55	15.52	705.96
250965	2.98	0.12	0.22	0.060	0.052	116.58	15.94	606.47
250966	3.14	0.14	0.24	0.072	0.045	116.44	17.60	617.14
250967	3.41	0.14	0.27	0.086	0.04	112.66	17.04	622.58

## Appendix J. (cont.)

NK Number	Body Mass (g)	Supra (g)	P.major (g)	Heart (g)	Average Lung (g)	Total Wing Span (mm)	Body Width (mm)	Average Wing Area (mm <sup>2</sup> )
250968	2.75	0.14	0.22	0.058	0.038	106.92	14.24	564.03
250969	2.91	0.15	0.23	0.072	0.04	99.21	16.76	492.41
250970	2.91	0.15	0.23	0.072	0.04	99.21	16.76	492.41
250974	3.51		0.22	0.082	0.044	119.63	19.90	736.71
250975	3.38	0.13	0.28	0.074	0.047	113.09	19.46	676.16
250976	3.51		0.27	0.090	0.044	117.39	21.76	697.92
250977	3.23		0.26	0.088	0.04	105.92	19.81	567.37
250978	2.74	0.14	0.24	0.078	0.037	106.31	17.53	529.95
250979	2.55	0.11	0.20	0.088	0.031	104.78	16.86	497.82
250980	2.89	0.14	0.27	0.094	0.029	118.70	18.90	581.45
250990	2.61	0.11	0.24	0.074	0.043	119.24	19.41	
275861	3.13	0.17	0.36	0.080	0.0445	121.17	19.24	695.46
275862	3.37	0.13	0.30	0.072	0.052	117.39	17.16	644.34
275863	4.75	0.14	0.30	0.076	0.042	120.78	19.18	666.80
275864	3.40	0.14	0.27	0.070	0.047	106.93	15.76	525.54
275865	3.84	0.18	0.29	0.082	0.045	104.55	16.66	529.98
275866	3.88	0.16	0.33	0.074	0.045	123.33	18.17	705.45
250967	3.41	0.14	0.27	0.086	0.04	112.66	17.04	622.58
275868	3.90	0.18	0.28	0.068	0.045	107.39	18.56	520.23
275869	3.16	0.15	0.36	0.076	0.054	117.67	15.49	639.29



## Appendix J. (cont.)

NK Number	Body Mass (g)	Supra (g)	P.major (g)	Heart (g)	Average Lung (g)	Total Wing Span (mm)	Body Width (mm)	Average Wing Area (mm <sup>2</sup> )
275870	3.29	0.14	0.30	0.086	0.043	117.18	17.38	633.95
275891	3.00	0.13	0.29	0.084	0.037	109.49	16.79	576.03
275892	3.20	0.16	0.29	0.086	0.042	105.97	17.19	548.55
275893	3.51	0.16	0.29	0.096	0.052	109.23	18.59	544.18
275894	3.21	0.16	0.32	0.082	0.046	107.28	15.38	568.53
275895	3.20	0.13	0.29	0.086	0.04	106.57	16.52	524.98
275896	3.20	0.15	0.30	0.078	0.043	114.98	16.23	585.79
275897	3.10		0.31	0.080	0.041	123.47	15.59	709.69
275898	3.37	0.14	0.29	0.078	0.048	104.53	16.48	505.35
275899	3.25	0.15	0.29	0.076	0.048	124.37	17.15	656.94
275900	3.32	0.15	0.29	0.080	0.0485	105.63	17.86	535.72
276051	3.06	0.13	0.22	0.086	0.036	114.93	16.17	661.63
276052	4.88	0.15	0.33	0.088	0.048	120.10	16.28	622.95
276053	4.08	0.14	0.29	0.078	0.04	121.56	17.09	639.98
276054	3.55	0.16	0.34	0.082	0.046	123.21	16.80	718.86
276055	3.69	0.15	0.31	0.068	0.047	125.34	15.49	760.98
276057	3.53	0.15	0.32	0.090	0.044	119.36	17.04	656.17
276058	4.33	0.18	0.35	0.080	0.047	122.81	17.02	750.02
276059	3.35	0.14	0.29	0.088	0.04	120.98	17.29	680.79
276060	3.06	0.12	0.23	0.064	0.04	99.28	14.10	437.70
276111	4.46	0.14	0.29	0.088	0.044	122.08	17.40	637.58

Appendix K. Museum of Southwestern Biology Bird Division NK numbers, measurements of an individual's average wing length (mm<sup>2</sup>), aspect ratio, skull length(mm), scapula length (mm), femur width (mm), femur length (mm), humerus with (mm), and humerus length (mm).

NK Number	Average Wing length (cm)	Aspect Ratio	Skull length (mm)	Scapula length (mm)	Femur width (mm)	Femur length (mm)	Humerus width (mm)	Humerus length (mm)
222332								
222333								
222334								
222335								
222336								
222337								
250954	52.53	8.73	10.27	11.55	0.41	7.02	0.72	4.25
250955	41.88	7.43						
250956	41.26	7.45	9.87	10.71	0.48	7.46	0.66	3.77
250957	42.81	7.68						
250958	54.23	8.60	10.13	11.63	0.46	7.47	0.73	4.22
250959	52.86	7.87						
250960	46.98	8.33	9.88	11.19	0.45	7.18	0.72	3.72
250961	43.93	7.85	9.77	11.00	0.46	7.07	0.65	3.80
250962	44.94	7.98	9.89	11.17	0.46	7.43	0.63	3.83
250963								
250964	53.52	8.11						
250965	50.32	8.35	9.95	11.46	0.48	6.71	0.72	4.15
250966	49.42	7.92						
250967	47.81	7.34	9.94	10.70	0.48	7.30	0.72	

## Appendix K. (cont.)

NK Number	Average Wing length (cm)	Aspect Ratio	Skull length (mm)	Scapula length (mm)	Femur width (mm)	Femur length (mm)	Humerus width (mm)	Humerus length (mm)
250968	46.34	7.61	10.11	11.20	0.49	7.36	0.69	3.85
250969	41.22	6.90	9.76	10.66	0.44	7.21	0.67	3.88
250970	41.22	6.90						
250974	49.86	6.75						
250975	46.82	6.48	10.25	11.50	0.45	7.26	0.67	4.12
250976	47.81	6.55	10.00	10.96	0.44	7.59	0.70	4.12
250977	43.06	6.53	9.61	10.81	0.46	7.54	0.64	3.78
250978	44.39	7.44	9.86	10.67	0.50	7.12	0.69	3.81
250979	43.96	7.76						
250980	49.90	8.57						
250990								
275861	50.97	7.47	9.83	11.10	0.41	7.44	0.66	4.17
275862	50.12	7.80	9.58					
275863	50.80	7.74	10.17	11.33	0.47	7.18	0.75	4.12
275864	45.59	7.91	9.86	10.00	0.48	7.26	0.67	3.76
275865	43.95	7.29	10.15	10.35		6.96	0.64	3.86
275866	52.58	7.84						
275868	44.42	7.58	9.89	10.68				
275869	51.09	8.17	9.96	11.27	0.44	6.45	0.67	4.21

## Appendix K. (cont.)

NK Number	Average Wing length (cm)	Aspect Ratio	Skull length (mm)	Scapula length (mm)	Femur width (mm)	Femur length (mm)	Humerus width (mm)	Humerus length (mm)
275870	49.90	7.86	10.15	11.51	0.46	7.24	0.72	4.26
275891	46.35	7.46	10.25	10.99	0.45	6.90	0.67	3.75
275892	44.39	7.18	10.28	10.92	0.47	7.03	0.66	3.77
275893	45.32	7.55	9.60	11.17	0.47	7.28	0.63	3.71
275894	45.95	7.43	9.67	10.77	0.47	7.25		3.80
275895	45.03	7.72	9.94	10.44	0.44	6.87	0.64	3.75
275896	49.37	8.32	9.84	10.66	0.38	7.31	0.76	4.16
275897	53.94	8.20	9.82	11.13	0.39	6.92	0.64	3.97
275898	44.03	7.67	9.83		0.52	7.13	0.67	3.68
275899	53.61	8.75	9.72	10.97	0.49	7.00	0.63	4.15
275900	43.88	7.19	9.87		0.46		0.68	3.77
276051	49.38	7.37	9.82	10.96	0.48	7.07	0.65	4.08
276052	51.91	8.65		11.03				
276053	52.23	8.53	9.80	11.20	0.49	6.87	0.70	4.24
276054	53.20	7.88	10.16	11.00	0.48	7.30	0.67	4.21
276055	54.92	7.93	10.13	11.66	0.43	7.10		
276057	51.16	7.98	9.91	10.90	0.37	6.73	0.67	4.27
276058	52.89	7.46	10.17	11.21	0.48	7.27	0.74	4.14
276059	51.85	7.90	9.66		0.47		0.68	4.05
276060	42.59	8.29	10.28	10.68	0.41	7.03		3.79
276111	52.34	8.59	9.97	10.53	0.47	7.13	0.74	4.14

## Appendix L. URLs for each specimen collected.

NK Number	MSB Arctos URL
222332	<a href="http://arctos.database.museum/guid/MSB:Bird:45350">http://arctos.database.museum/guid/MSB:Bird:45350</a>
222333	<a href="http://arctos.database.museum/guid/MSB:Bird:45351">http://arctos.database.museum/guid/MSB:Bird:45351</a>
222334	<a href="http://arctos.database.museum/guid/MSB:Bird:45352">http://arctos.database.museum/guid/MSB:Bird:45352</a>
222335	<a href="http://arctos.database.museum/guid/MSB:Bird:45353">http://arctos.database.museum/guid/MSB:Bird:45353</a>
222336	<a href="http://arctos.database.museum/guid/MSB:Bird:45354">http://arctos.database.museum/guid/MSB:Bird:45354</a>
222337	<a href="http://arctos.database.museum/guid/MSB:Bird:45355">http://arctos.database.museum/guid/MSB:Bird:45355</a>
250954	<a href="http://arctos.database.museum/guid/MSB:Bird:45356">http://arctos.database.museum/guid/MSB:Bird:45356</a>
250955	<a href="http://arctos.database.museum/guid/MSB:Bird:45357">http://arctos.database.museum/guid/MSB:Bird:45357</a>
250956	<a href="http://arctos.database.museum/guid/MSB:Bird:45358">http://arctos.database.museum/guid/MSB:Bird:45358</a>
250957	<a href="http://arctos.database.museum/guid/MSB:Bird:45359">http://arctos.database.museum/guid/MSB:Bird:45359</a>
250958	<a href="http://arctos.database.museum/guid/MSB:Bird:45360">http://arctos.database.museum/guid/MSB:Bird:45360</a>
250959	<a href="http://arctos.database.museum/guid/MSB:Bird:45361">http://arctos.database.museum/guid/MSB:Bird:45361</a>
250960	<a href="http://arctos.database.museum/guid/MSB:Bird:45362">http://arctos.database.museum/guid/MSB:Bird:45362</a>
250961	<a href="http://arctos.database.museum/guid/MSB:Bird:45363">http://arctos.database.museum/guid/MSB:Bird:45363</a>
250962	<a href="http://arctos.database.museum/guid/MSB:Bird:45364">http://arctos.database.museum/guid/MSB:Bird:45364</a>
250963	<a href="http://arctos.database.museum/guid/MSB:Bird:45365">http://arctos.database.museum/guid/MSB:Bird:45365</a>
250964	<a href="http://arctos.database.museum/guid/MSB:Bird:45366">http://arctos.database.museum/guid/MSB:Bird:45366</a>
250965	<a href="http://arctos.database.museum/guid/MSB:Bird:45367">http://arctos.database.museum/guid/MSB:Bird:45367</a>
250966	<a href="http://arctos.database.museum/guid/MSB:Bird:45368">http://arctos.database.museum/guid/MSB:Bird:45368</a>
250967	<a href="http://arctos.database.museum/guid/MSB:Bird:45369">http://arctos.database.museum/guid/MSB:Bird:45369</a>

## Appendix L. (cont.)

NK Number	MSB Arctos URL
250968	<a href="http://arctos.database.museum/guid/MSB:Bird:45370">http://arctos.database.museum/guid/MSB:Bird:45370</a>
250969	<a href="http://arctos.database.museum/guid/MSB:Bird:45371">http://arctos.database.museum/guid/MSB:Bird:45371</a>
250970	<a href="http://arctos.database.museum/guid/MSB:Bird:45372">http://arctos.database.museum/guid/MSB:Bird:45372</a>
250974	<a href="http://arctos.database.museum/guid/MSB:Bird:45373">http://arctos.database.museum/guid/MSB:Bird:45373</a>
250975	<a href="http://arctos.database.museum/guid/MSB:Bird:45374">http://arctos.database.museum/guid/MSB:Bird:45374</a>
250976	<a href="http://arctos.database.museum/guid/MSB:Bird:45375">http://arctos.database.museum/guid/MSB:Bird:45375</a>
250977	<a href="http://arctos.database.museum/guid/MSB:Bird:45376">http://arctos.database.museum/guid/MSB:Bird:45376</a>
250978	<a href="http://arctos.database.museum/guid/MSB:Bird:45377">http://arctos.database.museum/guid/MSB:Bird:45377</a>
250979	<a href="http://arctos.database.museum/guid/MSB:Bird:45378">http://arctos.database.museum/guid/MSB:Bird:45378</a>
250980	<a href="http://arctos.database.museum/guid/MSB:Bird:45379">http://arctos.database.museum/guid/MSB:Bird:45379</a>
250990	<a href="http://arctos.database.museum/guid/MSB:Bird:45380">http://arctos.database.museum/guid/MSB:Bird:45380</a>
275861	<a href="http://arctos.database.museum/guid/MSB:Bird:45381">http://arctos.database.museum/guid/MSB:Bird:45381</a>
275862	<a href="http://arctos.database.museum/guid/MSB:Bird:45382">http://arctos.database.museum/guid/MSB:Bird:45382</a>
275863	<a href="http://arctos.database.museum/guid/MSB:Bird:45383">http://arctos.database.museum/guid/MSB:Bird:45383</a>
275864	<a href="http://arctos.database.museum/guid/MSB:Bird:45384">http://arctos.database.museum/guid/MSB:Bird:45384</a>
275865	<a href="http://arctos.database.museum/guid/MSB:Bird:45385">http://arctos.database.museum/guid/MSB:Bird:45385</a>
275866	<a href="http://arctos.database.museum/guid/MSB:Bird:45386">http://arctos.database.museum/guid/MSB:Bird:45386</a>
275868	<a href="http://arctos.database.museum/guid/MSB:Bird:45387">http://arctos.database.museum/guid/MSB:Bird:45387</a>
275869	<a href="http://arctos.database.museum/guid/MSB:Bird:45388">http://arctos.database.museum/guid/MSB:Bird:45388</a>

## Appendix L. (cont.)

NK Number	MSB Arctos URL
275870	<a href="http://arctos.database.museum/guid/MSB:Bird:45389">http://arctos.database.museum/guid/MSB:Bird:45389</a>
275891	<a href="http://arctos.database.museum/guid/MSB:Bird:45390">http://arctos.database.museum/guid/MSB:Bird:45390</a>
275892	<a href="http://arctos.database.museum/guid/MSB:Bird:45391">http://arctos.database.museum/guid/MSB:Bird:45391</a>
275893	<a href="http://arctos.database.museum/guid/MSB:Bird:45392">http://arctos.database.museum/guid/MSB:Bird:45392</a>
275894	<a href="http://arctos.database.museum/guid/MSB:Bird:45393">http://arctos.database.museum/guid/MSB:Bird:45393</a>
275895	<a href="http://arctos.database.museum/guid/MSB:Bird:45394">http://arctos.database.museum/guid/MSB:Bird:45394</a>
275896	<a href="http://arctos.database.museum/guid/MSB:Bird:45395">http://arctos.database.museum/guid/MSB:Bird:45395</a>
275897	<a href="http://arctos.database.museum/guid/MSB:Bird:45396">http://arctos.database.museum/guid/MSB:Bird:45396</a>
275898	<a href="http://arctos.database.museum/guid/MSB:Bird:45397">http://arctos.database.museum/guid/MSB:Bird:45397</a>
275899	<a href="http://arctos.database.museum/guid/MSB:Bird:45398">http://arctos.database.museum/guid/MSB:Bird:45398</a>
275900	<a href="http://arctos.database.museum/guid/MSB:Bird:45399">http://arctos.database.museum/guid/MSB:Bird:45399</a>
276051	<a href="http://arctos.database.museum/guid/MSB:Bird:45400">http://arctos.database.museum/guid/MSB:Bird:45400</a>
276052	<a href="http://arctos.database.museum/guid/MSB:Bird:45401">http://arctos.database.museum/guid/MSB:Bird:45401</a>
276053	<a href="http://arctos.database.museum/guid/MSB:Bird:45402">http://arctos.database.museum/guid/MSB:Bird:45402</a>
276054	<a href="http://arctos.database.museum/guid/MSB:Bird:45403">http://arctos.database.museum/guid/MSB:Bird:45403</a>
276055	<a href="http://arctos.database.museum/guid/MSB:Bird:45404">http://arctos.database.museum/guid/MSB:Bird:45404</a>
276057	<a href="http://arctos.database.museum/guid/MSB:Bird:45405">http://arctos.database.museum/guid/MSB:Bird:45405</a>
276058	<a href="http://arctos.database.museum/guid/MSB:Bird:45406">http://arctos.database.museum/guid/MSB:Bird:45406</a>
276059	<a href="http://arctos.database.museum/guid/MSB:Bird:45407">http://arctos.database.museum/guid/MSB:Bird:45407</a>
276060	<a href="http://arctos.database.museum/guid/MSB:Bird:45408">http://arctos.database.museum/guid/MSB:Bird:45408</a>
276111	<a href="http://arctos.database.museum/guid/MSB:Bird:45409">http://arctos.database.museum/guid/MSB:Bird:45409</a>

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