

Bill Morphology and Niche Partitioning in *Selasphorus platycercus*

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Abstract

It has often been stated that the bill shape of hummingbirds is an example of ecological adaptation, as the morphology of their bills are strongly associated with flower nectar resources (Bleiweiss 1999). Broad-tailed hummingbirds (*Selasphorus platycercus*) are considered the most characteristic hummingbird of the Rocky Mountain region (Grant & Grant 1968). At the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, the ecology and behavior of Broad-tailed hummingbirds have been studied, but the reason behind why Broad-tailed hummingbirds visit specific flowers has yet to be thoroughly examined. To explore this question, we caught a total of 198 Broad-tailed hummingbirds in the summer of 2016 and measured their bill length, width, and curvature. We found that females have significantly longer, wider, and curvier bills. In addition, we examined the association between sex and flower visitation using pollen swabs as a proxy for flower visitation. A total of 13 species of pollen were found on bodies of hummingbirds. Through examining the visitation rate across these 13 species of pollen, we found potential for niche partitioning between sexes.

Introduction

Interactions between animal pollinated plants and pollinators often exhibit mutualistic relationships (Temeles & Kress, 2003). For example, hummingbirds provide pollination services that cultivate floral biodiversity while, in turn, specific flowers contribute energy-rich nectar (Potts et al. 2010 & McKinney et al. 2012). Hummingbirds have also been known to exhibit feeding specialization and choose nectar resources based on both energy requirements and correspondence of their bill shape to nectar resources (Temeles & Kress, 2003). For example, it was found that hummingbirds with shorter bills have shorter handling times at flowers with narrower corollas compared to hummingbirds with longer bills (Temeles, 1995). The association between hummingbird bills and their nectar resources has been stated as a “paradigm of ecological adaptation” (Bleiweiss 1999). Furthermore, interactions between hummingbirds and flowers may also contribute to intersexual differences in bill morphology, as feeding ecology has been known to contribute to the evolution of sexual differences, specifically sexual dimorphism (Temeles 2009).

Selasphorus platycercus (Broad-tailed hummingbirds) are migratory hummingbirds that feed primarily on nectar and insects (Waser 1976). In the spring, Broad-tailed hummingbirds migrate from Mexico and Central America to the Rocky Mountains to feed on flowers that bloom sequentially throughout the summer. In the Rocky Mountains, there have been anecdotal accounts on intrasexual and intersexual differences in populations of Broad-tailed hummingbirds (BTLHs), but little evidence has been comprised to support these observations.

At the Rocky Mountain Biological Laboratory in Gothic, Colorado, we examined 1. Morphological differences in bill length, width, and curvature between male and female BTLHs 2. Association between sex and flower visitation 3. Association between bill morphology and flower visitation. Through measuring the variation in bill shape between male and female BTLHs, examining flower visitation via pollen swabs, and measuring corolla length, width, and curvature in hummingbird visited flowers, we have begun to examine the adaptive qualities exhibited through the bill morphology of Broad-tailed hummingbirds.

Materials and Methods

Study Species and Sites

Adult Broad-tailed hummingbirds (*Selasphorus platycercus*) were captured and measured during the summer of 2016. Our two study sites were located at the Rocky Mountain Biological Laboratory in Gothic, Colorado (RMBL: 38.96° N, 106.99° W, 2900 m above sea level) and Washington Gulch (WG: 38.52°N 106.57°W, 2705 m above sea level). Since Broad-tails are the most characteristic hummingbird in the Rocky Mountain region, especially in June and early July, it was chosen as our study species (Grant & Grant, 1968). There are several features that distinguish adult male and female Broad-tailed hummingbirds. Males have a rose-red gorget while females have white chests dotted with green and/or bronze spots (Cornell Lab of Ornithology). In order to court females and defend territories, males perform aerial displays and use their wing tips to produce loud trilling noises (Cornell Lab of Ornithology).

Flowers were also collected and measured during the June and July of 2016. All flowers were measured within the Gothic Townsite, with the exception of *Erythronium grandiflorum* and *Chamerion angustifolium*, which were measured at Mexican Cut and Emerald Lake respectively. Plants were collected based on whether they were known to be hummingbird visited. Hummingbird visited plants were determined by referencing previous literature. For example, *Delphinium nuttallianum*, *Ipomopsis aggregata*, and *Erythronium grandiflorum* have all been stated as hummingbird visited flowers (Waser 1978, Waser 2001, McKinney et al. 1978). Other hummingbird visited flower species were determined from referencing *Hummingbirds and Their Flowers*, where hummingbird visited flowers were recorded through field observations (Grant & Grant, 1978).

Capturing Broad-tailed Hummingbirds

Broad-tailed hummingbirds were caught using a mesh butterfly net. A total of n=198 hummingbirds were caught in the summer of 2016, 97 of which were male and 101 female. A red plastic hummingbird feeder was used to attract the hummingbirds. The net would be strategically placed directly beneath the feeder and swept over individuals that came in close proximity to the feeder. Once captured, each bird was gently extracted from the net. Following removal from the net, we conducted several procedures in the following order: 1. Pollen swab 2. Side profile picture 3. Bill shape measurements 4. Blood collection

Pollen Swab

When removing birds from the net, we were careful not to contact the bills in order to prevent contamination. A 1mm x 1mm piece of fuchsin gel was used to swab the forehead, chin, and upper and lower bills, as these are areas that normally come in contact with the anthers of visited flowers. Once swabbing was complete, the gel would be placed in clear capillary tubes and labeled with the order in which the hummingbirds were caught. At a later time, the pieces of fuchsin gel would be placed on a glass slide and melted over a 95% ethanol flame. The slide would be labeled with the date of collection, band number, and whether the individual was a male or female. Instructions on preparation of fuchsin gel can be found in the appendix.

Pollen Species Richness

Pollen slides were analyzed using a compound microscope and microscope camera. Every pollen slide would be analyzed starting in the top left corner, where the slide would then be scanned from left to right. Pollen grains were compared side by side to a pollen reference collection. The genus and/or species would be recorded. Once the first row was examined, the slide would be shifted down by 3 mm (the length of the hemocytometer in the lens of the compound microscope) and then examined from right to left. The above procedure would be repeated until the whole slide was analyzed.

Calculating Curvature

Once the pollen swab was complete, a bird would be placed parallel to a 60mm calibration line, where we captured a side profile picture. Three pictures were taken of each individual. These pictures were later used in ImageJ to determine the curvature of individual bills. In ImageJ, a Three Point Circular ROI was used to calculate the curvature of each bill. For every bird, three points were selected along the midline of the bill where a perfect circle would then be fitted around the bill. Curvature was determined as $1/\text{radius}$ of the circle in mm.

Bill Shape Measurements

Bill length, bill width, and wing chord were measured for every hummingbird. Calipers were used for all measurements. We had two measurements of bill length: full gape and exposed culmen. Full gape was measured from the mandible to the tip of the bill while exposed culmen was measured from where the feathers impinge on the bill to the tip of the bill. Both full gape and exposed culmen were used as measurements of bill length, as it has been shown that bill feathering may affect an individual's ability to extend their bill into flowers, especially flowers with narrower corollas (Temeles 1996). However, hummingbirds with feathers on their bills have also been observed to fully insert their bills into flowers as well (Temeles 1996). We measured bill width at the widest point of the bill, or the base of the bill. Wing chord was taken by measuring from the right shoulder blade to the right wing tip.

Blood Collection

Prior to collecting blood, each hummingbird would be wrapped in a mesh bag. A weight would be placed adjacent to the bird (on top of the bag) to prevent excessive movement during blood collection. Blood was taken from each hummingbird by clipping a single toenail, preferably the middle toenail. Heparinized capillary tubes were used to collect blood from each bird; the toes of each bird would usually fit into the heparinized tubes. For every hummingbird, no more than 10 μl of blood was taken. Once 2-10 μl of blood was taken, the clipped toenail was covered in cornstarch to stop bleeding. Before releasing the bird, we would ensure that blood flow had ceased and would attempt to feed them an approximately 50% sucrose solution.

Flower Collection

We collected 16 species of hummingbird visited flowers (a complete list can be found in the appendix). We collected 10 flowers per species and ensured that plants were at least 10 feet apart from each other, as to not generate spatial bias. In order to systematically measure the corolla lengths, widths, and curvatures of each flower, we categorized the hummingbird-visited flowers into several groups. The groups consist of: 1. Cup shaped 2. Open 3. Short and Curvy 4. Long and Curvy 5. Short and Straight 6. Long and Straight. A side profile of every measured flower was taken along a 60 millimeter calibration line. Digital calipers were used to measure each flower.

Cup-shaped flowers

When measuring cup shaped flowers, the corolla length was measured from the nectary to the anthesis. The corolla widths were measured by gently pressing down on the flower from the top and bottom and measuring its maximum corolla width. Curvature measurements were not taken for cup shaped flowers, as there was no curvature present. An example of a cup shaped flower species would be *Mertensia fusiformis*.

Short and Long Straight Flowers

Short and straight as well as long and straight flowers were measured in the same way as cup shaped flowers. In the case of *Aquilegia elegantula*, spurs were measured instead. Spurs were chosen randomly by holding up the flower and choosing the top most spur. In the next measured flower, the flower would be held in a random position and the second spur to the right would be chosen, etc. An example of a short and straight flower would be *Ribes montigenum*. An example of a long and straight flower would be *Aquilegia caerulea*.

Open flowers

In open flowers, corolla length was absent as the nectary was exposed. Corolla width was taken by observing the flower head on and measuring the natural opening of the flower (not from the tips of the petals). There were no curvature measurements for open flowers. An example of an open flower would be *Erythronium grandiflorum*.

Short and Long Curvy Flowers

In short and curvy as well as long and curvy flowers, the corolla length was measured as a straight line from the nectary to the anthesis. The corolla widths were measured by gently pressing down on the flower from the top and bottom and measuring its maximum corolla width. Curvatures were measured in image J, where the 60 mm line was used as a calibration reference. Through using a three point circular ROI, Image J was able to create a perfect circle from the curvature of the flower. The three points were selected along the midline of each flower. Image J would then calculate the radius of the circle, and subsequently the curvature as $1/\text{radius}$ in millimeters. An example of a short and curvy flower would be *Pedicularis bracteosa*. An example of a long and curvy flower would be *Castilleja miniata*.

Flower Curvature Measurements

Flower curvature was also measured in ImageJ using the Three Point Circular ROI. In ImageJ, a Three Point Circular ROI was used to place three points along the midline of the flower where the radius (in mm) of a perfect circle would then be fit around the flower or spur. Curvature was determined as $1/\text{radius}$ in mm.

Results

Bill Morphology

Broad-tailed hummingbirds exhibited intersexual differences in bill morphology. Prior to analyzing intersexual differences in bill morphology, the bill lengths of $n=198$ hummingbirds were plotted against wing chord. Wing chord measurements were used as a proxy for body size. Approximately 7% of the variation in bill length was explained by body size regardless of sex (Fig 1). In addition, approximately 0.022% of variation in bill curvature was explained by bill length (Fig 2).

Female Broad-tailed hummingbirds had significantly longer bills than males (ANOVA, $F=89.81$, $df=202$, $p<0.001$, $\alpha=0.05$) (Fig. 3). Full gape and exposed culmen measurements were highly correlated (RSquared: 0.691) (Fig. 4). Therefore, full gape measurements were used for bill length. Female Broad tails also had significantly wider (ANOVA, $F=119.43$, $df=202$, $p<0.001$, $\alpha=0.05$) (Fig 5) and curvier (ANOVA, $F=8.20$, $df=202$, $p<0.0046$, $\alpha=0.05$) (Fig. 6) bills than males.

Within the $n=198$ population of Broad-tailed hummingbirds, measurements of bill length, width, and curvature were independent from each other (Principle Components Analysis) (Fig. 7). In addition, longer and wider bills were more representative of female broad-tails than curvier bills. Shorter, narrower, and straighter bills were more representative of male broad-tails (Discriminate Analysis) (Fig 8).

Sex and Flower Visitation

Pollen swabs were used as a proxy for flower visitation. In the summer of 2016, 126 out of 198 pollen slides were analyzed. Pollen samples from analyzed pollen slides were collected from June 6th to July 21st.

Thirteen different types of pollen were found on the bodies of Broad-tailed hummingbirds. Visitation rate to these 13 species of flowers were varied among males and females (Fig. 9). No definitive conclusions can be formed from looking at differences in visitation among 126 hummingbirds, but some results are worth noting. Overall, in both males and females, there was a low visitation rate to *Aquilegia caerulea*. Interestingly, on the analyzed pollen slides, *A. caerulea* pollen was only found on females. In addition, more females visited *Ipomopsis aggregata*, *Delphinium barbeyi*, and *Castilleja* compared to males. Males visited *Ribes* (*montigenum* and *cereum*), *Salix*, and *Mertensia* (*fusiformis* and *ciliata*) more than females. Overall, there were also low visitation rates for *Campanula parryi*, *Erythronium grandiflorum*, and *Hydrophyllum* (Fig. 9). Moreover, *Salix*, *Ribes*, and *Delphinium nuttalianum* pollen were found abundantly on the bodies of Broad-tailed hummingbirds, with abundant defined as the majority pollen species found on a particular slide.

Bill Morphology and Flower Visitation

Analysis has not yet been performed to determine whether there is a significant difference in flower visitation based on bill morphology. However, we plan on performing a Pianka analysis to determine whether differences in flower visitation are due mostly to bill morphology or some other behavior across our population of Broad-tailed hummingbirds. By analyzing whether intersexual differences in bill morphology and flower visitation also occur within sexes, we could differentiate behaviors due to bill morphometrics.

Blood Collection

Blood will be used to sequence genomes of Broad-tailed hummingbirds. Sequencing has not yet begun, but we plan on isolating the broad-tails with the most extreme bill shapes and sequencing their genomes to determine genes responsible for differences in bill length, width, and curvature.

Discussion

There was a significant difference in bill length, width, and curvature between male and female Broad-tailed hummingbirds. Regardless of sex, variation in bill length only explained 7% of the variation in body size. This indicates that bill length is strongly independent of body size in BTLHs. Subsequently, there may be different selective pressures acting separately upon bill length and body size. In many hummingbird species, sexual dimorphism is a common occurrence (Bleiweiss 1999, Colwell 2000). In our study, Broad-tailed females had significantly longer, wider, and curvier bills than males. These morphological differences may be due to several factors. For example, differences in bill morphology could be due to competitive exclusion. Since male Broad-tails are territorial, they may be selective on the types of females that feed within their territories (Bleiweiss 1999). Consequently, females may have developed morphological traits that allow them to take advantage of nectar resources that territorial males cannot easily access, thus resulting in niche differentiation (Bleiweiss, 1999).

There could also be several other factors that explain similarities and differences in flower visitation between male and female Broad-tails. As mentioned previously, there was a low visitation rate to *A. caerulea*, and only females visited this flower. *Aquilegia caerulea* spurs are especially long compared to other hummingbird visited flowers, with spur lengths averaging 46.45 mm. Since females have significantly longer bill lengths compared to males, females may be able to access *A. caerulea* nectar easier, which is thus reflected in a higher female visitation rate to *A. caerulea*. In addition the corolla lengths of *Ipomopsis aggregata* (23.62mm), *Delphinium barbeyi*(18.83), *Castilleja miniata* (36.52), and *Castilleja sulphurea* (21.38) while the bill length of females averaged 21.53 mm and males 20.39 mm. *Castilleja* flowers are also curvy, which may create an additional barrier for males to access the nectar resources of these flowers. In the future, a larger population of hummingbirds should be captured to determine a greater significant difference in visitation rate between flowers. On the other hand, within our population of hummingbirds, males visited *Salix* and *Ribes* more frequently. Since *Salix* and *Ribes* are shorter flowers, males may have easier access to these nectar resources compared to flowers with longer corolla lengths. In this case, females may also have easier access to short flowers, but since they may have other equally accessible options to choose from, they may not visit *Ribes* and *Salix* as often as males.

Often, hummingbirds align their nesting season with peak flowering abundance (Waser 1976). Both males and females frequently visited *Delphinium nuttallianum*, *Salix*, and *Ribes* in June of 2016. At RMBL, it has been observed that once the first *D. nuttallianum* appeared, both males and females began establishing habitats, indicating the importance of *D. nuttallianum* for nest initiation (Waser 1976). *Salix*

could also be used as nest material for females as well. Different species of *Ribes* (montigenum and cereum) have been found on Broad-tailed hummingbirds, which may also indicate that it is a vital nectar resource in the early summer. Also, since both *Ribes* species have a slight pink to red tinge, their color alone may be an enticing to hummingbirds (Rodriguez-Girones & Santamaria, 2004).

There was a striking difference in visitation rate to *Lonicera involucrata* between male and female hummingbirds. From the 126 pollen slides, *Lonicera* was found on 11 of them, 10 being male and 1 female. Visitation rate to *Lonicera* may be due to behavioral differences between males and females (rather than differences in bill shape). On average, females fill their crops with 0.32 grams of nectar per visitation while males fill their crops with 0.11 grams per visitation (unpublished). This may indicate that it is costlier for females to make more trips to obtain their nectar sources as shown by their larger volume of nectar obtained per visit (Calder & Calder 1992). *Lonicera* bushes tend to be more dispersed spatially. Moreover, more pollen slides need to be analyzed in order to determine a greater significance in visitation to *Lonicera* between males and females.

Mertensia pollen was found on 29/126 analyzed slides, 10 of which were female and 19 male. *Mertensia* has been known to be visited less than flowers such as *Ipomopsis aggregata* and *Castilleja miniata*, which are known hummingbird visited flowers (Grant & Grant, 1968). Subsequently, why are hummingbirds visiting *Mertensia* when there are other, potentially better, resources out there? Hummingbirds are known as “periscopic foragers,” or foragers that have knowledge on different rewards within a variety of flowers (Waser 1997). Hummingbirds may visit flower “X” contingent upon the type and quality of flowers surrounding flower “X” (Mitchell 1989). It has been observed that hummingbirds that visit high quality resources, tend to also visit poor resources to obtain nectar. This phenomenon may be due to the fact that hummingbirds can determine the quality of a flower patch by primarily visiting a poor resource and determining nectar presence in a poor flower (Mitchell 1968).

Aquilegia elegantula is primarily pollinated by Broad-tailed hummingbirds and three species of bumblebees; it also secretes 44% sucrose nectar (Miller, 1978). From analyzing 126 pollen slides, *A. elegantula* was found on 65 slides, 35 of which were female and 30 male. *A. elegantula* has long been known to be attractive to hummingbirds, and Broad-tails have been known to be effective pollinators of *A. elegantula* (Miller, 1978). *A. elegantula* was found abundantly (i.e. was the majority species) on the 65 slides. Since *A. elegantula* is typically located in shady areas and streambanks, it could be difficult to find these flowers and may even cost more energy to find them compared to other flowers. Therefore, there is a possibility that we may have a biased sample of Broad-tailed hummingbirds. Since the majority of our samples were obtained at David Inouye’s cabin, particular populations of hummingbirds may be closer to *A. elegantula* than others. Our samples from Washington Gulch were obtained later in the summer (7/21/16), so populations of *A. elegantula* may not be as abundant. In the future, we aim to sample more study sites within a similar time period from May to late July.

In the future, we hope to also consider other factors that may affect flower visitation within our observational experiments. The annual difference in spatial distribution of flowers may affect visitation rates, especially since climate change has affected first flowering dates and created phenological mismatches (McKinney et al, 2012). For example, early-flowering nectar resources in the Rocky Mountains, such as *Erythronium grandiflorum* and *Delphinium nuttallianum*, have had increasingly advanced flowering dates (McKinney et al. 2012). Implications of this phenological mismatch include a reduction of resource availability for Broad-tailed hummingbirds, increase in competition for nectar resources, a forced change in breeding range towards lower latitudes (McKinney et al, 2012). Eventually, if the interval between arrival of Broad-tailed hummingbirds and first flowering dates shortens, populations of Broad-tailed hummingbirds could be in danger of significant decline (McKinney et al. 2012).

Oftentimes, hummingbird visited flowers also contain specific communication channels meant to attract hummingbirds (Castellanos 2004). For example, color significantly improve attractiveness of

legitimate pollinators to *I. aggregata* (Melendez-Ackerman et al, 1997). It is likely that the morphological traits of flowers are not the only factor that determines whether an individual hummingbird visits a flower. Studying the interaction of flower color and morphology on attracting hummingbirds would create a clearer picture of why Broad-tailed hummingbirds are visiting certain flowers. Also, anecdotally, many have assumed that hummingbirds favor flowers that contain nectar with higher sucrose contents. However, flowers such as *I. aggregata* have especially “thin” or “dilute” nectar compared to flowers visited by pollinators such as bees (Baker 1976, Meeuse 1961). It has been hypothesized that hummingbirds prefer thinner nectar due to the ease in which the nectar moves up their tongues via capillary action (Grant & Grant 1968). Interactions between nectar content and bill morphology have also yet to be explored.

The pollination syndrome theory has also been debated frequently among the scientific community. However, over evolutionary time, many flowers seemed to have developed traits that deter some pollinators and attract others (Castellanos 2004). Therefore, apart from hummingbird specific flower adaptations, other adaptations (i.e. anti-bee adaptations) have also been important in shaping hummingbird and flower relationships (Castellanos 2004).

Figures

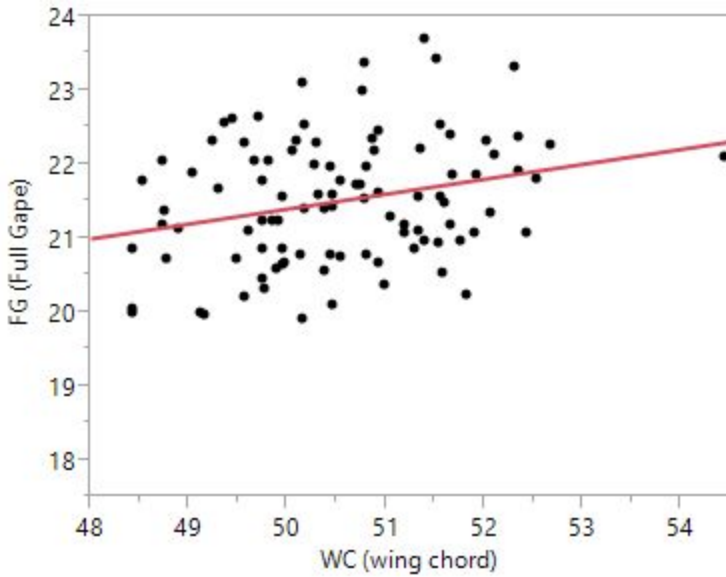


Figure 1. . Bivariate analysis on wing chord and full gape of n=198 Broad-tailed hummingbirds in millimeters. Red line represents line of best fit (Bivariate, RSquared:0.072326).

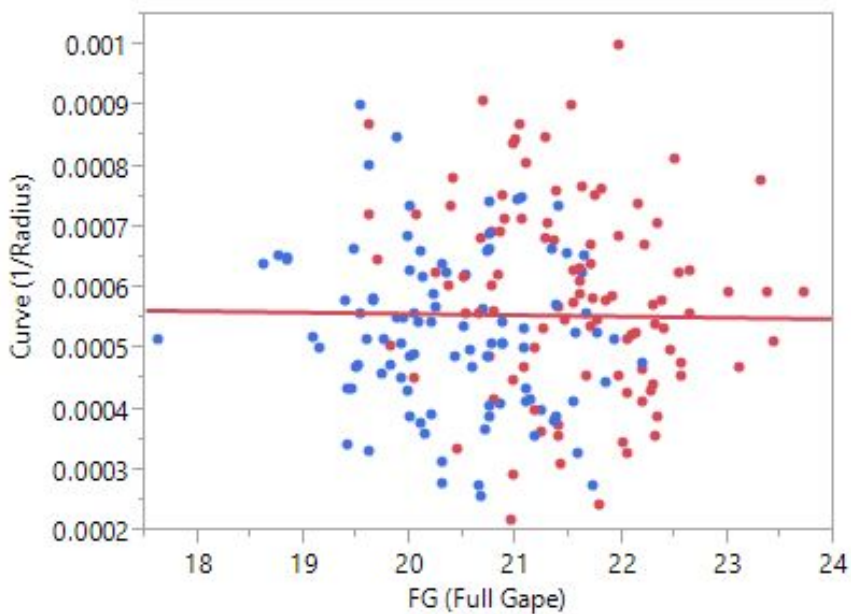


Figure 2. Bivariate analysis on curvature and bill length of n=198 broad-tailed hummingbirds in millimeters. Blue dots represent males and red females. Red line represents line of best fit (Bivariate, RSquared: 0.00022).

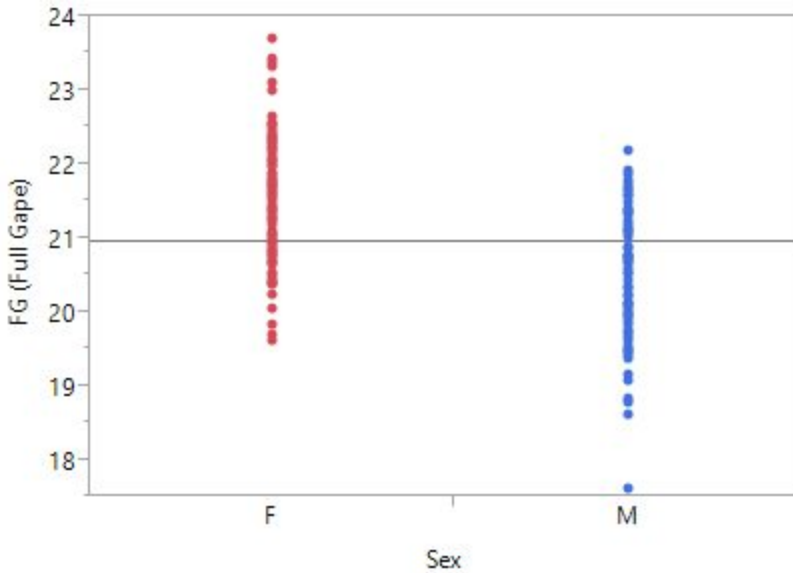


Figure 3. Average bill length (in mm) of 97 female and 101 male Broad-tailed hummingbirds. Females and males were significantly different, with females and males averaging 21.53 and 20.39mm respectively. Each dot represents an individual bird. Black line represents the mean of all data, regardless of sex (ANOVA, $F=89.81$, $df=197$, $p<0.001$, $\alpha=0.05$)

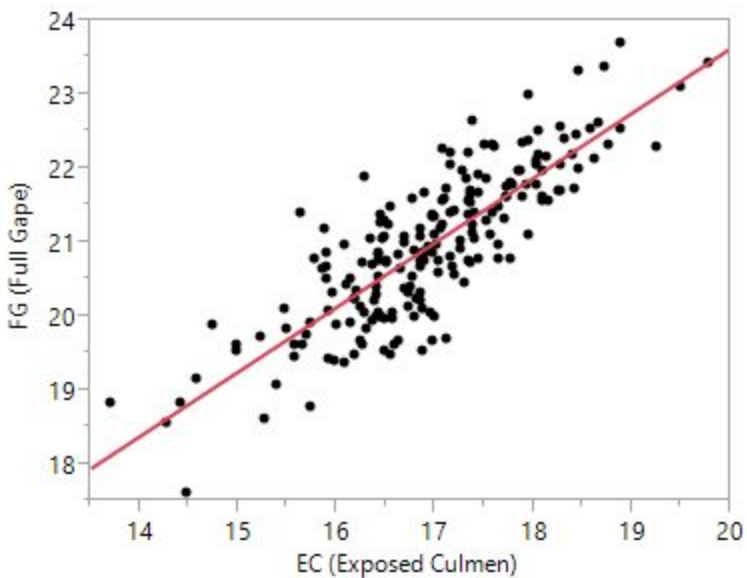


Fig 4. Bivariate Fit of full gape and exposed culmen in millimeters. Red line represents line of best fit. Each point on the graph represents an individual hummingbird (Bivariate, $RSquared: 0.691$).

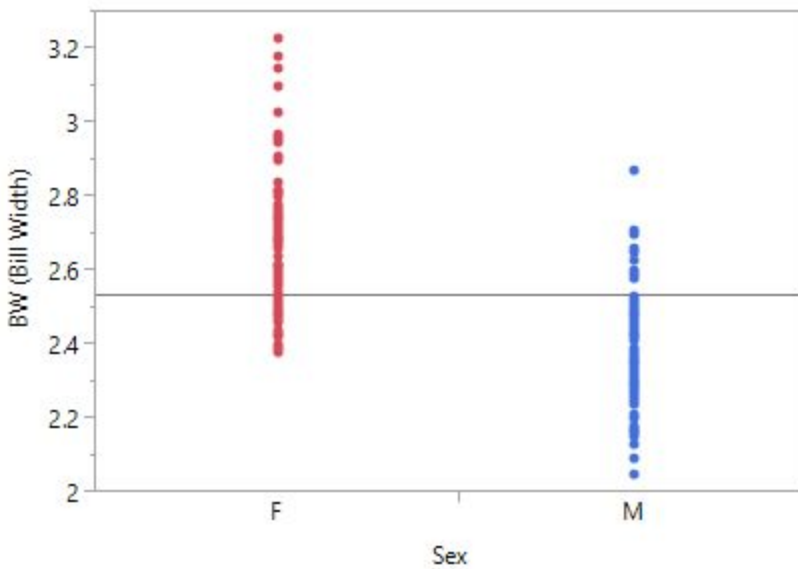


Figure 5. Average bill width (in mm) of 97 female and 101 male Broad-tailed hummingbirds. Females and males were significantly different, with females and males averaging 2.66 and 2.40 mm respectively. Black line represents the mean of all data, regardless of sex. Each dot represents an individual bird. (ANOVA, $F=119.43$, $df=197$, $p<0.001$, $\alpha=0.05$)

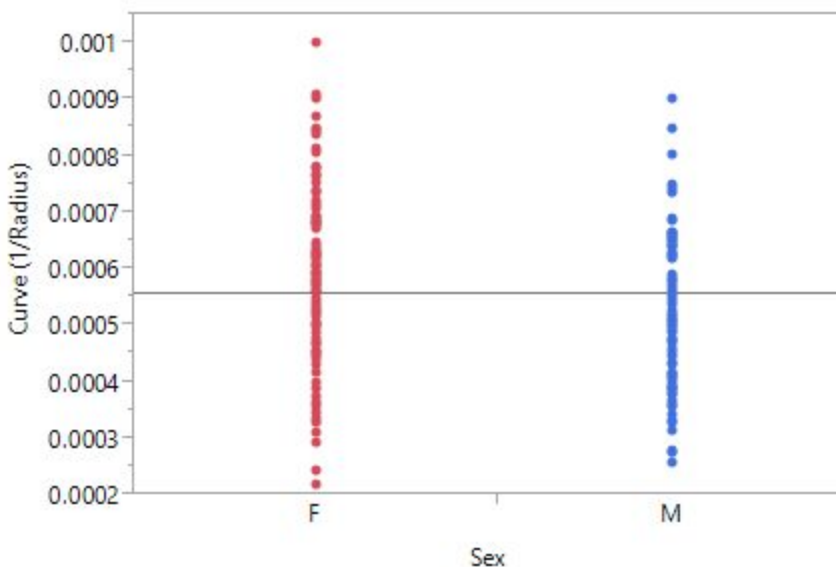


Fig. 6 Average bill curvature (in mm) of 97 female and 101 male Broad-tailed hummingbirds. Females and males were significantly different, with females and males averaging 0.000587 and 0.000525 mm respectively. Curvature was taken using three-point circular ROI in ImageJ. Once three points were chosen along the midline of each bill, a perfect circle was fit on the bill. Radius of the circle was calculated using ImageJ and curvature was calculated as $1/\text{radius}$ in mm. Black line represents the mean

of all data, regardless of sex. Each dot represents an individual bird (ANOVA, $F=8.20$, $df=197$, $p<0.0046$, $\alpha=0.05$).

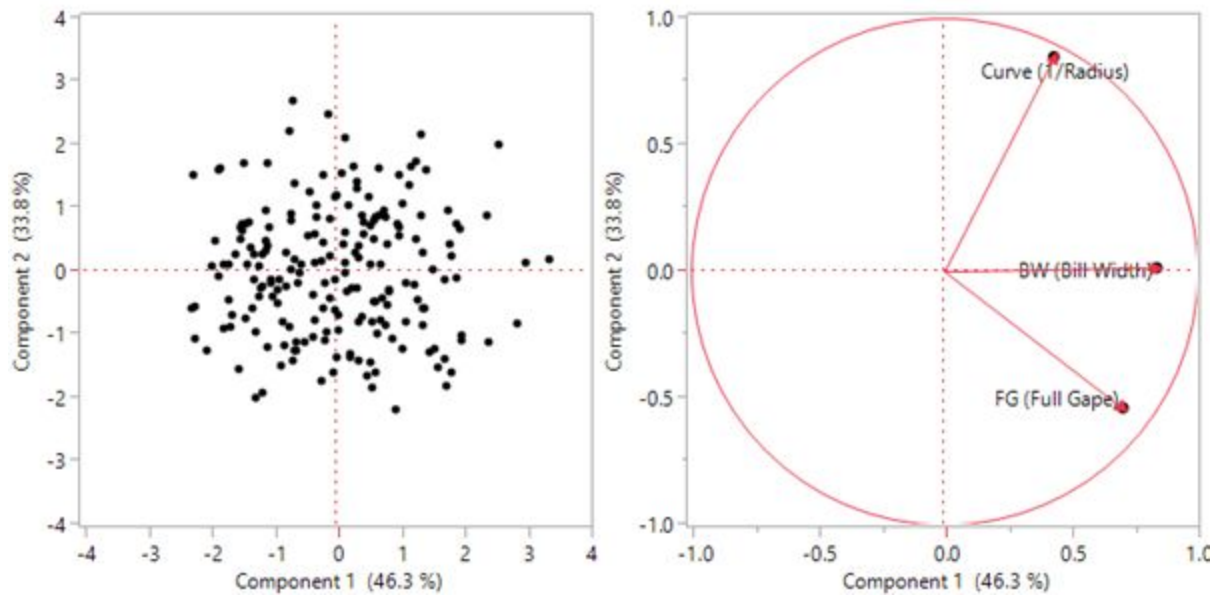


Fig 7. Principle Components Analysis of n= 198 population of Broad-tailed hummingbirds on three aspects of bill morphology: bill length, width, and curvature. The x and y axis represent data points with the highest and second highest variability respectively.

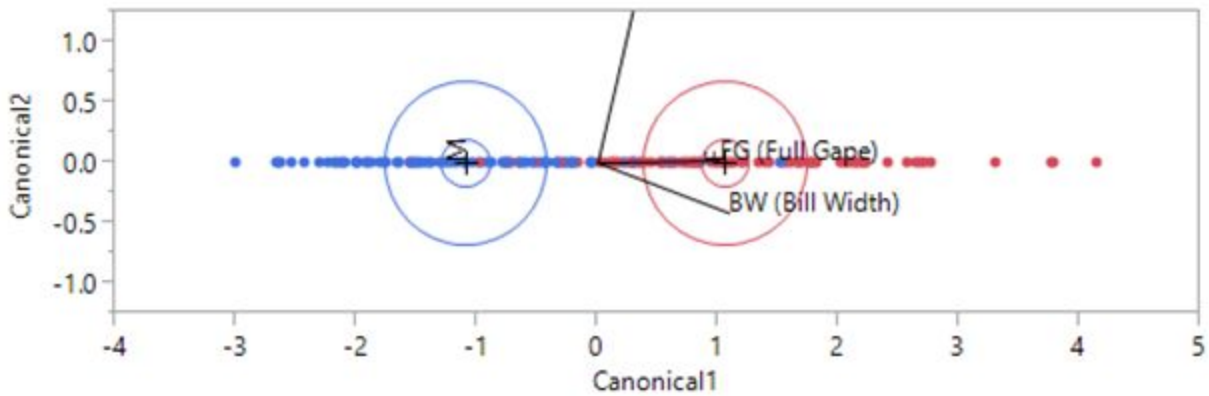


Fig. 8 Discriminate Analysis of n= 198 population of Broad-tailed hummingbirds on three aspects of bill morphology: bill length, width, and curvature. A priori knowledge on whether a bird was male or female was used to categorize birds into male or female based on bill length, width, and curvature. Approximately 10.6% were misclassified as the wrong sex.

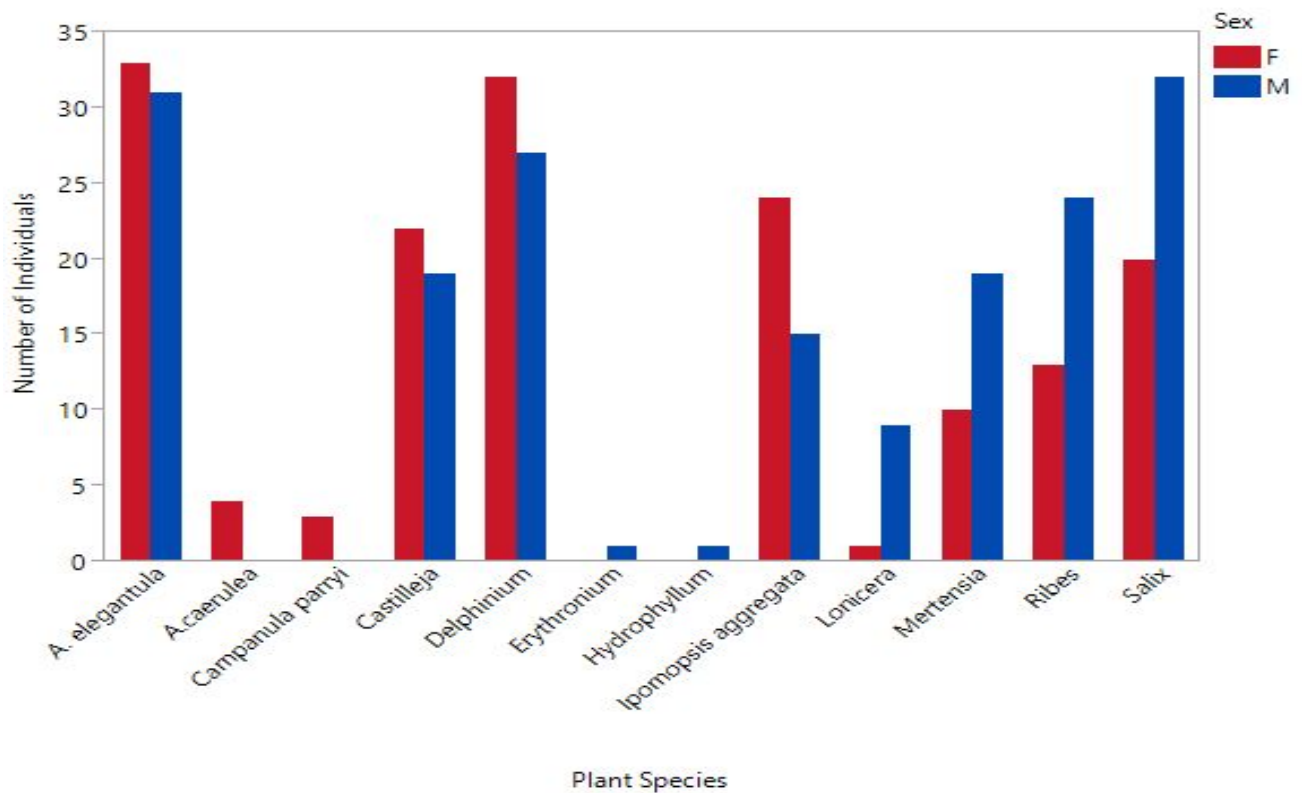


Fig 9. Visitation rate of n=198 Broad-tailed hummingbirds across 13 types of wildflowers. Pollen slides were analyzed from June 6th to July 21st. Blue bars represent the number of male hummingbirds that visited each type of wildflower and red represents females. Pollen identification down to species was not always achieved, in those cases the only the genus would be recorded.



Fig 10. Primary study site located at David Inouye's cabin in Gothic, Colorado (RMBL: 38.96° N, 106.99° W, 2900 m above sea level).



Fig 11. Hummingbird visited flowers collected within Gothic townsite with the exception of *Erythronium grandiflorum* and *Chamerion angustifolium* which were collected at Mexican Cut and Emerald Lake respectively.

Appendix

Preparation of Fuchsin gel

To prepare the Fuchsin gel, add 50 grams of gelatin to 175cc of distilled water in a beaker and heat until the mixture dissolves. Next, add 150cc of glycerin and 50 grams of crystalline phenol. In order to create the desired color of the gel, add as many crystalline basic fuchsin gels as necessary (Kearns & Inouye, 1993).

Flower species collected in Gothic Townsite, Mexican Cut, and Emerald Lake

1. *Mertensia ciliata*
2. *Polemonium foliosissimum*
3. *Erythronium grandiflorum* (Mexican Cut)
4. *Frasera speciosa*
5. *Chamerion angustifolium* (Emerald Lake)
6. *Lonicera involucrata*
7. *Pedicularis procera*
8. *Pedicularis bracteosa*
9. *Aquilegia elegantula*
10. *Delphinium barbeyi*
11. *Aconitum columbianum*
12. *Castilleja miniata*
13. *Castilleja sulphurea*
14. *Ipomopsis aggregata*
15. *Aquilegia caerulea*
16. *Ribes montigenum*

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