

Variation in bill morphology and pollen prevalence in the Broad-Tailed
Hummingbird (*Selasphorus platycercus*)

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Abstract

We found that the Broad-Tailed Hummingbird (*Selasphorus platycercus*) exhibits significant intraspecies variation across several morphological traits, including those important in foraging. However, the effects of these morphological variation on flower visitation is not well understood. To begin to understand the effects morphological variation on flower foraging, we quantified morphological variation across a total of 521 Broad-Tailed Hummingbirds across two summers at the Rocky Mountain Biological Laboratory. We also quantified prevalence of *Ipomopsis* spp. pollen in this population in order to test for a link between variation in bill morphology and variation in *Ipomopsis* visitation. We found that females are significantly larger in full gape, exposed culmen, bill width, bill depth, mandible width than males. There was no difference in curvature of bill and wing chord between males and females. We found a significant and positive association between bill length in males and the presence of *Ipomopsis* spp. pollen in males and a negative association between curvature and *Ipomopsis* spp. presence in both males and females. Overall, our analysis suggests that the considerable variation in bill traits in the Broad-Tailed Hummingbird at RMBL may be functionally important. These patterns may mirror patterns across hummingbird species, which show tight linkages between bill length and curvature and morphological traits of nectar plants.

Introduction

Organisms must maintain a high caloric intake in order to survive and reproduce. However, organisms face several constraints while foraging for food, including handling time, and morphology (Banbura 1999, Tucker *et al.* 1995, Wainwright 1988). To overcome these constraints species implement different foraging strategies (Chapman *et al.* 2004). Differentiation within a species based on foraging constraints and patterns may lead to niche partitioning. For example, the African finch (*Pyrenestes ostrinus*) has two prevailing bill size morphs, large and small (Smith 1990). While large billed birds eat seeds of a wide size range, they have an increased handling time with small seeds (Smith 1990). In contrast, small-billed

birds are able to utilize small seeds with no handling time penalty, but cannot eat large seeds (Smith 1990). This difference in handling time may contribute to the presence of both bill size classes. Similarly, the Ruby-throated Hummingbird (*Archilochus colubris*) exhibits a similar morphological constraint while feeding at nectar sources; longer-billed birds were able to feed at greater depths than shorter-billed birds, whereas shorter-billed birds made few insertion errors at narrow flowers than longer-billed birds (Temeles 1996). This is surprising because such interspecies variation in foraging efficiencies may lead to resource partitioning within a species.

The Broad-Tailed Hummingbird (*Selasphorus platycercus*) (BTH) is a migratory bee hummingbird that winters in Mexico and migrates up the length of the Rocky Mountains in summer (Bendire 1895). In the summer months, BTHs follow sequentially blooming flowers of varying corolla dimensions (Waser 1976). Similar morphological variation has been observed in the BTH to the Ruby-Throated Hummingbird (Chen 2016). Therefore, the BTH may also face feeding efficiency trade-offs when feeding on different flowers. However, given the phenotypic intra- and intersexual variation, there is evidence for some level of floral use specialization within and between the sexes (Chen 2016). The variation observed within a sex may also lead to intersexual variation floral specialization.

At the Rocky Mountain Biological Laboratory in Gothic, Colorado, we measured 1) variation in morphological traits of BTH, including sex, wing chord, and of bill shape, 2) we also measured *Ipomopsis* spp. pollen prevalence by identifying pollen using microscopy. We then examined associations between morphological trait variation and pollen prevalence. This allows to begin to explore the potential for the adaptive significance of within-species morphological variation in the BTH.

Methods

Study Species and Site

Adult BTH were sampled at several sites in Gunnison County, Colorado during the summer of 2017. BTH are the most abundant hummingbird species at our study sites during

June and early July. Males and females several features distinguishing males and females. Females have white gorgets with green spots while males have red iridescent gorgets. Males also produce a high pitched trilling sound that is used for mating displays and territory defense. These features distinguish the BTH from other species of hummingbirds.

Capture of Broad Tailed Hummingbirds

BTH were captured using a bonnet trap, which suspends a hummingbird feeder in its center and uses a gravity-based drop-net that is released around the feeder onto a platform (Ruschi 2009). A total of 400 BTH were captured in the summer of 2017 (male n=205 female n=195). After capture, each BTH was subject to several procedures in the following order: 1) pollen collection 2) side profile picture 3) morphological measurements.

Pollen Collection and Analysis

Pollen was collected from the bill and head of each bird using two collection methods. First a 1mm² piece of sticky tape was used on half of the bird's forehead, gorget, upper and lower parts of the bill for future pollen sequencing. After this process was repeated with a piece of 1mm³ piece of fuchsin gel on the other half of the bird's head. At a later date, the gel was melted onto a microscope slide. Each slide was examined underneath a compound light microscope and microscope camera. Starting at the top left corner the entire slide was scanned. A reference collection of pollen grains were used to identify pollen present to genus or species when possible. A list of flower species and genera that can be visually identified can be found in the appendix.

Bill measurements

Each bird had bill length, exposed culmen, bill width, bill depth, mandible, and wing chord measured. Hand calipers with a digital display were used for each measurement. Each set of measurement was performed twice by different researchers and later averaged. Full gape was measured from the back of the mandible to the tip of the bill. Exposed culmen was measured from the end of the feather on the upper bill to the tip of the bill. Bill depth is the

distance across the bill where the feathers end on the upper bill. Bill depth is the distance from upper and lower portion of the bill from the end of the feather on the upper part of the bill.

Calculating Bill Curvature

Each bird had a side profile picture taken alongside a 60mm calibration line. ImageJ was later used to calculate the curvature of the bill. A three point ROI was used in ImageJ to create a perfect circle using three points along the midline of the bill. The radius of each bill was calculated twice and average. The curvature of the bill was calculated by using $1/\text{radius}$.

Statistical Analysis

Statistical Analyses were restricted to samples collected in Almont, Colorado and to presence absence of *Ipomopsis* spp. A Chi-squared test was performed to assess the prevalence of *Ipomopsis* spp. between sexes. Birds were then binned *Ipomopsis* spp. presence or absence. An Analysis of Variance test was performed in which birds from each class (Pollen present or absent) differ in load score using two principal component obtained from a principal component analysis of all morphological traits. The morphological traits driving the PCA were then parsed. T-test were performed across sexes for each morphological traits measured.

Results

BTH Morphological Variation

Variation was quantified between males and females in BTH. Full gape, exposed culmen, bill width, bill depth, mandible, wing chord and curvature. Wing chord was used as a proxy for body size due to the high positive correlation with body mass across hummingbird species (Brown and Bowers 1985). Based on linear regression approximately 3% of the variations in full gape was explained by wing chord.

A two tailed student's t-test with equal variance was performed for each morphological trait measures by sex. Females were significantly larger ($p < 0.01$) in Full gape, exposed

culmen, bill width, bill depth, and mandible (**Fi. 2a-e**). There was no difference in curvature and wing chord between sex ($p>0.05$) (**Fig. 2f-g**).

Within a population of BTH curvature, wing chord, mandible were decoupled from each other and from full gape and exposed culmen (Principal Component analysis) (**Fig.3**) (n=94).

Ipomopsis spp. Pollen Prevalence Variation Between and Within Sexes

Data from 2016 was pooled with 2017 data to increase statistical power. In a population sample of 294 BTH (n=165 M; n=129 F) *Ipomopsis* spp. prevalence did not vary between sexes. The usage of *Ipomopsis* spp. was nearly equal for the two sexes. However, females did have slightly higher prevalence.

Two-tailed student's t-tests (equal variances) were performed for each trait and presence or absence of *Ipomopsis* spp. (**Fig. 4-6**). *Ipomopsis* spp. use did not vary significantly in females for any trait. In a population of males (n=114) there was an interaction between curvature and *Ipomopsis* spp. pollen presence and absence. The average curvature with *Ipomopsis* spp. was significantly smaller than the average curvature without *Ipomopsis* spp.. There was also a significant correlation between full gape in and presence or absence of *Ipomopsis* spp. pollen in a population of males n=132. The average length of bills with The average curvature with *Ipomopsis* spp. was significantly smaller than the average curvature without *Ipomopsis* spp. present were longer than the average bill length with The average curvature with *Ipomopsis* spp. was significantly smaller than the average curvature without *Ipomopsis* spp. absent. In a population of females n=122 and found an interaction between *Ipomopsis* spp. prevalence and mean curvature. The same interaction observed in males was found.

Discussion

Bills of female BTH, are on average, larger than males in all measured traits. Wing chord

and bill curvature did not differ between males and females. Considerable variation was found within each sex for all traits. Wing chord only explained 3% of the variation in full gape. This indicated that body size and bill length are independent of each other. This is surprising because in other species of birds the body size strongly predict the variation in bill length (Hughes 2014). This pattern is also surprising because it is much weaker than the pattern discovered across species of hummingbirds (although across species, the pattern is weaker than for other birds) (Brown and Bowers 1985). This independence could be due to environmental constraints on wing size due to altitude (Ortega-Jimenez *et al.* 2016). However, in hummingbird species sexual dimorphisms is a common occurrence (Bleiweiss 1999). Foraging also appears to play an important role in determining bill length across hummingbird species (Tavares 2016). Therefore, the differences in bill size could be the result of several different pressures acting on males and females.

One possible explanation for the variation in bill size for males and females is that males are more territorial than females (Miller and Inouye 1983). Males may be selective on which females may breed in their territories and could limit the flowers that are available to them. Consequently, this limitation in resource availability females BTH may have developed traits that allows them to access floral resources that males may not as easily access. Another reason for differences in bill shape is that females are the only caretaker of young and have to devote time and energy requirements to these activities (Calder 1973). The time and energy budget required for parental care may limited the range at which the females can forage. Since males are freed from this restraint they may have larger foraging ranges. These difference in ranges and flower availability to males and females could lead to the differences in in bill shape.

No significant difference in *Ipomopsis* spp. prevalence was found between sexes. This indicates that *Ipomopsis* spp. is being used equally among males and females. Given that did bills not vary in curvature this may indicate that curvature is an important bill trait in the use of *Ipomopsis* spp. since *Ipomopsis* spp. flowers tend to have straight corollas. This could be an

example of phenotype matching that has been observed across hummingbird species (Maglianesi 2014). In other species of hummingbirds there are strong associations between hummingbird bill shape and flower shape. Similar matching may be occurring within the BTH.

Within sex we found associations between two bill traits in both males and females and pollen prevalence. Male *Ipomopsis* spp. pollen prevalence was associated with both average full gape and average bill curvature. The average bill length with *Ipomopsis* spp. present was longer than the average bill length without *Ipomopsis* spp. present. The average curvature with *Ipomopsis* spp. pollen present was smaller while the average curvature without *Ipomopsis* spp. pollen was larger. However, in females only curvature was associated with *Ipomopsis* spp. pollen prevalence. The association found in males between *Ipomopsis* spp. pollen and full gape and was not found in females could be explained by the variation in bill length between sexes. Females have significantly longer bills than males, and bill length appears to be an important factor in *Ipomopsis* spp. Therefore, female may not be restricted in *Ipomopsis* spp. use by full gape the same way males are restricted by full gape. A possible explanation for the difference in bill length and *Ipomopsis* spp. pollen prevalence could be feeding efficiencies at long flowers. It was shown in Ruby-Throated hummingbirds that long-billed birds were able to feed at greater depths while short-billed birds made few insertion errors at narrow flowers (Temeles 1996). This trade off in feeding efficiencies could also be occurring in BTH, and could be causing differential flower usage.

There was very little variation in average curvature between male and female BTH. There was also associations with average curvature and *Ipomopsis* spp. pollen prevalence and absence in both sexes.. This may indicate that curvature plays an important role in *Ipomopsis* spp. flower visitation. Since there was variation in *Ipomopsis* spp. pollen prevalence in association with curvature that is similar to the variation observed with full gape, there may be a feeding efficiency trade off also occurring with curvature.

Data were pooled for statistical analysis from 2016 and 2017. These data sets were

collected from different sites and may be problematic. However, when each data set was analysed individually and trends were in the same direction. Due to this similarity, in results from each year we felt it appropriate to pull the data sets to increase power. Analysis was also limited to *Ipomopsis* spp. because other pollen that was able to be visually identified were in low abundance and did not have high enough power for meaningful analysis.

In future studies, we hope to examine other factors influencing floral visitation of the BTH. Replicating the Ruby-Throated Hummingbird study on feeding efficiency in the BTH would provide greater insight into the trade-offs of different bill shapes. Understanding the underlying genomic variation would provide a better understanding of the maintenance of the morphological variation in BTH.

By examining the morphological variation within the BTH we have begun to unravel more complex intraspecies variations. The variation discovered in BTH may help us to understand pollinator plant interactions and how some aspects of pollinator networks operate. Understanding the variation within a species we may determine the evolutionary potential of a species. Through understanding the variation within the BTH we may also begin to understand the process by which across species variation has occurred. Within species variation is necessary for across species variation to arise.

Appendix

Pollen genus and species that can be visually identified

- 1) *Campanula parryi*
- 2) *Chamerion angustifolium*
- 3) *Erythronium grandiflorum*
- 4) *Ipomopsis* sp.
- 5) *Lonicera involucrata*

6) *Mertensia ciliata*

7) *Ribes montigenum*

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Figures

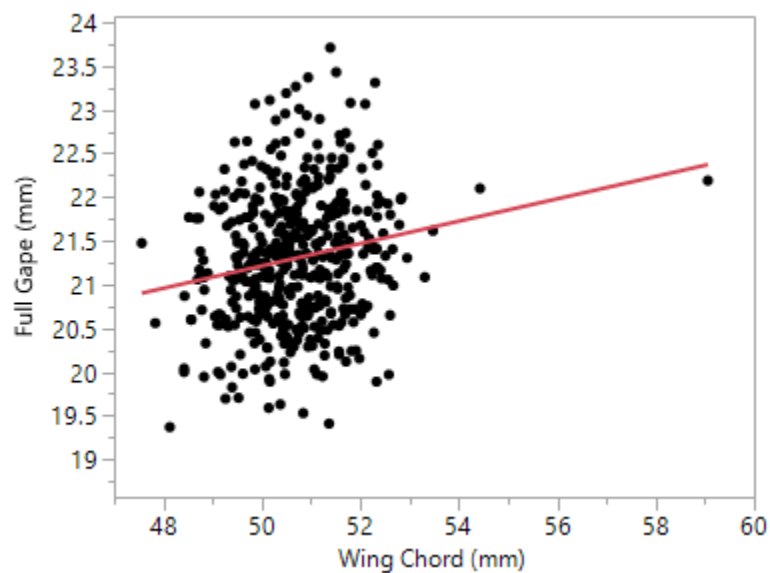


Figure 1. Linear regression analysis of Full Gape and Wing Chord in millimeters. Red line represents the best fine line. Slope: $FG = 14.817063 + 0.1279357 \cdot WC$ $P < 0.0001$ There is a positive correlation between wing chord and full gape. $R^2 = 0.033366$

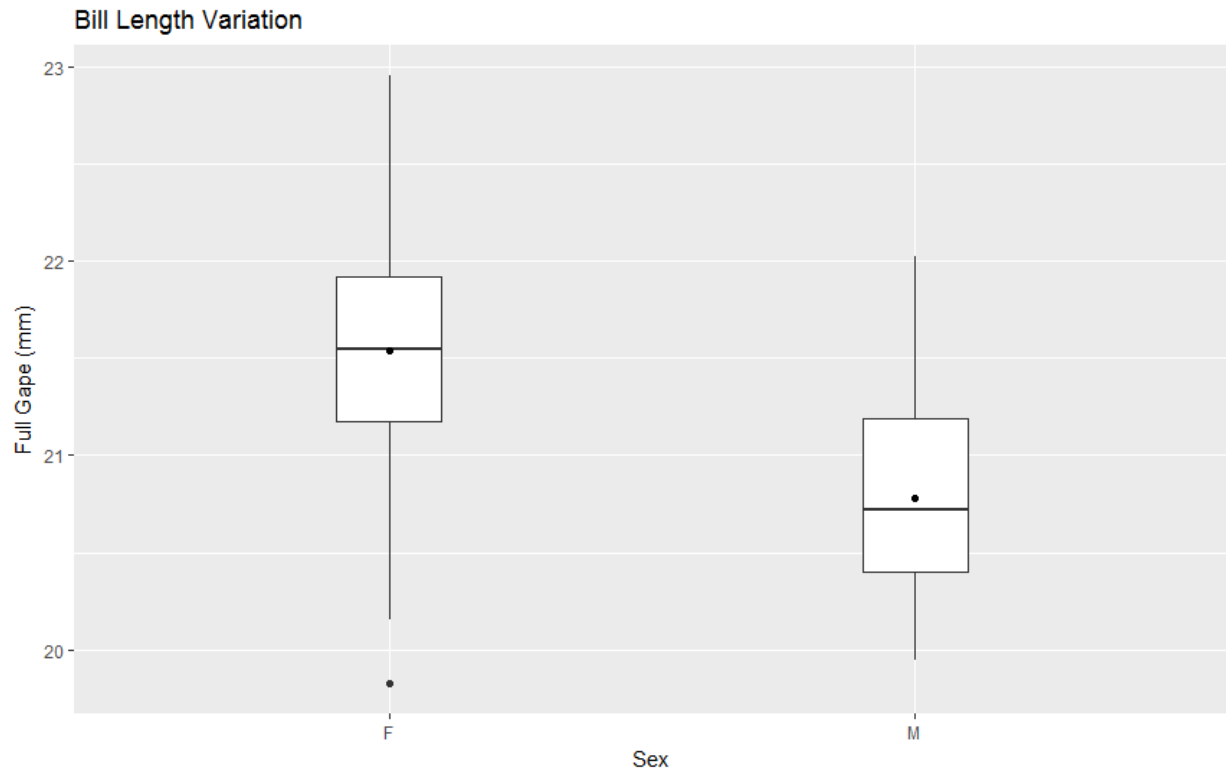


Figure 2a. T Test of Full Gape by Sex. Average full gape of females were on larger than males. $t = 15.435$, $df = 575.72$, $p\text{-value} < 2.2e-16$

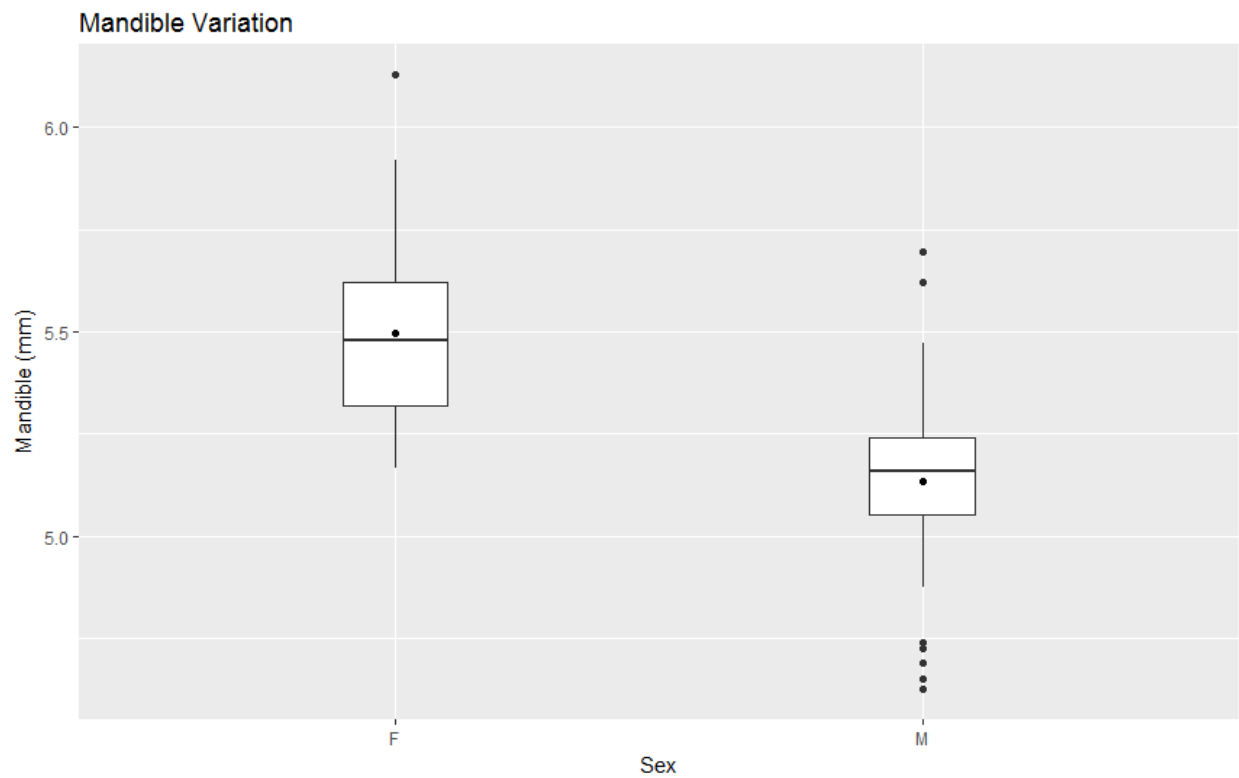


Figure 2b. T Test of Mandible by sex. Average mandible width of females were on larger than males.

$t = 6.6269$, $df = 51.451$, $p\text{-value} = 2.037e-08$

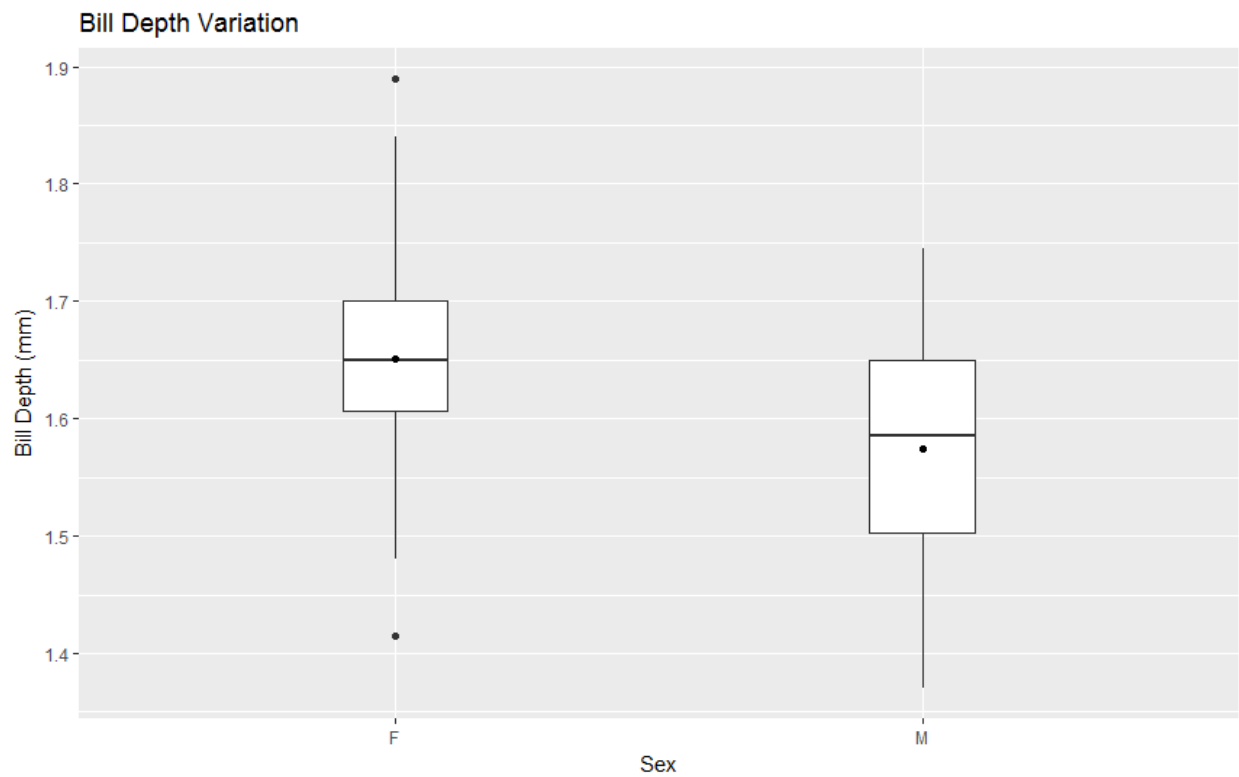


Figure 2c. T Test of Bill Depth by sex. Average bill depth of females were on larger than males. $t = 3.8447$, $df = 49.72$, $p\text{-value} = 0.0003439$

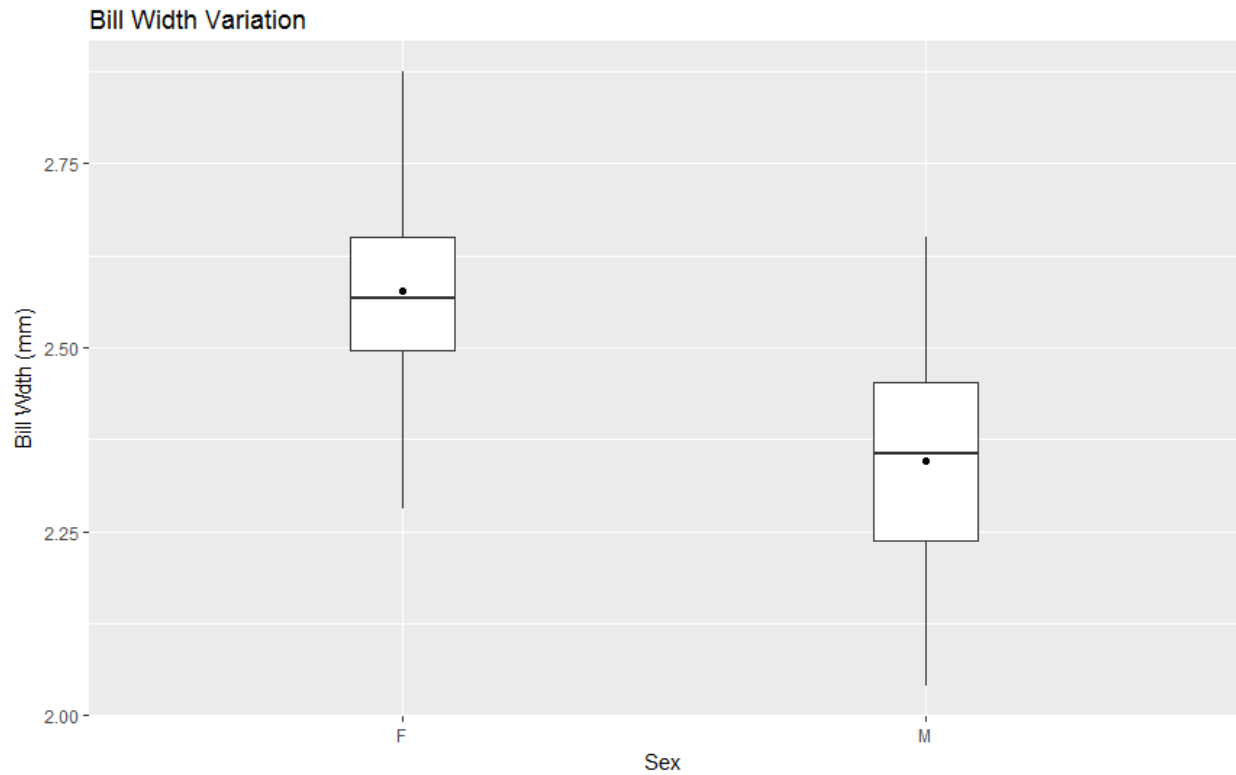


Figure 2d. T Test of Bill Width by sex. Average bill width of females were on larger than males.
 $t = 7.6035$, $df = 55.732$, $p\text{-value} = 3.582e-10$

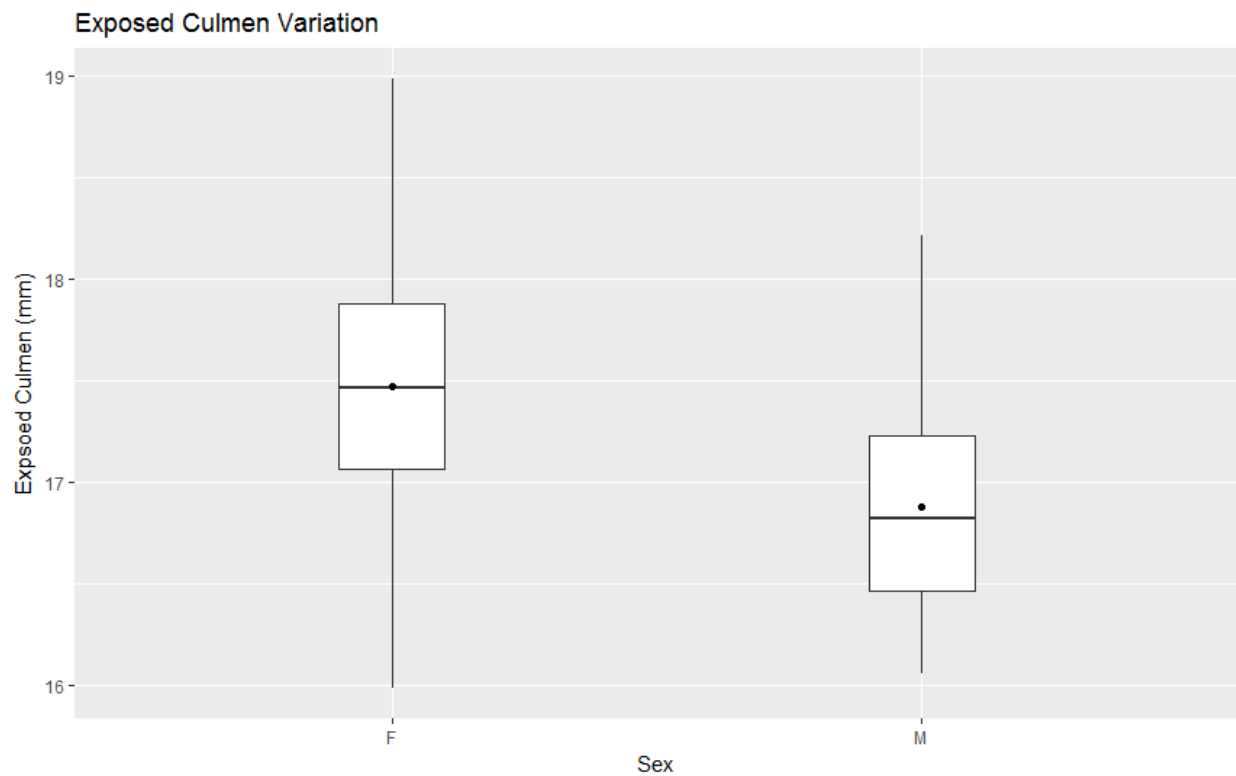


Figure 2e. T Test of Exposed Culmen by sex. Average exposed culmen of females were on larger than males. $t = 4.5823$, $df = 70.637$, $p\text{-value} = 1.931e-05$

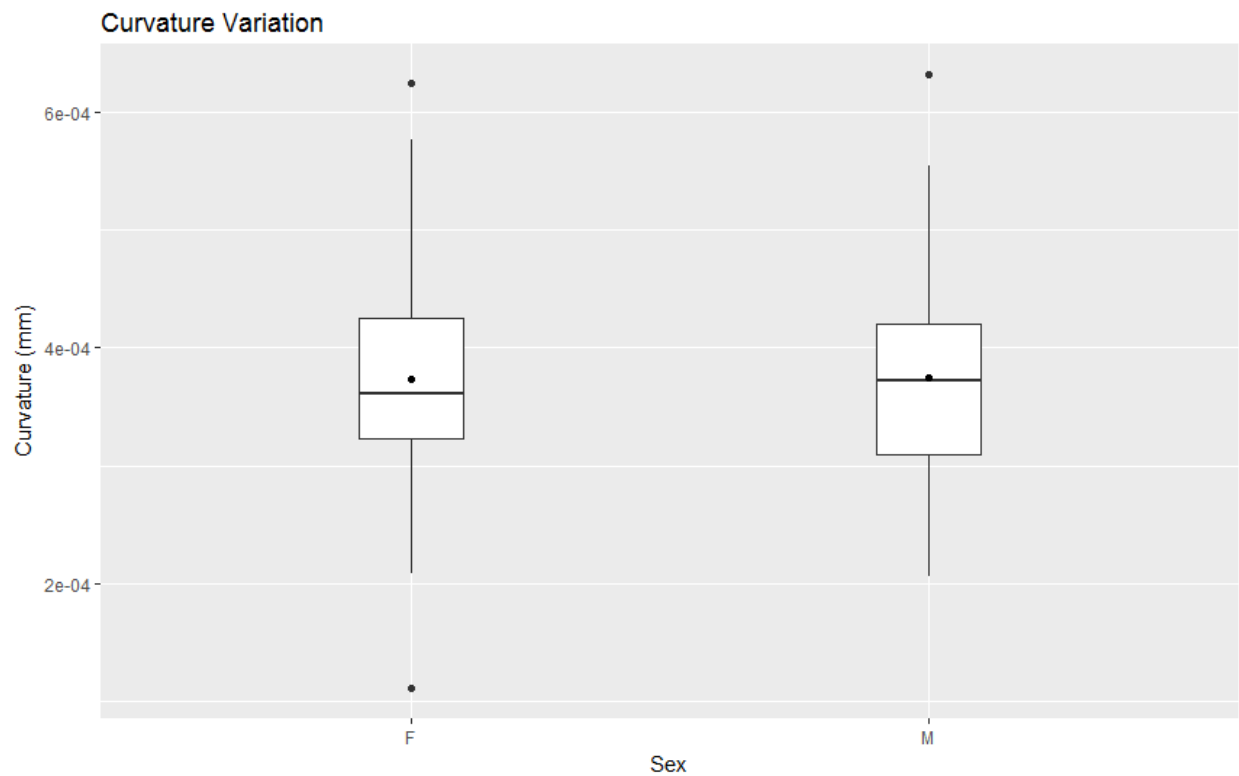


Figure 2f. T Test of Curvature by sex. No difference was found in average curvature values between females and males. $t = -0.028669$, $df = 53.324$, $p\text{-value} = 0.9772$

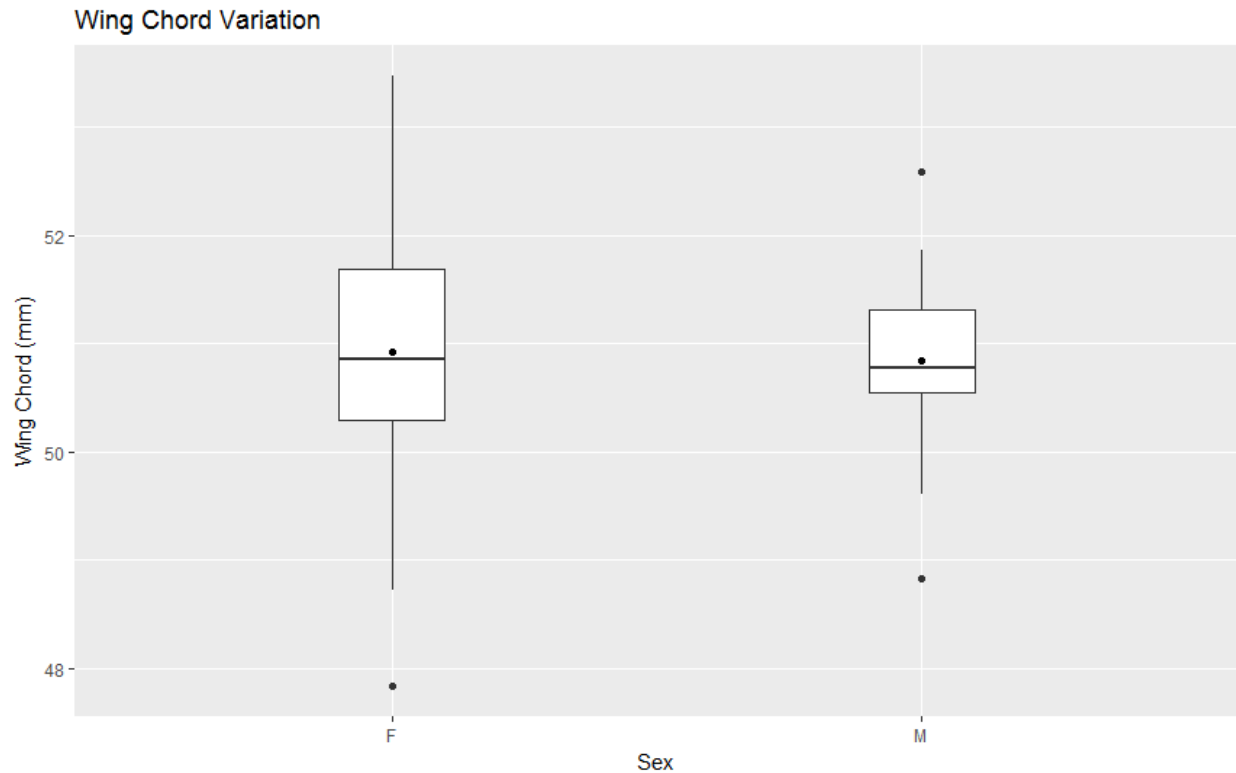


Figure 2g. T Test of Wing Chord by sex. No difference was found in average wing chord between females and males. $t = 0.3978$, $df = 83.526$, $p\text{-value} = 0.6918$

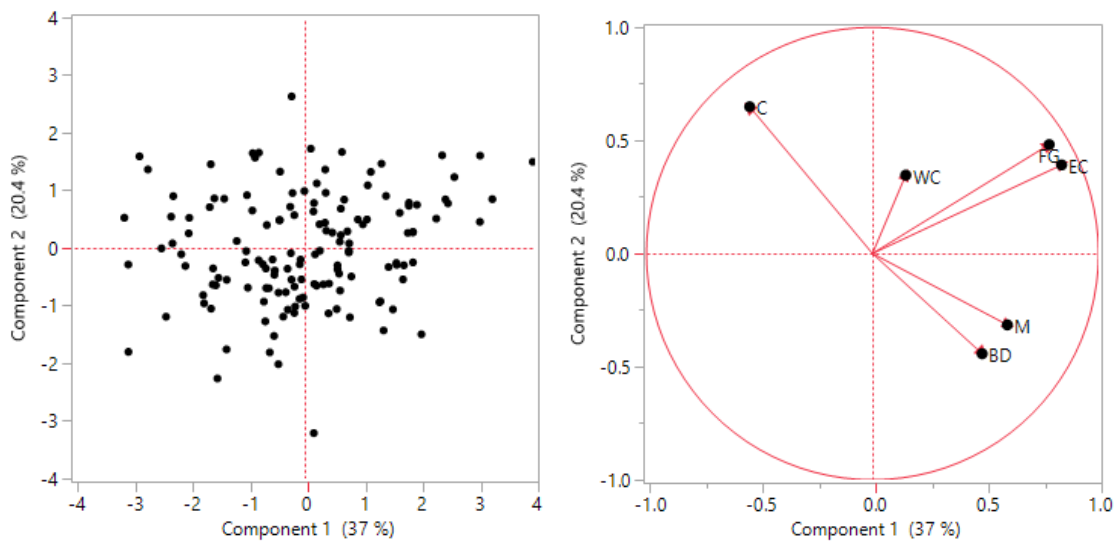


Figure 3. Principle Component analysis of n=94 Broad-Tailed Hummingbird captured in 2017. Six traits were analysed: Curvature, Wing Chord, Full Gape, Exposed Culmen, Mandible, and Bill Depth. The X and Y axis represent the data points that explain the most amount of variation.

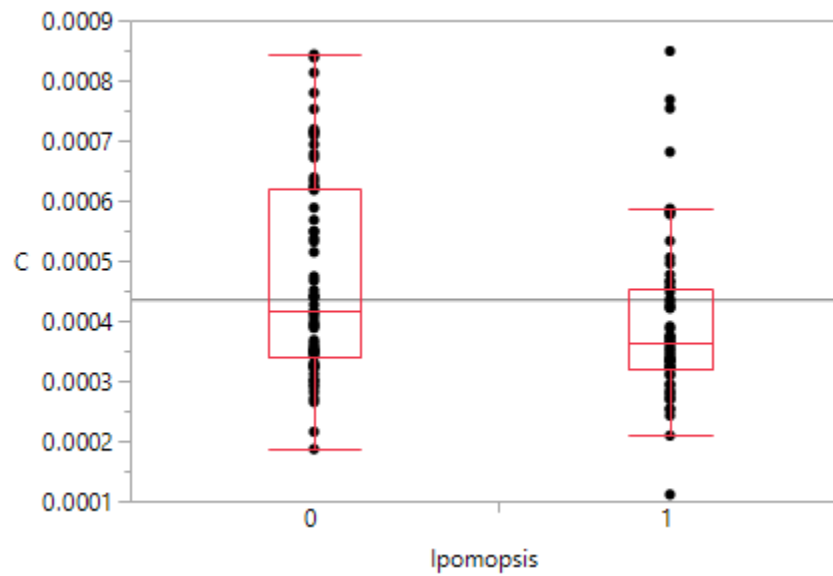


Figure 4. One Tailed Test *Ipomopsis* spp. prevalence in females by curvature. There was a difference in average curvature values in females and *Ipomopsis* spp. prevalence. *Ipomopsis* spp. pollen presence was associated with smaller average curvature values. *Ipomopsis* spp. pollen absence was associated with large average curvature values. $t=1.97993$ $p=0.0051$ $n=99$ (absent=63, present=36)

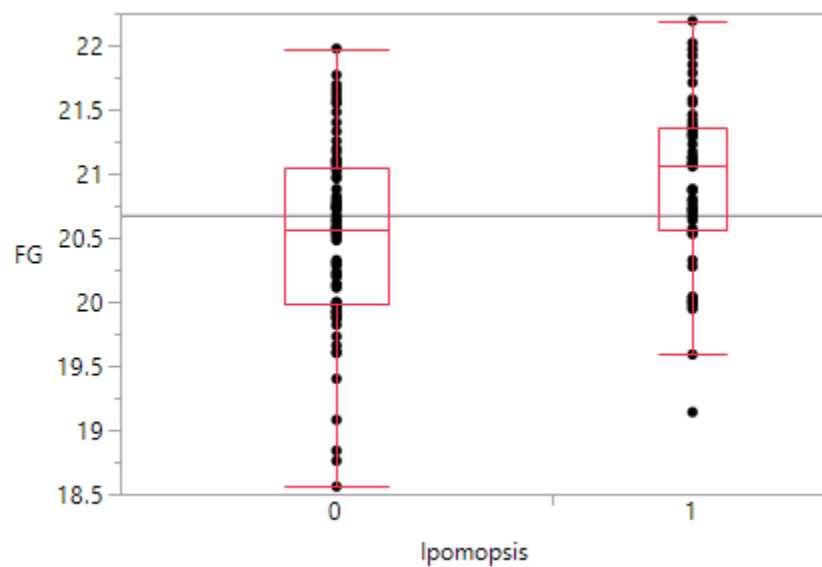


Figure 5. *Ipomopsis* spp. prevalence in males by full gape. There was a difference in in average full gape values and *Ipomopsis* spp. prevalence. *Ipomopsis* spp. pollen presence was associated with longer average full gape. *Ipomopsis* spp. pollen absence was associated with shorter average full gape values. $t=1.997867$ $p=0.0005$ $n=132$ (absent=80, present=52)

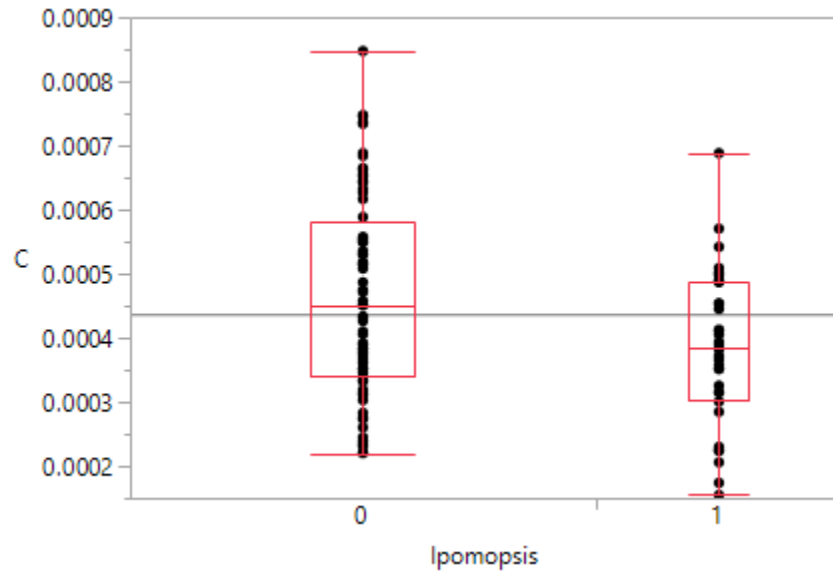


Figure 6. *Ipomopsis* spp. prevalence in male curvature. There was a difference in average curvature values in females and *Ipomopsis* spp. prevalence. *Ipomopsis* spp. pollen presence was associated with smaller average curvature values. *Ipomopsis* spp. pollen absence was associated with large average curvature values. $t=1.98350$ $p=0.0034$ $n=105$ (absent=67, present=38)

Gunnison County

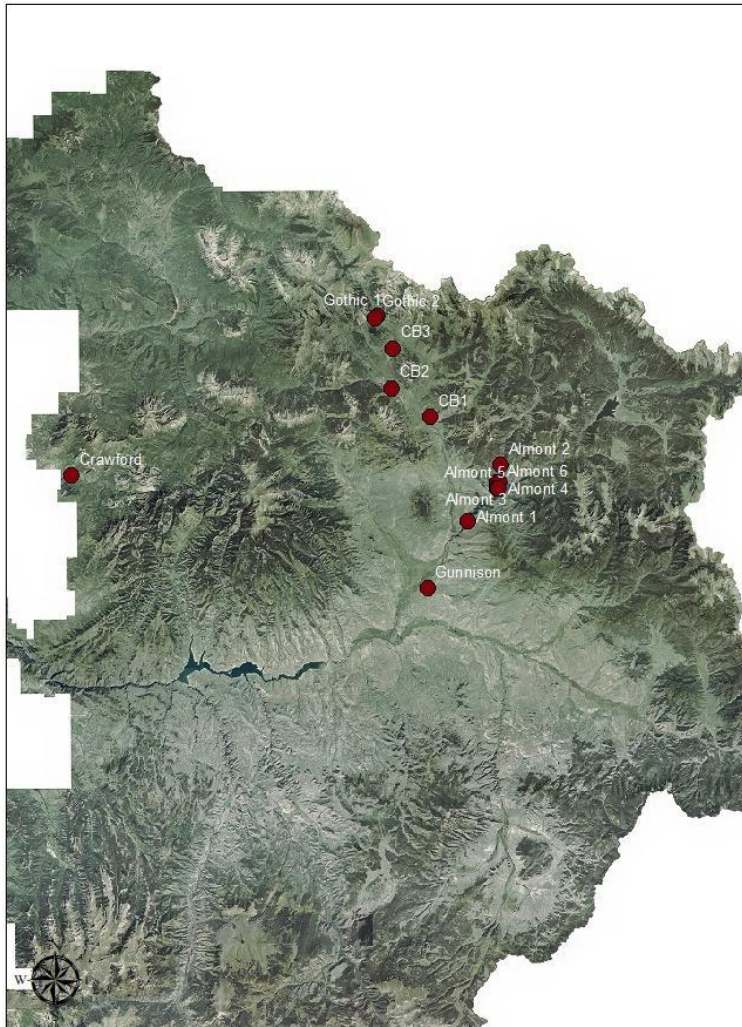


Figure 7. Map of Gunnison County, Colorado with data collection sites marked.

Almont Sites

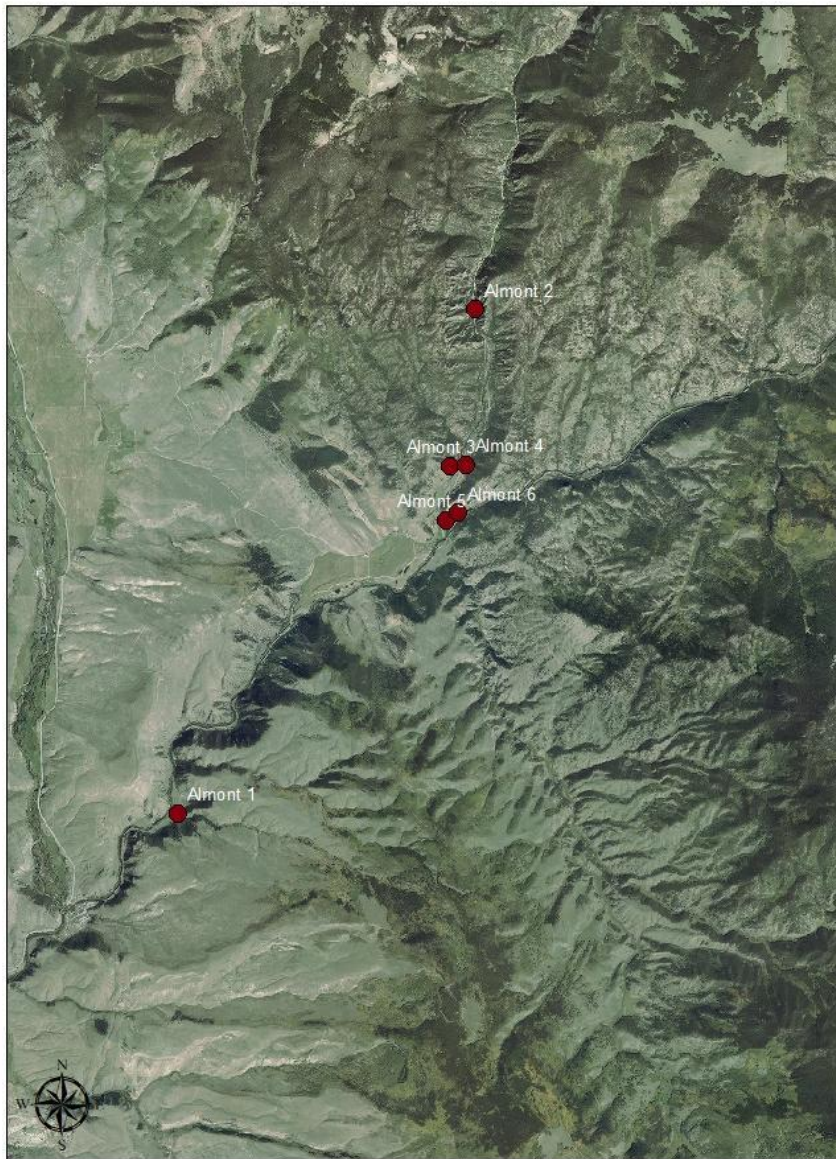


Figure 8. Map of Almont with Almont collection site plotted. All pollen data analyzed from 2017 is from these sites. Most of 2017 data collection was conducted here.

Gothic Sites

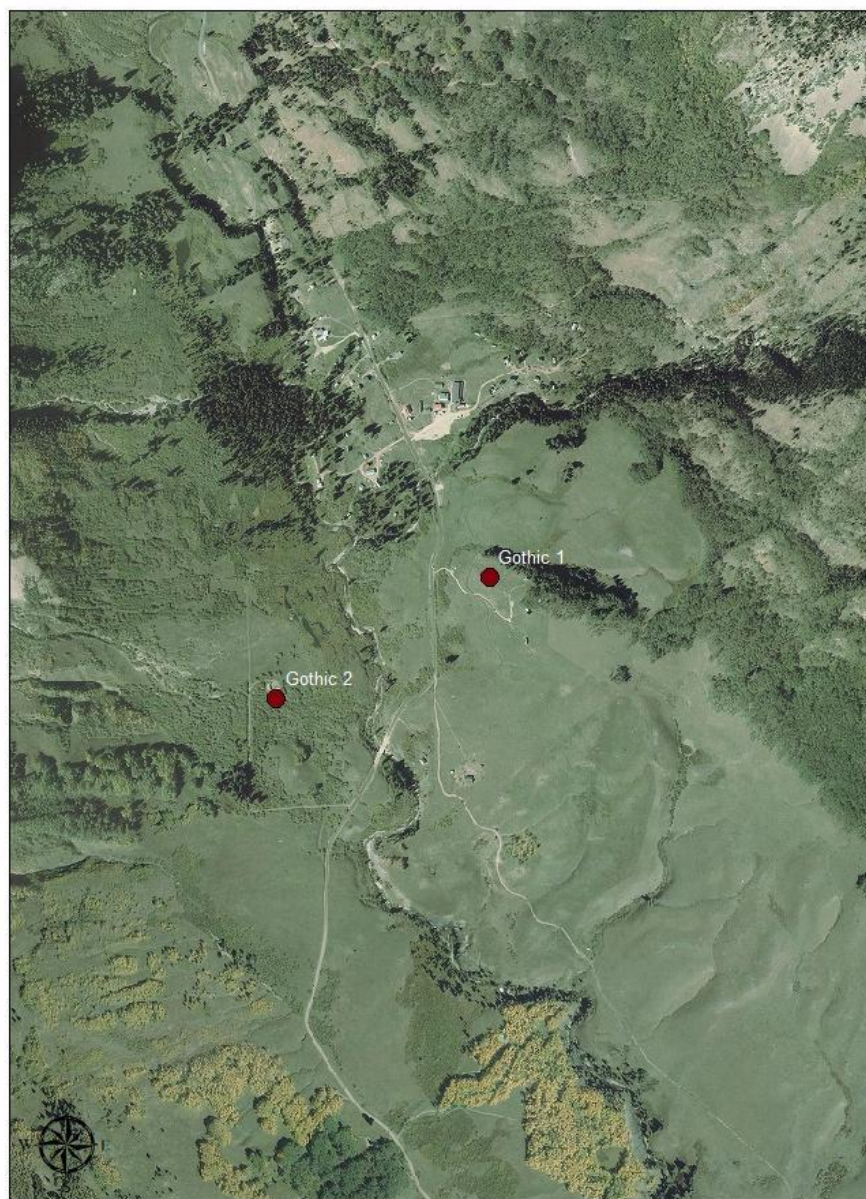


Figure 9. Map of data collection sites in Gothic. Most of 2016 data was collected at Gothic 1.

Crawford Site

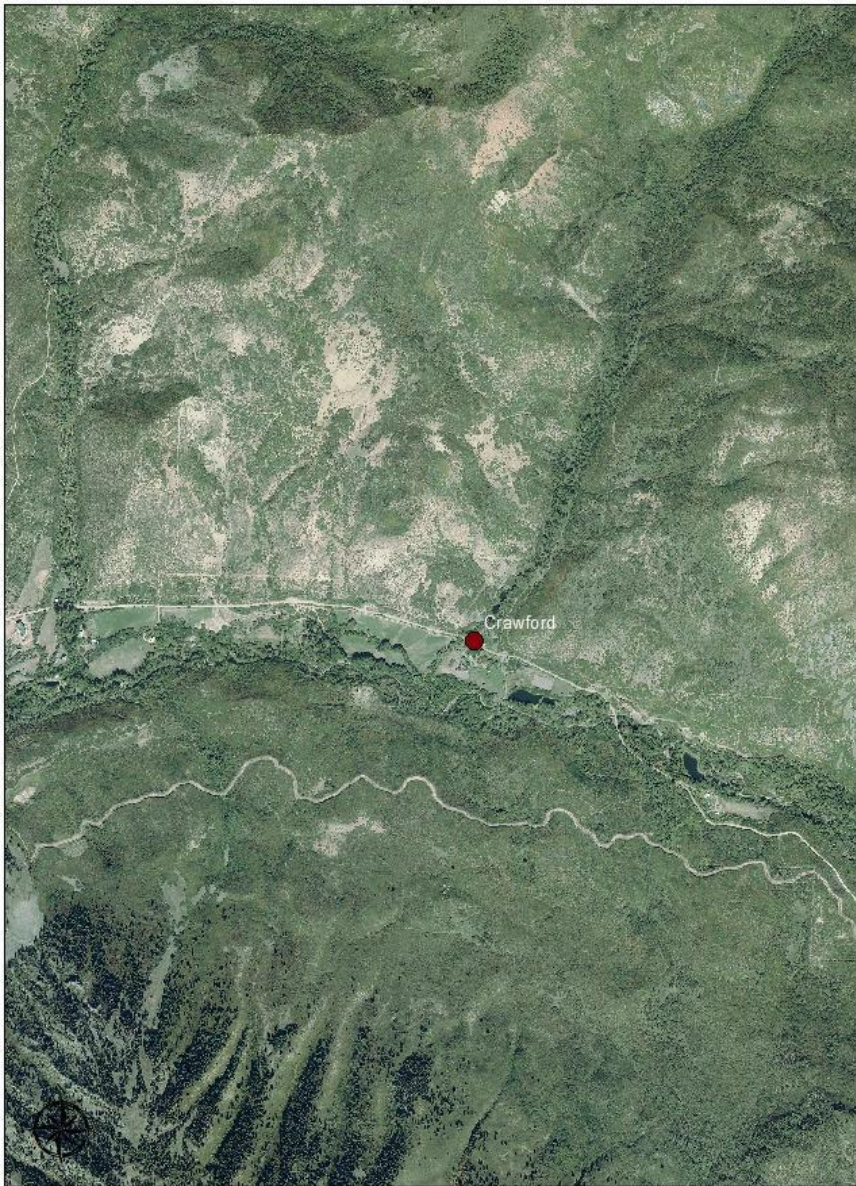


Figure 10. Crawford collection site plotted.

Crested Butte Sites



Figure 11. Data collection sites in Crested Butte are plotted.