

Across Caste Variation of Proboscis Length and Within Caste Phenotype Matching
of Subalpine Bumble Bees (*Bombus* spp.)

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Abstract

Bumble bees (*Bombus* spp.) are eusocial insects that typically consist of three castes, with each caste emerging and foraging at different times. Bumble bee queens and workers differ morphologically in several measurable traits, but morphological variation within species and within caste has not been well characterized. For the purposes of this study traits that are functionally significant are of particular interest, such as the length of the bumble bee's proboscis, which bees use to extract nectar from flowers. Variation in the length of the bumble bee's proboscis may affect their foraging behavior, and may inform what flowers they prefer to, or are capable of, feeding on. Variation of the length of the proboscis of five species of subalpine bumble bees was observed using 150 specimens of each species, comparing between species and between caste within those species. This study found that the queens of five of the five species have significantly longer proboscis than workers. Corolla length of several flowers that the bumble bees visited were measured and compared to the proboscis length. Phenotype matching, meaning flower preference according to proboscis length, was observed between species, but was not significant between castes of the same species.

Introduction

The bumble bee has captured many scientists' interest, including Darwin himself. Darwin noted in his book, *On the Origin of Species*, that the bumble bees were beneficial to the clovers growing in England. He goes on to note that the bumble bee alone visits the common red clover and speculates that the extinction of the bumble bee would cause the common red clover to become extinct as well (Darwin 1859). Our understanding of plant-pollinator interactions has become vital to understanding how climate change will affect the world's ecosystems (Potts *et al.* 2016). However, a finer understanding of within-species variation of pollinators, particularly of bumble bees who are abundant pollinators of wild plants and agricultural crops (Goulson 2010), is needed to better understand their role in ecosystems and how a changing climate may affect them.

Bumble bees are eusocial insects that are typically made up of three distinct castes: Queens, workers, and males (Kearns and Thomson 2001). Queens typically emerge in early spring once the early flowers have started to bloom, and during this time for a period of a few days to several weeks, queens forage and search for a nesting site. After an appropriate nesting site has been located the queen produces workers that take over the foraging duties. Workers spend much of their time foraging and maintaining the nest. As the season progresses the queen switches from producing workers to producing males and new queens. Broadly the members of *Bombus* have been placed into three categories based on their proboscis length: short, medium, and long (Pyke 1982, Williams *et al.* 2014).

However, the queens are typically much larger than the workers of the same species, meaning it is possible that their proboscis could be of a different size (Kearns and Thomson 2001). Phenotype matching has been observed across species of *Bombus* meaning particular species prefer certain flowers (e.g., Inouye 1980, Pyke 1982), but the within species variation in foraging has not been well characterized, nor has the effect on foraging behavior by the morphological variation in proboscis length.

At the Rocky Mountain Biological Laboratory in Gothic, Colorado, USA the across caste differences in proboscis length in common species of *Bombus* (*B. appositus*, *B. flavifrons*, *B. mixtus*, *B. bifarius*, *B. kirbiellus*) was quantified and this variation was related to differences in floral resource use of each caste. Queen's were found to have proboscis that were on average longer than the workers of the same species. However, it is unclear if the differences observed in proboscis length has any effect of the foraging preferences of the bumble bees within a caste.

Methods

Study Species and Site

Five species of subalpine bumble bees were collected for this study: *Bombus appositus*, *Bombus bifarius*, *Bombus flavifrons*, *Bombus kirbiellus*, and *Bombus sylvicola*. These were five species were the most abundant species at the collection site during this study. Five additional species were encountered during collection but had low sample size. Each species has distinctive markings in their abdomen allowing for easy field identification. Caste identification was based on the size of the individual; large bees were determined to be queen while small bees were workers. If the bee's caste was difficult to determine the bee was placed into the queen caste. Queen and workers were collected from seven site near the Rocky Mountain Biological Laboratory, Gothic, Colorado, USA.

Capture and Measurement of Bumble Bees

Bumble bees were captured using an insect net placed over the bee while the bee was foraging on a flower. The bumble bee will then be allowed to fly to the top of the net before being placed into a bee squeezer for measurements (Kearns and Thomson 2001). The caste and flower species collected on was recorded. After measurements are taken the bumble bee was released on or near the flower on which it was captured.

Measurement of the proboscis directly is impossible to do in the field. It requires careful dissection of the mouth parts. Fortunately, intertegular distance has been shown to be a good proxy for tongue length and will be used to estimate bumble bee tongue length (Cariveau *et al.* 2016). The bumble bee is temporarily immobilized in a bee squeezer and hand calipers were used to measure intertegular

distance (the distance between the wing bases).

Data Analysis

Intertegular length was converted into proboscis length using an R package provided by Cariveau et al. (2016). In order to assess the difference in proboscis length across queens and workers a two sample t-test was used to compare the mean proboscis length for each caste of each species measured. A one tailed t-test was used because queens have been shown to be larger than workers, and allometric trends are expected to be consistent across caste.

Results

Proboscis length across caste

Variation in queen and worker proboscis length across five species of *Bombus* was quantified. Intertegular distance was used as a proxy for proboscis length due to the high correlation between intertegular distance and proboscis length. Intertegular distance was measured for 150 individuals across ten species. Five of the ten species collected did not have a high enough sample to perform analysis ($n < 3$ for either caste).

A one tailed t-test was performed for each caste for each species. The mean proboscis length for workers and queens of the same species were compared using this test. Across the five species the queen proboscis length was found to be significantly longer than worker proboscis length of the same species (**Fig. 1a-e**).

There also appears to be overlap in the proboscis length of the queens of one species and the proboscis length of the workers on another. The queens across all species observed appear to be longer than the workers (**Fig. 1f**)

Association of proboscis length and foraging preference

No significant association was found between corolla length and proboscis length within a species. No difference in floral preference based on caste was observed in any of the *Bombus* species tested. Nor was there a significant relationship between corolla length and proboscis length within a caste for any of the study species. However, previously observed trends in floral preferences based on proboscis length and corolla length across species were observed.

Discussion

Across the five species studied queens consistently had longer proboscis lengths than the workers of the same species. The means of the queen proboscis length across species are also longer

than the workers. However, the shorter proboscis of workers does not appear to affect the foraging preferences of the workers of a species. This is surprising because handling time has been shown to be affected by flower depth when it exceeds the length of the bumble bee's glossa (Harder 1983), but the types of flowers the workers of *Bombus appositus* have been observed to visit exceeds the length of their corollas. Bumble bees have also been observed to visit a wide breadth of flowers (Ogilvie, unpublished data) and may explain, in part, why a close correlation between corolla length and proboscis length was not found.

One possible explanation for the difference in proboscis length is that the variation does not affect the fitness of the bumble bee, and is a product of the resources given to the individual while developing. In captive colonies of bumble bees it has been shown that worker size is correlated with the amount of resource the colony receive (Sutcliffe 1988). Another possible explanation for the lack of variation in floral preferences is that each species is excluded from visiting flowers by other bumble bee or other pollinators. It has been shown that when a species of bumble bee is excluded from an area other bumble bees will utilize the flower species that the excluded bumble bee tends to visit (Inouye 1978). Rather than being limited through morphological traits competition for resources and niche partitioning may be forcing bumble bees to visit flowers that are not ideal for their morphology.

Close matching of proboscis length and corolla length may not have been observed through the duration of this study as it was not a year with normal flowering. At the Rocky Mountain Biological Laboratory where this study took place flowing an abnormally low amount of snowfall the previous winter and likely affected the amount of flowers during the summer this study took place. The diminished amount of flowers therefore may have affected the health and development the bumble bees that were collected. Queens that were collected this year experienced a normal year of floral resources while developing before overwintering. While under stress from lack of resources bumble bees may not express normal foraging behaviors and modify their foraging behavior (Cartar 1990). This may have prevented this study from detecting the normal preferences of the bumble bees while being studied. Repeating this studying following a winter with normal snowfall may more accurately reflect the typical foraging preferences of each species.

Potential improvements to this study would include sampling of species that did not have a high enough sample size this summer for analysis, as well as expanding the number for bumble bee species included. Five of the ten species encounter this summer had very few workers encountered during sampling, and analysis was not performed. Increasing the number of species examined in this study could better characterize the variation that occurs in *Bombus*. Another potential improvements is to use effective flower depth as a replacement for corolla length. This measurement would use the distance

from the corolla opening to the nectar in the flower instead of the entire length of the corolla. Effective flower depth instead of corolla length as it may better reflect the distance to resources a pollinator experiences while interacting with a flower (van Rijn 2016). Using this measurement instead of corolla length may also better reflect the relationship between proboscis of the bumble bee and the distance the bumble bee must extend to reach its nectar.

In future studies a closer examination of feeding efficiency of queens and workers within the same species may better explain why queens and workers visits similar flowers. It has been shown that shorter tongued bumble bees are more efficient at handling flowers of shorter corolla lengths (Plowright 1997), and expanding on this in order to examine how longer tongued bumble bees of the same species overcome this difference in efficiency may begin to explain the similarities if floral preferences. If extraction depth and handling time trade-offs allow for the same or similar energetic pay offs then proboscis length may not play an important role in within species variation of floral preferences. Understanding the energetic investment and payoff as it relates to proboscis length may shed light as to why queens and workers appear to visit similar flowers despite predicted trade offs in handling times, based on proboscis length alone.

Examining the within species variation of bumble bees and how it relates to foraging preferences may prove to be useful as climate change continues to threaten the existence of these species. Understanding the restrictions on the foraging of a bumble bee species may allow us to better understand how a community may be affected by the loss of that species. As well as the potential for another bumble bee species to fill that niche.

Appendix

Bombus species encountered during collection and sample size of each caste

B. appositus (Q=17, W=15)

B. bifarius (Q=14, W=33)

B. feridus (Q=1, W=3)

B. flavifrons (Q=7, W=22)

B. kirbiellus (Q=5, W=4)

B. mixtus (Q=0, W=1)

B. nevadensis (Q=7, W=0)

B. occidentalis (Q=1, W=1)

B. rufocinctus (Q=3, W=0)

B. sylvicola (Q=3, W=13)

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Figures

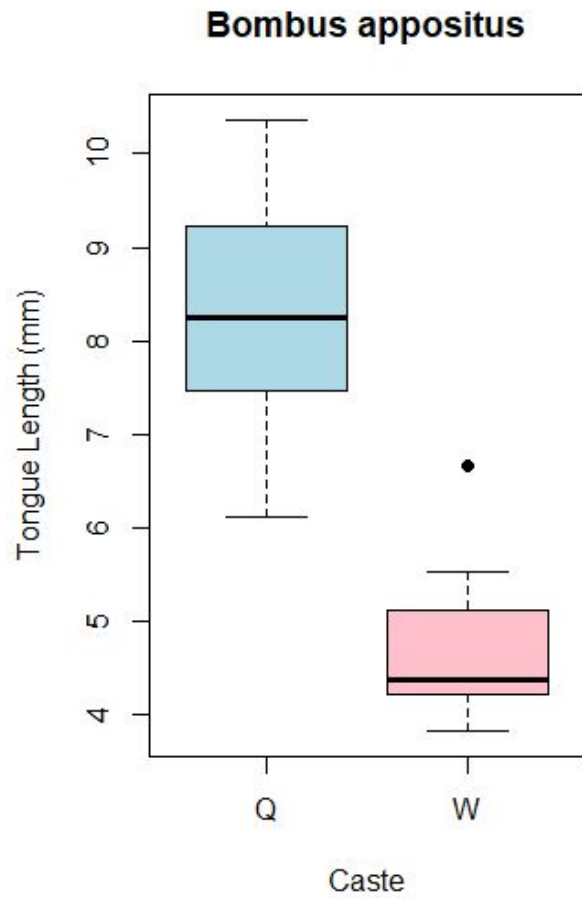


Figure 1a: T test of Queen and Worker caste of *Bombus appositus*. A significant difference was found in the means proboscis length of queens and workers of *Bombus appositus*. $t= 4.5666$, $df= 152.59$, $p\text{-value} = 1.015e-5$

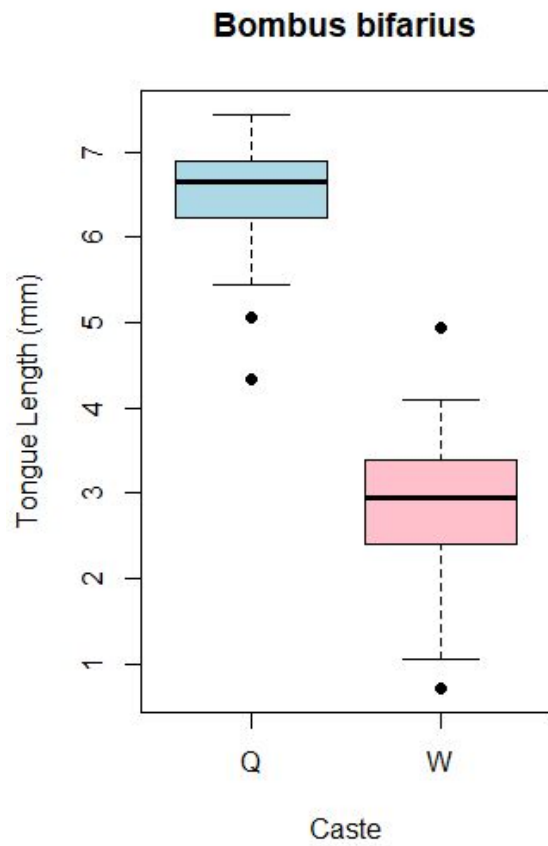


Figure 1b: T test of Queen and Worker caste of *Bombus bifarius*. A significant difference was found in the means proboscis length of queens and workers of *Bombus bifarius*. $t = 5.1774$, $df = 114.34$, $p\text{-value} = 9.743\text{e-}07$

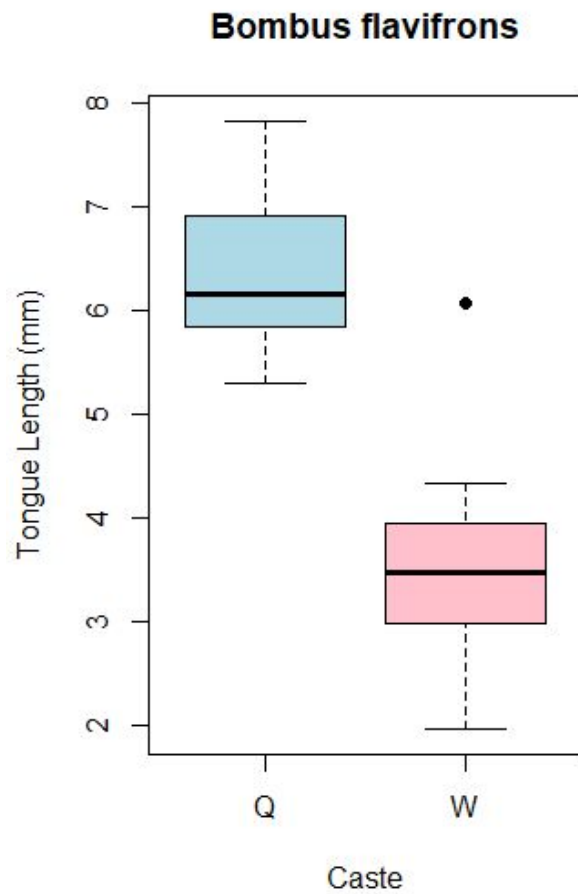


Figure 1c: T test of Queen and Worker caste of *Bombus flavifrons*. A significant difference was found in the means proboscis length of queens and workers of *Bombus flavifrons*. $t = 3.1817$, $df = 57.79$, $p\text{-value} = 0.002356$

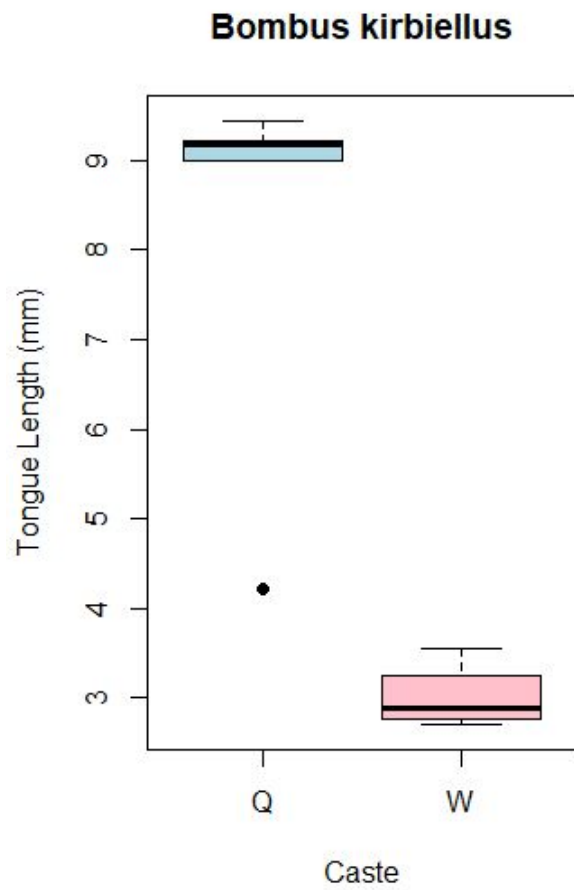


Figure 1d: T test of Queen and Worker caste of *Bombus kirbiellus*. A significant difference was found in the means proboscis length of queens and workers of *Bombus kirbiellus*. $t = 3.0715$, $df = 39.484$, $p\text{-value} = 0.003847$

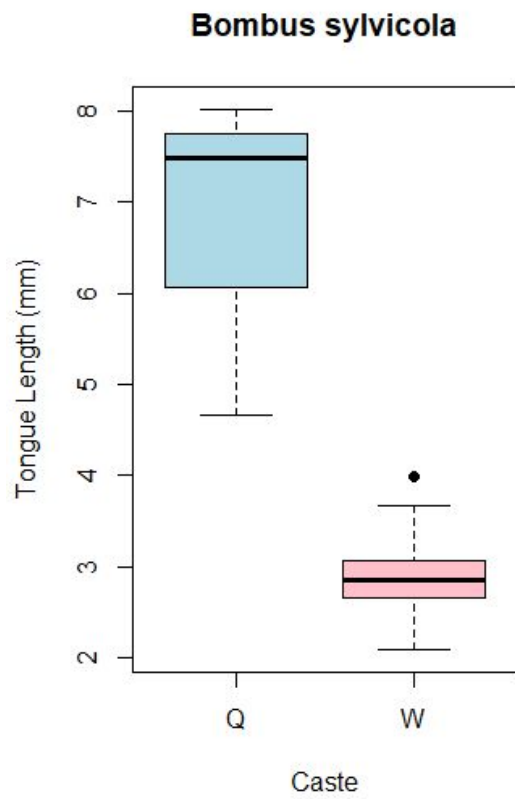


Figure 1e: T test of Queen and Worker caste of *Bombus sylvicola*. A significant difference was found in the means proboscis length of queens and workers of *Bombus sylvicola*. $t = 2.259$, $df = 23.15$, $p\text{-value} = 0.0336$

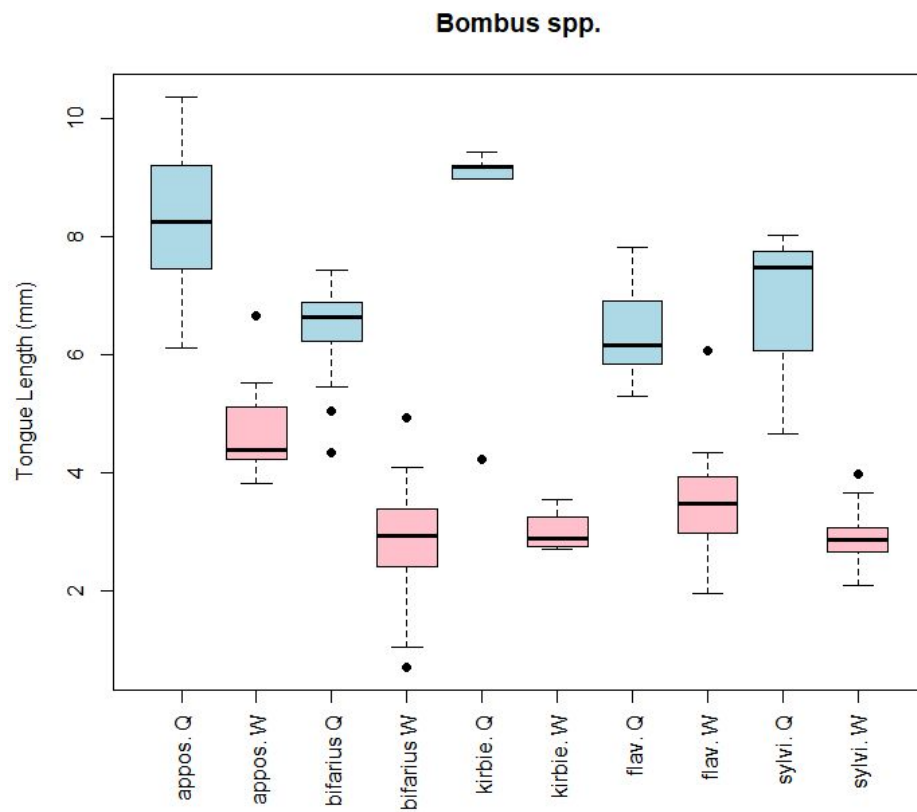


Figure 1f: Box plot with the mean proboscis length of all species of *Bombus* examined. Blue boxes are the mean proboscis lengths for the queens of each species. Pink boxes are the mean proboscis lengths for the workers of each species.

Collection Sites in Gunnison County



Figure 2: Map of Gunnison County where samples were collected

Collection Sites in East River Valley

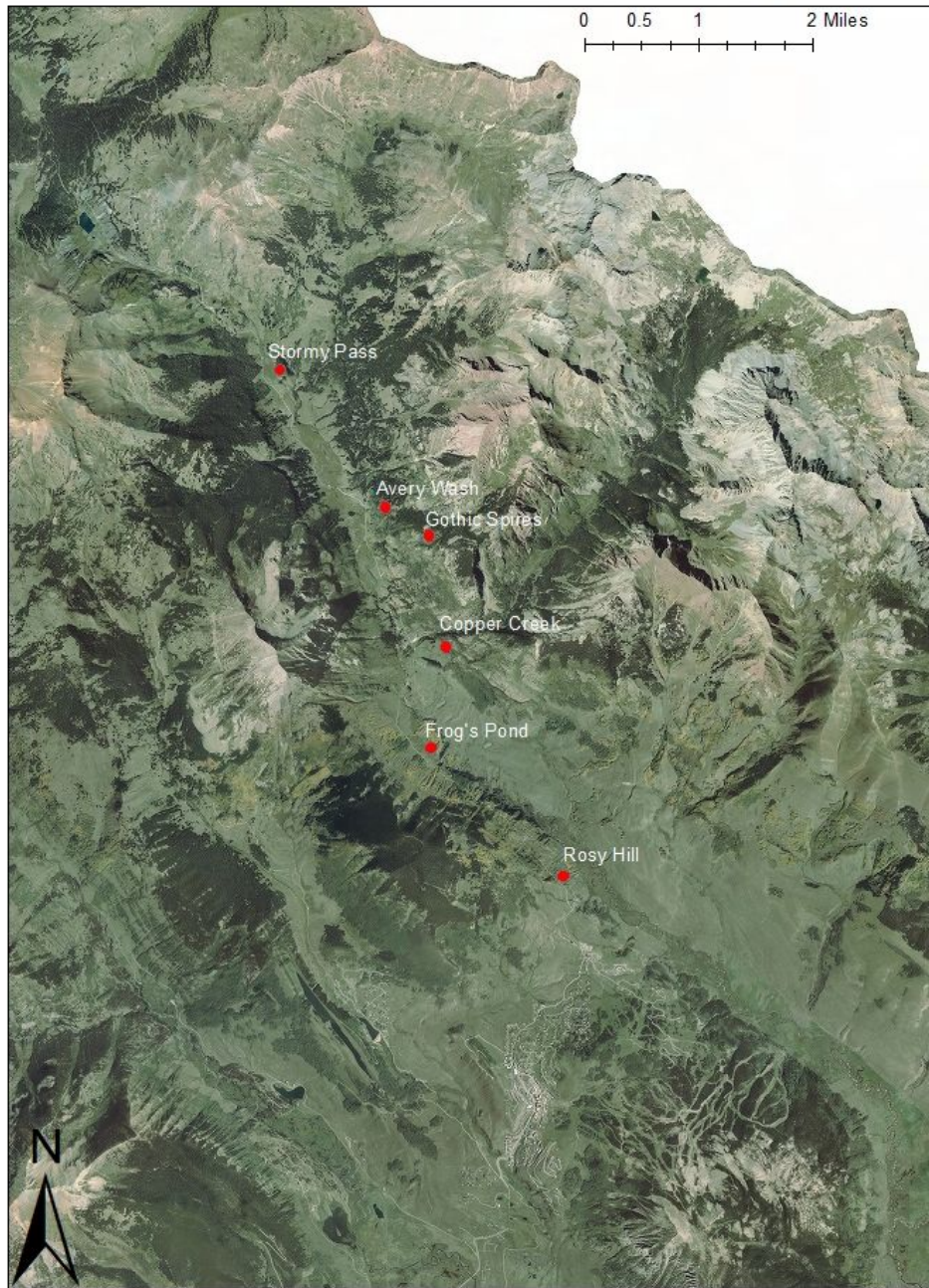


Figure 3: Map of collection sites within the East River Valley

Collection Site in Paradise Basin



Figure 4: Map of collection site within Paradise Basin