

Functional Morphology of Pollen Capture in Syrphid Flies (Diptera: Syrphidae): The Effects of Pile Density and Body Region

Abstract:

This paper will focus on Syrphidae, an understudied but potentially impactful pollinator. Specifically, I will investigate how the density of the pile (hair) affects the amount of pollen grains carried. This research has the potential to significantly contribute to the field of entomology, as it addresses the controversy about the efficiency of Syrphidae as pollinators, especially in the context of the global decline of primary pollinators such as bees. To conduct this, I will gather Syrphids using the established protocol from the Berry Brosi Lab. The Syrphids will be microscopically examined to determine their pile density and the number of pollen grains present. I expect that as the pili are more dense, more pollen grains will be gathered by a Syrphid fly. Additionally, I will be examining six body parts and comparing the differences in the amount of pollen grains they can carry. I expect essential body parts for pollination, such as the face and ventral thorax, will contain more pollen grains than passive body parts. This research is significant due to the lack of comprehensive studies on Syrphidae, particularly regarding their roles as pollinators.

Introduction:

Syrphids are heavily understudied, despite functioning as both biological control agents and pollinators (Dunn et al. 2020). Both of these roles are crucial for the health of the environment, as pollinators facilitate the sexual reproduction of plants and crops, and biological control agents naturally reduce harmful insect herbivore populations, promoting the health of the environment. Due to larvae not depending upon pollen feeding, adult Syrphids are able to cover larger landscapes and disperse more pollen (Gervais et al. 2018; Dunn et al. 2020). Syrphids are of interest as bees are in decline due to climate change, and they are the prominent pollinators in high elevations and cooler temperatures, compared to bees (Sommaggio et al. 2022; Gervais et al. 2018). Moreover, Syrphidae are present worldwide, an impressive feat compared to other flower-visiting insects (Chisausky et al. 2020). Additionally, syrphids are often mistaken for bees or wasps due to their resemblance to these insects. Both

flies and bees contain body hairs used to collect pollen both passively and for feeding (Stavert et al. 2016; Ollerton, 2021). Despite their mimicry, syrphids are not harmful to humans. Furthermore, there is a larger debate about whether syrphids are efficient pollinators, as they are classified as “lesser” pollinators compared to Hymenoptera despite having pili and visiting flowers exclusively.

It is known that Gothic, Colorado, is visited by a variety of Syrphidae genera. Specifically, since Syrphids typically visit flowers for a relatively brief period, their morphology is of interest. Syrphids can vary in body size and hairiness between genera. In addition, there have been findings that body size is correlated with pollination effectiveness (Roquer-Beni et al. 2020; Phillips et al. 2018). Larger insects can make more contact with the stigmas and stamens of a flower at the same time (Phillips et al. 2018). This hairiness, however, is known as pile when referring to insects (Syrphidae Foundation, Schaepmanlaan 2, Baarn, the Netherlands et al. 2023) and can vary in its characteristics. Specifically, the characteristics of the pile are compelling, as hair is thought to affect pollen uptake and, therefore, pollination success (Roquer-Beni et al. 2020). Additionally, pili are an important feature in bees, allowing pollen capture. Even more specifically, insect body hairs are thought to be of importance as they allow insects to gather food (pollen) (Stavert et al. 2016; Ollerton, 2021), on which they intrinsically survive off of. Nevertheless, despite acknowledging that pili are an essential feature for pollination, little is known about Syrphidae morphology.

The morphological characteristics of syrphids are not well understood, nor have they been extensively studied. Typically, when research is conducted on the hairiness of insects, bees are the primary focus of study (Ollerton, 2021; Roquer-Beni et al., 2020; Stavert et al. 2016). It is believed that the pile evolved in pollinators to collect and transport pollen. For instance, parasitic bees that do not need to collect pollen for their young are not covered in hair. For this reason, it is paramount that this study is conducted on syrphids, as there is a literature gap on this topic, preventing us from knowing if Syrphids are efficient pollinators. This raises the question: How does the density of pile on syrphids affect the amount of pollen carried? The predictor variables will be the presence and the characteristics of those pili. Therefore, I predict that the more dense pile is on a syrphid, the more pollen will be gathered by that fly, leading to greater pollination. Furthermore, I am curious about, how different body parts differ in the amount of pollen grains they carry? The predictor variable will be the specific body part. I predict that important body parts for pollination will carry more pollen grains than passive body parts.

Methods:

- Field sites

We collected syrphids at five different sites, respectively known as: Gothic Town, Avery Picnic, Rustler's Gulch, Virginia Basin, and Washington Gulch. These sites are located within the East River valley near Gothic, CO. Each site has four transects, each 1 meter by 20 meters. Each of these transects is divided into ten sections, each measuring 1 meter by 2 meters.

- Syrphid Fly Collection Methods

Regarding the sampling methods, I have adopted the established protocol of the Brosi Lab, under which I am working. RMBL has approved this established protocol. Before starting rounds, we conducted a flower count for each section of each transect to track the available resources, allowing us to determine what pollinators will forage on. We each received a randomized sheet, with usually one to three rounds. This randomizer is designed to prevent biases (such as focusing on one flower species) and to avoid becoming too comfortable with a plot segment. At each plot segment, I spent 3 minutes there, dividing it in half for either side of the section, as different flowers can be viewed from different angles. We only collected beetles, butterflies, moths, solitary bees, syrphid flies, bee flies, and other flies. We typically ignored grasshoppers, aphids, ants, and ladybugs. However, I would like to point out that we did collect bumble bees, but placed them in a no-kill vial and released them as soon as they were identified. That being said, a pollinator is only collected when it makes contact with the reproductive parts of a flower, ie. the stamens and anthers. We did not collect the pollinator if it was on the petals or leaves, and typically waited for it to make contact with the reproductive parts. Ensuring that the syrphids make contact with the reproductive parts is essential, as it will verify the presence of pollen grains on the fly. Once a pollinator made contact with the anthers and stamens of a flower, we placed a bug net over the flower. Usually, the pollinator flew up the net; however, I would like to point out that syrphids typically fly down, so in this case, the bug net needed to make contact with the ground. For smaller pollinators, such as beetles and flies, it was often easier to place a kill vial over the insects and then tip the flower over to let the pollinator fall into the vial. These kill viles have small paper napkin pieces that are soaked in one to two drops of ethyl acetate. This form of collecting is considered one of the most ethical methods of killing insects, as it minimizes suffering. Once an insect is in a vile, it

is vital for that vile to have the following information: Observer and number, scientific name of plant, and gross ID of the pollinator. Additional information we include is the date, the site initials, if it is a control or manipulation day, and which round, and finally, the transect and section in which the insect was collected in.

- Additional Opportunistic Sampling

Additionally, after quantifying the amount of each morphotype of Syrphid collected, I may collect outside of our protocols in the field so that there is an even number of Syrphids in each pile density category to compare with each other. There is a risk of possible biases as I can identify Syrphidae based on coloring (ie. black and yellow patterning) and flight behavior. The identification bias can impact my data, as I would be excluding Syrphids that do not have the striking coloring or that need to be examined under the microscope to be identified as Syrphidae. Additionally, the Syrphidae I had collected at the beginning of the sampling season outside of rounds were not on the reproductive parts of a flower. However, after consulting with my lab, we had agreed that it would be best to wait for a syrphid to land on a flower to determine if it is pollinating and to ensure there are pollen grains on the individual.

- Lab work

- Insect ID

From there, in the lab, I usually took all of the syrphids that we had collected that day to pin them. It is vital to pin these syrphids on the day of collection, as I risked them drying out and body parts coming off if done later. Each syrphid collected during the rounds has a unique ID number, which includes information such as the flower it was pollinating, the date and site, as well as the transect and section from which it was collected. As for the syrphids I had been collecting outside of rounds, to differentiate them from those collected through the QNEST protocol, instead of using a pound (#) symbol, I will be using a star (*) symbol. Additionally, a majority of the Syrphids collected through QNEST have ID numbers starting in the ten thousands.. However, so far, I have the date, site, the closest transect and section from which it was sampled, as well as the flower it was collected from, if that is true.

- Developing “hairiness” Bins

After pinning and drying, the Syrphid is now ready for microscopic observation. This observation aims to determine the presence and density of the pile. The density determination

will be categorized into three levels: low, medium, and high. I would also like to emphasize that hair density is determined from the dorsal thorax only. This was due to time constraints and the inability to code software for image analysis within ten weeks.

- Quantifying Pollen Loads

Additionally, I quantified the number of pollen grains present on each syrphid fly. I examined the various body parts of each syrphid, including the dorsal thorax, the ventral thorax, the dorsal and ventral abdomen, as well as the legs and face (Tepedino et al. 2011). My lab and I have determined it would be worth counting each individual pollen grain with the assistance of a clicker. The amount of pollen from each body part will then be added together for a total pollen grain count for each Syrphid fly. Although this method may be more time-intensive, it facilitated later data analysis.

- Statistical Analysis

All models were conducted using Rstudio version 4.5.0. For statistical testing, since I had three categories I wanted to compare to one another, and could not use ANOVA as I did not meet the normality assumption, I utilized the Kruskal-Wallis Test (Stats R, n.d.). Another positive of the Kruskal-Wallis Test is that the sample size across the categories does not need to be the same. However, a downside of the Kruskal-Wallis Test is that when it gives the p-value for a significant difference, this only represents whether one of the groups is different from the others, and does not specify which group combinations are significantly different from one another. To see which group combinations are significantly different, I employed the Dunn Test, a multiple pairwise comparison test, which is the most common post-hoc test used for the Kruskal-Wallis Test.

Results:

To answer my main question of how the density of pili on Syrphidae affects the amount of pollen grains a syrphid can carry, I placed my pile densities along the x-axis against the total pollen grains counted. As a categorical variable is being used against a numerical one, a boxplot was used to visualize the data. Each point represents a single syrphid fly that was sampled ($n = 46$), 18 of those flies falling under the low pile density category, and 14 flies falling under each of the medium and high pile density categories (Table 1). The Kruskal-Wallis test

shows that there is a significant difference in pollen grain counts across the three hair density categories (p -value = 0.02). When conducting the Dunn Test, however, low pile density vs. medium pile density was not significantly different (p -value = 0.97, Table 2).

As for my second question, how do different body parts differ in the amount of pollen they carry, I again used a boxplot, as each of the six body parts (Face, Legs, Dorsal and Ventral Abdomen, and Dorsal and Ventral Thorax) is a category that went against pollen grain count on the y-axis. The Kruskal-Wallis Test showed that there is a significant difference between the body parts (p -value = 4.908×10^{-5}). When I ran the Dunn Test, there was a significant difference between group combinations: Face - Ventral Abdomen (p -value = 0.0004293062), Dorsal Abdomen - Face (p -value = 0.0054076304), Ventral Abdomen - Ventral Thorax (p -value = 0.0152376953), Legs - Ventral Abdomen (p -value = 0.0192738622), and Dorsal Thorax - Ventral Abdomen (p -value = 0.0322112750). The only body part not significantly different from the Ventral Abdomen was the Dorsal Abdomen (Table 3).

Next, I considered the relationship between pile density and the amount of pollen grains gathered across different body parts. Again, the low and medium pile densities did not have much difference along the six different body parts, which is not surprising, as my Dunn Test did not show a significant difference (Table 2). Additionally, there was a lot of variance for the high pile density along all six body parts. Interestingly, the Leg body part exhibited considerable variance across all three pile density categories.

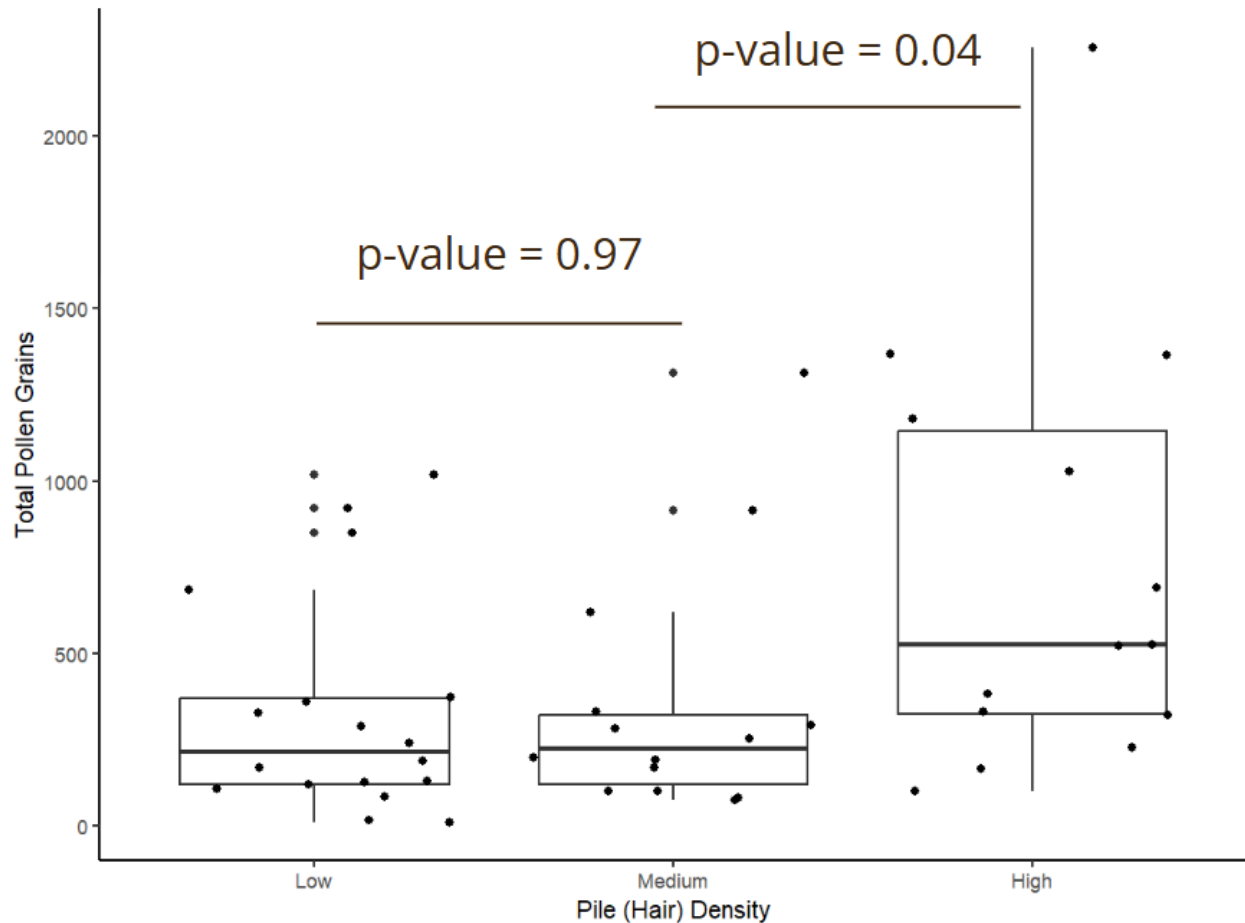


Figure 1.) The relationship between Pile Density, across three categories (Low, Median, and High), and the total pollen grains collected. Each point on this graph represents a single Syrphid fly ($n = 46$). Overall, there is a general positive relationship. There is a significant difference among the pile density categories (Kruskal-Wallis Test, $p\text{-value} = 0.02801$). Multiple pairwise comparisons showed that High pile density was significantly different from categories medium pile density (Dunn Test, $p\text{-value} = 0.04853836$) and low pile density (Dunn Test, $p\text{-value} = 0.04706989$).

Table 1.) The number of syrphids sampled that fall under each Pile Density category.

Hair_Density	n
Low	18
Median	14
High	14

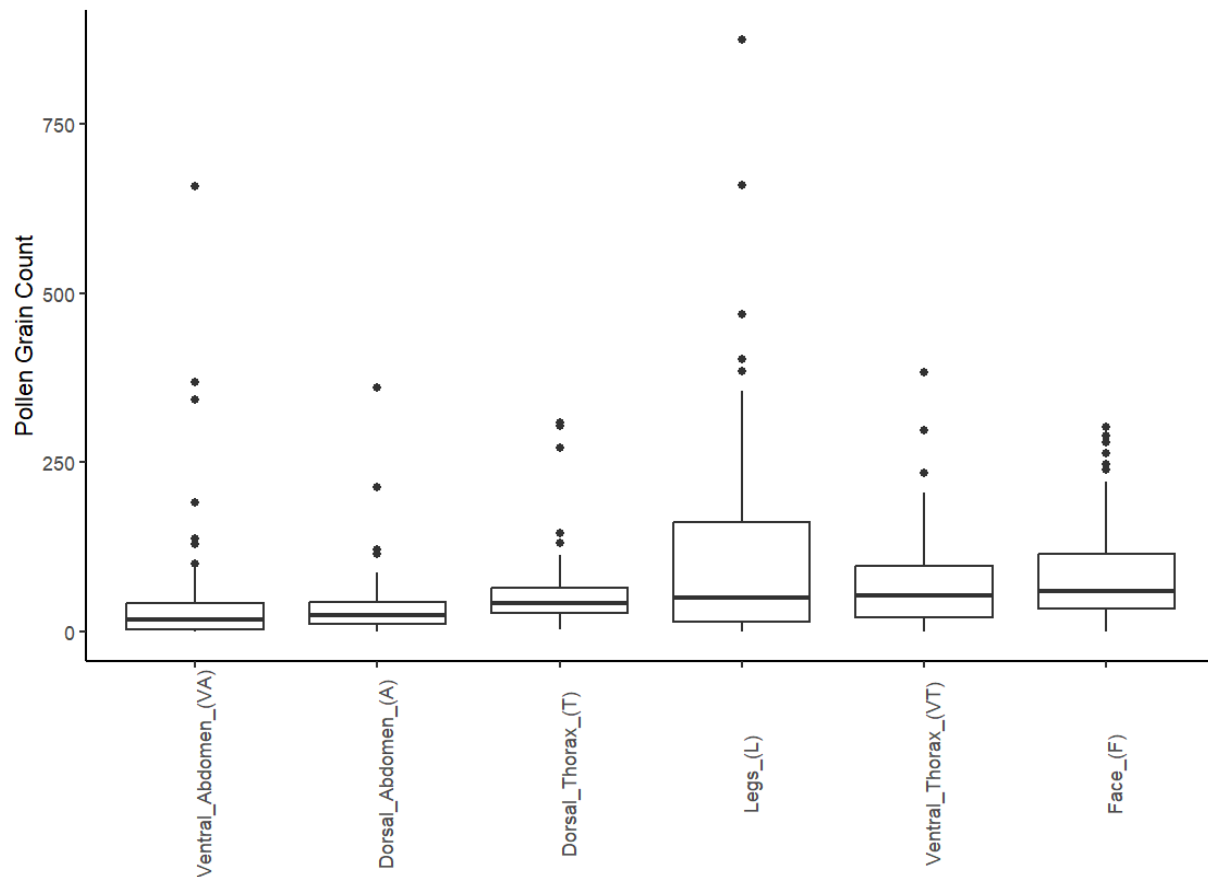


Figure 2.) The amount of pollen grain counts under each different body part of a Syrphid. There is a significant difference between the body parts (Kruskal-Wallis Test, p-value = 4.908e-05). All the results from the Dunn Test for the group combinations are listed in Table 3.

Table 2.) Results from running the Dunn test, where we compare each hair density category to one another and see the correlated P-value to see if the difference is significant or not.

Comparisons	P-value Adjusted
High-Low	0.04706989
High-Median	0.04853836
Low-Median	0.97881867

Table 3.) Results from the Dunn test, showing each body part combination significant difference from the smallest to the largest value.

Comparisons	P-value Adjusted
Face - Ventral Abdomen	0.0004293062
Dorsal Abdomen - Face	0.0054076304
Ventral Abdomen - Ventral Thorax	0.0152376953
Legs - Ventral Abdomen	0.0192738622
Dorsal Thorax - Ventral Abdomen	0.0322112750
Dorsal Abdomen - Ventral Thorax	0.0903570550
Dorsal Abdomen - Legs	0.1056462635
Dorsal Abdomen - Dorsal Thorax	0.1541501862
Legs - Ventral Thorax	0.9276595149
Dorsal Thorax - Face	1.0000000000
Dorsal Thorax - Legs	1.0000000000
Face - Legs	1.0000000000
Dorsal Abdomen - Ventral Abdomen	1.0000000000
Dorsal Thorax - Ventral Thorax	1.0000000000
Face - Ventral Thorax	1.0000000000

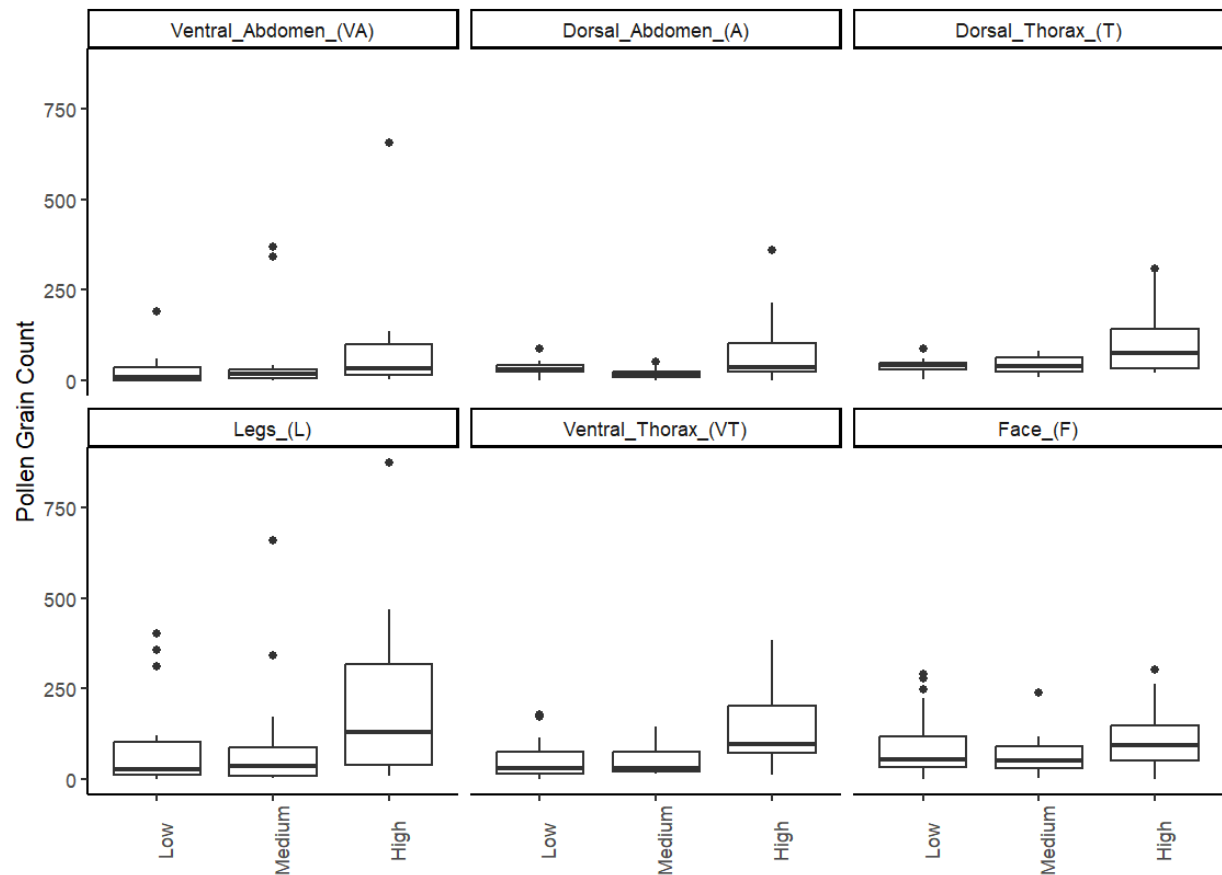


Figure 3.) Pollen grain counts against the six body parts of a Syrphid, with pile density also being considered.

Discussion:

When answering my main question, in Figure 1, my study suggests that Syrphids with denser pile carry more pollen. However, there is a lot of variance in the high pile density category (Figure 1). There are some Syrphids in the High pile density category that scored about the same total pollen grain counts as the low and medium pile density Syrphids. The variance may be due to not accounting for and not knowing where flies are in their foraging bouts (Denny and Gaines 2007). The variation witnessed in the high pile density category may be attributed to the lower flies barley starting on foraging for the day, while the outliers are further along in their foraging bout. Is the fly on flower two or flower fifteen of the day? Outliers can show the potential that high pile density Syrphids can reach the further along they are in their foraging boat.

As I predicted, I found essential body parts for pollination carried more pollen grains than passive body parts. Such important body parts include the face and ventral thorax, as both of these body parts make the most contact with the reproductive parts of a flower, as the Syrphid is foraging for flower resources (Roquer-Beni et al. 2020; Stavert et al. 2016). Additionally, the legs will also be a critical body part, as they are utilized in the grooming and feeding behavior of Syrphidae. The grooming behavior allows Syrphids to gather pollen grains from hard-to-reach places onto their frontal legs, which they are then able to feed off of (Holloway 1976). In Figure 2, these three important body parts had the most pollen grain counts of all six body parts, as expected.

To conclude, this study provides supporting evidence that Syrphidae are also good pollinators. Every single sample of Syrphid flies had pollen grains present. Furthermore, all of the Syrphids had more pollen grains present than needed for fruit production, which is only eight pollen grains (Gervais et al. 2018). Additionally, there was a concentration of pollen grains on the face and ventral thorax, which is necessary for pollination.

- Future Directions

I initially aimed to identify the genera of the Syrphidae I had collected; however, with only ten weeks, I did not have enough time to go through the 46 flies that I gathered and identify each one of them. Additionally, along with identifying the Syrphidae, measuring the body lengths would have been interesting as well, as it has been found that body length and pile density are heavily correlated (Phillips et al. 2018; Roquer-Beni et al. 2020). I also believe that comparing body length to genera would further add to our knowledge gap on Syrphidae. Furthermore, I would have liked to go deeper into the characteristics of pili, such as pile length and geometric properties, rather than just the density of the pili, and investigate how these additional characteristics affect the number of pollen grains the pili can carry.

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