

Diptera Behavioral Response to Targeted *Bombus* Removal

Student: Julia Brokaw

Mentor: Heather Briggs

Independent Research

Summer 2011

Introduction:

Bombus pollinators are highly studied, while pollinators in the diptera order are largely ignored. These pollinators are often overlooked because of their inefficiency compared to bumblebees. However, they are an integral part of high altitude alpine pollination systems where bees are less active. In fact, many alpine flower species depend on these insect pollinators to produce seeds (Kevan 1972).

There are important differences between diptera and *Bombus* pollinators. Because they do not return to a nest, diptera flight time, flight distance, and energy requirements are lower than that of bumblebee species. Also, diptera pollinators absorb heat from their environment for thermoregulation and therefore can spend much of their time basking, whereas bee pollinators must forage for metabolic and colony needs (Kearns et al. 2008). Diptera species range from specialist to generalist pollinators and tend to prefer flowers that are small and bowl-shaped (Goldblatt et al. 1995). These flowers allow diptera to forage, take advantage of the warm microclimate, and provide mating arenas (Herrera 1995).

When individuals of different species compete for the same resource, it has the potential to alter population, community dynamics, and the evolution of the interacting species (Crombie 1947). This phenomenon is extensively documented in the literature, ranging from plants to mammals. A classic study involving nectar-consuming parrots found that when two species were co-occurring, they occupied a smaller altitudinal range than when they occurred individually (Diamond 1978).

In pollinator systems, interspecific competition can lead to short-term specialization (STS) within a single foraging trip (Sowig 1989, Fontaine et al. 2008, Heinrich 1976, Morse 1977). This behavior is also termed “flower constancy” and occurs when a pollinator visits multiple individuals of the same plant species in succession (Waser 1986). STS by bumblebees is influenced by a variety of factors such as short-term memory, acquired and innate preferences, and plant distributions (Ishii 2005).

STS is also strongly influenced by resource partitioning based on morphological and behavioral characteristics. Optimal foraging theory predicts that pollinators will stay faithful to specific flowers and patches where they receive the most energy and resources (Fenster et al. 2004). Some bumblebee species are specialized for certain flowering species but become generalist when other resources are available (Robinson and Wilson 1998). Inouye (1980) studied a system of two plant species with shallow and deep corollas and two bumblebee species with short and long proboscises. When co-occurring, each species foraged on the flower specific to their morphology; the short proboscis bees foraged on the flowers with the shallow corollas and the long proboscis bees foraged on the flowers with deep corollas. However, when either species were removed from the system, the remaining pollinator foraged on both plant species, leading to decrease in STS (Inouye 1980).

STS plays an important role in diverse plant communities. When a pollinator species is faithful to one species of flower during a foraging trip, conspecific pollen is transferred between individuals and there is a high rate of cross-fertilization (Waser 1986). In contrast, generalist pollinators that visit multiple flower species during a foraging trip transfer heterospecific pollen, thus, lowering the plant’s reproductive fitness (Gomez et al. 2007). The transfer of heterospecific pollen (low STS) lowers levels of

floral attractants, can lead to physical clogging and biochemical blockage and potentially lower plant fitness. One study found direct evidence that pollinator generalization has direct effects on plant fitness. In populations with high generalization, plants produced less seeds than in populations with intermediate generalization (Gomez et al. 2007).

The importance of pollinator species is well documented in many plant community systems, from agriculture to alpine habitats. Currently there is concern about the loss of pollinators and how it will affect community structure (Kearns 1990). A study in Britain and the Netherlands found that bee diversity is decreasing (Biesmeijer et al. 2006), a phenomenon that may have direct effects on plant fitness. While some perennial plants can persist despite lack of sexual reproduction, it is becoming increasingly important to understand the implications of species losses to pollination systems.

The objective of this study was to determine if diptera foraging behavior changes in response to the removal of the most abundant *Bombus* species in alpine sites in Gothic, Colorado. I hypothesized that the removal of the most abundant *Bombus* species would reduce interspecific competition and STS in diptera foraging. Without the dominant *Bombus* species, there will be more flower availability and foraging options for the diptera species. Because of this, the diet breadth and pollen types carried by the diptera should increase due to the increase in foraging options. This study provides insight to understanding the consequences of pollinator losses and further knowledge of pollination model systems.

Methods:

The primary focus of this study was to understand the behavioral changes of the diptera pollinators. Preliminary observations determined the behavioral differences between flower visits, basking, foraging, and pollinating. These observations also determined diptera flower species preferences.

Site

I studied 8 subalpine meadow sites in areas around the Rocky Mountain Biological Laboratory (RMBL) townsite with guidance from Heather Briggs and other research assistants. Each site was 20m x 20m. The different plots were separated by at least 1 km. The abundance and diversity of the plants within the plots was measured with two parallel transects and the number and species of flowers were counted within 0.5 m of either side of transect lines. “Fly” flowers were determined with fly pollination syndromes, and ongoing observations. I classified “generalist” flowers as those that were used by both *Bombus* and diptera species. The proportions of fly and generalist flowers within each site was determined by multiplying the transect counts by 10.

Treatment

Each site was used as the control and the manipulation. First, we visited each site as a control and determined the most common *Bombus* species with a timed netting period. Two field members walked throughout the plot and netted *Bombus* species for 20 minutes. The handling time from net to vial was not included in the 20 minutes to compensate for handling differences between field members. The species of *Bombus* was recorded and the most abundant species was determined.

Two days later, we returned to the same site and conducted the manipulation by removing the most common *Bombus* species with non-destructive, targeted netting. These bees were placed into vials and put into a cooler containing ice during manipulation and released unharmed after observations and netting. We took as much time as necessary to remove all of the target species from the plot (approximately 3-4 hours). I conducted foraging observations approximately 30 minutes after the bee removal to ensure the flies returned to their natural foraging behaviors after disturbance. The edges of the plots were patrolled and the target bee species was netted out if necessary.

Foraging Observation

Diptera foraging behavior was observed in the plots for a minimum of 40 minutes in both the control and manipulation conditions. I walked five horizontal transects within the plot at 4 meter intervals. Where there were many fly pollinated flowers, such as *Potentilla gracilis*, *Erigeron* sp., and *Heracleum sphondylium*, I would observe the flowers and surrounding area for a minimum of 5-10 minutes. I recorded both the number of flowers and species visited by diptera pollinators in one foraging trip.

For each sampling event, diptera foraging behavior was directly observed for a minimum of 40 minutes, during which each plant species and number of flowers visited was recorded. These observations lasted until the fly was lost from sight, or moved more than 25 m away from plot edge. Unfamiliar plant species were recorded and identified after the observation period. All observations from the control or manipulation site were pooled to prevent pseudoreplication.

Netting

After the foraging observations, the flies within the plot were netted. The netting lasted for 20 minutes in the first three plots and then was increased to 40 minutes in order to increase the number of flies caught. The netted flies were placed into a prepared kill jar containing a small paper towel and drops of ethyl acetate. The handling time from net to vial was not included in order to maximize the time for sighting the diptera. Each vial contained one fly. The flies were pinned, labeled, and identified to family using Kauffman's Field Guide to Insects of North America (2007), and the reference collection in the RMBL library.

Pollen

Pollen was removed from the body of the flies using cubes of fuschin jelly under a stereomicroscope in the lab. The cubes of fuschin jelly were placed onto the slide, covered with a cover slip and melted with a lighter. The pollen was analyzed in percentages of morphospecies.

Analysis

The observations of foraging sequences were analyzed separately from the pollen loads. The number of types of pollen found on the diptera were averaged in the control and manipulation and analyzed with a one-way ANOVA. Using a one-way ANOVA, the proportion of flower switches and flower constancy were compared between control and manipulation. The proportion of fly and generalist flowers visited were analyzed between

control and manipulation with an ANOVA. The effect of *Bombus* proboscis length was tested with an ANOVA.

Results:

Sites

The proportion of fly flowers were significantly different between site (Figure 1., $\chi^2=107.83$, $p<0.0001$, $df=7$). Because of this difference, the plots with a higher proportion of fly flowers were analyzed separately than the plots with low proportions of fly flowers.

The plots with a low proportion of fly flowers, the proportion of fly flowers visited was greater in the control than the manipulation. Conversely, the proportion of generalist flowers visited in the control is significantly less than in the manipulation (Figure 2., $\chi^2=33.84$, $p<0.0001$, $df=1$). In plots with higher proportions of fly flowers, the proportion of fly flowers visited is significantly greater in the control than the manipulation. Likewise, the proportion of generalist flowers visited in the control is significantly less than in the manipulation (Figure 3., $\chi^2=42.00$, $p<0.0001$, $df=1$).

Observational Analysis

The proportion of specialist foraging events in the control treatment was not significantly different from the proportion of specialist foraging events in the manipulation treatment. The proportion of generalist foraging events in the control treatment was not significantly different from the proportion of generalist foraging events in the manipulation treatment ($\chi^2=0.14$, $p=0.71$, $df=1$).

The proportion of fly flowers visited was significantly greater in the control than in the manipulation (Figure. 4, $\chi^2=120.66$, $p<0.0001$, $df=1$). Conversely, the proportion of generalist flowers visited was significantly less in the control than the manipulation treatment.

Proboscis Length

The proportion of fly and generalist flowers visited is significantly different between manipulations removing different tongue lengthed *Bombus* species (Figure 5., $\chi^2=18.99$, $p<0.0001$, $df=5$).

Pollen

There were 64 diptera flies caught and identified to 15 different families. The number of pollen types in the control treatment was not significantly different than the number of pollen types found in the manipulation treatment ($F_{62}=2.28$ $p=0.14$).

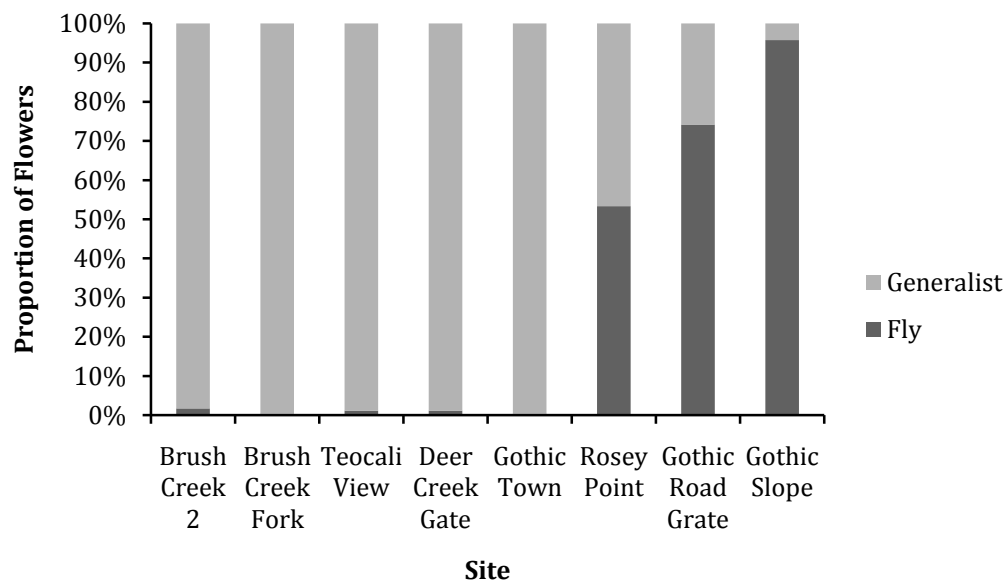


Figure 1. Proportion of fly and generalist flowers between sites ($\chi^2=67.70$, $p<0.0001$).

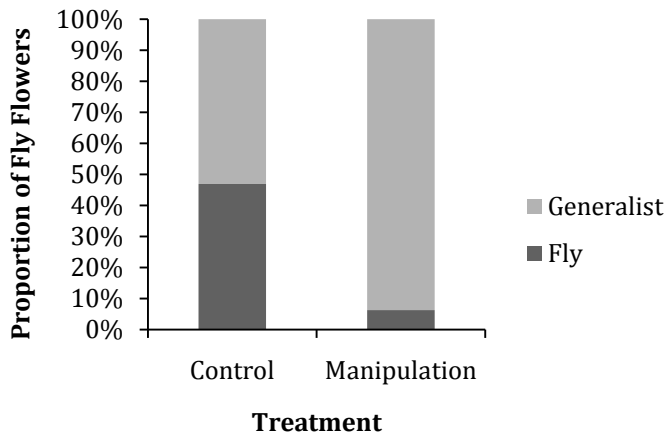


Figure 2. Proportions of fly and generalist flowers visited in control and manipulation treatments in plots with a low proportion of fly flowers ($\chi^2=33.84$, $p<0.0001$, $df=1$).

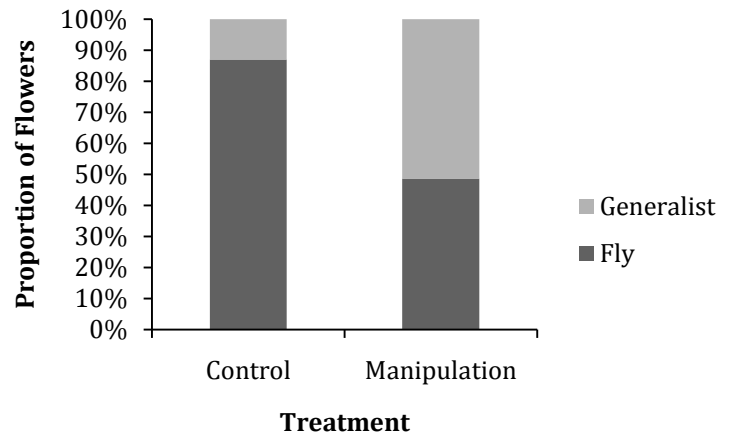


Figure 3. Proportions of fly and generalist flowers visited in control and manipulation treatments in plots with a high proportion of fly flowers $\chi^2=42.00$, $p<0.0001$, $df=1$).

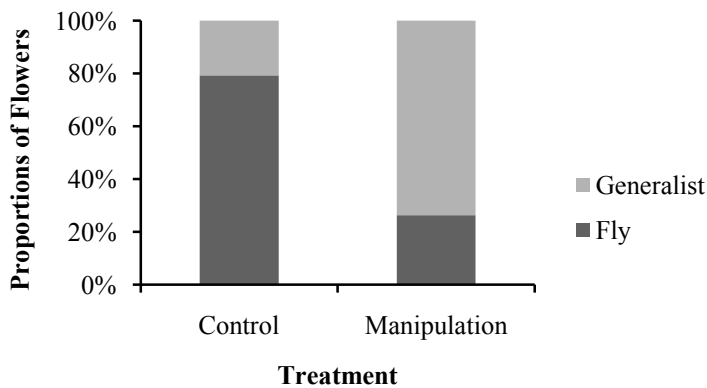


Figure 4. Proportions of fly and generalist flowers visited in the control and manipulation treatments ($\chi^2=120.66$, $p<0.0001$).

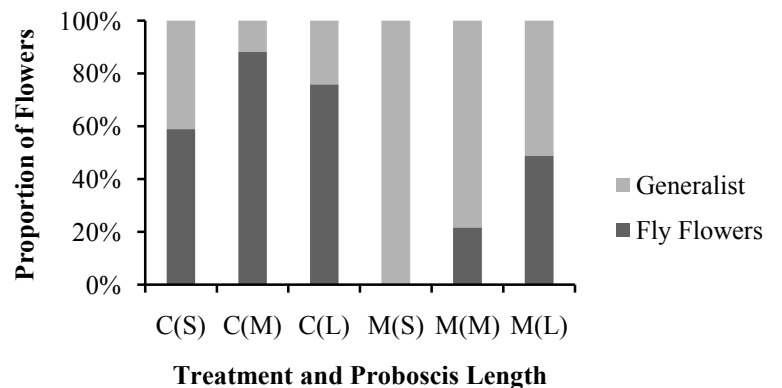


Figure 5. Proportions of fly and generalist flowers visited in the control (C) and manipulation (M) treatments that removed a short (S), medium (M), and long (L) tongued *Bombus* ($\chi^2=18.99$, $p<0.0001$).

Discussion:

The results demonstrate that the proportion of visits to generalist flowers significantly increases when the dominant *Bombus* species was removed. This result supports the hypothesis that the removal of dominant *Bombus* leads to a larger range of foraging options for the diptera pollinators.

Diptera species do not groom pollen from their bodies after foraging trips (Chapman 1998). Therefore, the pollen taken from the body may not be representative of their foraging trips during the *Bombus* removal treatment. Some diptera species have 'hairy' bodies that can carry copious pollen, whereas some species are smooth and carry very little pollen. Because pollen is small particles, the pollen found on the fly body and slide may be directly from the flower, picked up inside the net, or dispersed by the wind.

The proportions of switching flowers during one foraging trip were not significant between the control and manipulation. This is partially due to the foraging behavior of the diptera pollinators. These pollinators have short foraging bouts because of their low energy requirements and tend to spend much time resting and basking as opposed to foraging. Still, the diptera floral choices were significantly different between treatments. This demonstrates that they may be responding to the removal of the *Bombus* species because they visited a higher proportion of generalist flowers when the *Bombus* were not present. Diptera species are known to respond to intraspecific competition for certain resources (Bolnick 2001), and this result could demonstrate that they are also sensitive to interspecific competition with *Bombus* species.

There are many flowers that attract both bumblebee and diptera pollinators. Large *Bombus* species are adapted to forage on larger flowers with deeper corollas, whereas smaller *Bombus* species forage on smaller, open flower species. The diptera pollinators may not be competing with the larger *Bombus* species and may face a higher level of interspecific competition with smaller *Bombus* species. Because *Bombus* species differ in size, proboscis length, and floral preferences, these differences may play a role in the flower choice by the diptera species. Because we removed the most dominant pollinator in each site, tongue lengths of removed *Bombus* species varied from long (9.14-13.31), medium (5.39-9.84), or short (4.96-6.53) proboscises (Inouye 1976). The length of the proboscis is highly correlated to the types of flowers that the *Bombus* will forage. The range of proboscis length in dipteran species overlaps with the short and medium tongued *Bombus* (0.12-6.30) (Stang, Personal Communication 2011). The results comparing treatments and proboscis length reveal the interactions between the pollinators. When short-tongued bees were removed, the diptera were observed to forage on significantly more generalist than when the short-tongued bees were present. This demonstrates that there may be competition for floral resources between the diptera and short-tongued bee species (Inouye 1978). As the tongue length of the *Bombus* increased, the proportions of foraging on fly flowers also increased. This trend demonstrates that the competition decreased as *Bombus* tongue length increased.

There are other influences that may contribute to diptera flower choice. Because of tremendous diversity within the size, behavior, and morphology of diptera pollinators, the choices may be more influenced by individual species preferences than by *Bombus* presence (Kearns 2001, Goldblatt et al. 1995).

One major factor in diptera foraging behavior was the differences in floral composition between the plots. Because the proportions of fly flowers and generalist flowers were significantly different between the plots, the proportions of flowers visited in the overall study varied.

Future studies into diptera foraging behavior could standardize the timing of observations between sites and treatments. Diptera pollinators are most prominent in early morning and late afternoon, whereas *Bombus* pollinators are most active in late morning and early afternoon (Gilbert 1985; Herrera 1988). The peak foraging times of these insects slightly overlap and may have contributed to the results. Standardizing the observation time would demonstrate a stronger relationship and evidence of competition between the diptera and *Bombus* pollinators. Also, focusing on one family of diptera species would help to eliminate behavioral and morphological variables. While not accounted for in this study, site elevation is known to affect diptera abundance and activity. Future studies should also work to eliminate site differences that effect diptera flower visitation.

This study contributes to the understanding of pollination systems by demonstrating that diptera pollinators are involved with and affected by *Bombus* species. It also provides insight into how pollinator systems are affected by losses in *Bombus* species. The more we understand about this phenomenon, we can better predict the consequences and possibly prevent long-term damage to plant-pollinator interactions.

Diptera pollinators forage on a significantly higher proportion of both fly and bee flowers when the most abundant *Bombus* is removed. This demonstrates that losses in *Bombus* species affect many levels of pollinator networks and interactions.

Literature Cited

Biesmeijer, J. C., et al. "Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherland." *Science* 313 (2006): 351-354. PDF file.

Bolnick, D. I. "Intraspecific competition favours niche width expansion in *Drosophila melanogaster*." *Nature* 410.6827 (2001): 463-466. PDF file.

Brosi, Berry. "Pollinator Specialization and Diversity." *Unpublished* (2010): n. pag. PDF file.

Crombie, A. C. "Interspecific Competition." *Journal of Animal Ecology* Vol. 16.1 (1947): 44-73. PDF file.

Diamond, J. M. "Niche Shifts and Rediscovery of Interspecific Competition." *American Scientist* 66 (1978): 322-331. PDF file.

Charles B. Fenster, W. Scott Armbruster, Paul Wilson, Michele R. Dudash and James D. Thomson. "Pollination Syndromes and Floral Specialization". *Annual Review of Ecology, Evolution, and Systematics* 35 (2004): 375-403. PDF File.

Fontaine, C., C. L. Collin, and I. Dijoz. "Generalist foraging of pollinators: diet expansion at high density." *Journal of Ecology* 96 (2008): 1002-1010. PDF file.

- Gilbert, F. S. "Diurnal activity patterns in hoverflies (Diptera, Syrphidae)." *Ecology and Entomology* 4 (1895): 385-392. PDF file.
- Goldblatt, P., J. C. Manning, and P. Bernhardt. "Pollination biology of *Lapeirousia* subgenus *Lapeirousia* (Iridaceae) in Southern Africa; Floral divergence and adaptation for long-tongued fly pollination." *Ann. Missouri Bot. Gard.* 82 (1995): 517-534. PDF file.
- Heinrich, B. "Honeybee Thermoregulation." *Science* 276 (1997): 1015-1017. PDF file.
- Herrera, C. M. "Microclimate and individual variation in pollinators: flowering plants are more than their flowers." *Ecology* 76.5 (1995): 1516-1524. Print.
- Hiroshi, Ishii S. "Analysis of Bumblebee Visitation Sequences within Single Bouts: Implication of the Overstrike Effect on Short-Term Memory." *www.jstor.org*. N.p., 2005. Web. 5 Aug. 2011.
- Inouye DW , 1976. Resource partitioning and community structure: A study of bumblebees in the Colorado Rocky Mountains. 145 pages. Ph.D., University of North Carolina, Chapel Hill .
- Inouye, D. W. "Resource partitioning in bumblebees - Experimental studies of foraging behavior." *Ecology* 59 (1978): 652-678. PDF file.
- Kearns, C. A. *The role of fly pollination in montane habitats*. N.p.: n.p., 1990. N. pag. Print.
- Kevan, Peter G. "Insect Pollination of High Arctic Flowers." *Journal of Ecology* 60.3 (1972): 831-847. PDF file.
- Morse, D. H. "Resource partitioning in bumble bees: the role of behavioral factors." *Science* 4304 (1977): 678-680. PDF file.
- Robinson, Beren W., and David Sloan Wilson. "Optimal Foraging, Specialization, and a Solution to Liem's Paradox." *The American Naturalist* 151.3 (1998): 223-235. PDF file.
- Sowig, Peter. "Effects of Flowering Plant's Patch Size on Species Composition of Pollinator Communities, Foraging Strategies, and Resource Partitioning in Bumblebees (Hymenoptera: Apidae)." *Oecologia* 78.4 (1989): 550-558. PDF file.
- Stang, Martina. 2011. Personal communication.
- Waser, N. M. "Flower Constancy - definition, cause, and measurement." *American Naturalist* 127 (1986): 593-603. PDF file.