Development of a Regional Pollen Library to Investigate Foraging patterns in *Apis mellifera*

by

James Wolfin

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Degree of Bachelor of Science in Ecology with Distinction

Spring 2015

Development of a Regional Pollen Library to Investigate Foraging Patterns in *Apis mellifera*

by

James Wolfin

Approved: _____

Deborah Delaney, Ph.D.
Professor in charge of thesis on behalf of the Advisory Committee

Approved: _____

Judith Hough-Goldstein, Ph.D.
Committee member from the Department of Entomology and Wildlife Conservation

Approved: _____

Nicole Donofrio, Ph.D.
Committee member from the Board of Senior Thesis Readers

Approved: _____

Michelle Provost-Craig, Ph.D.
Chair of the University Committee on Student and Faculty Honors

ACKNOWLEDGMENTS

I would first like to thank my funding sources, the CANR SEED grant and MAAREC, the Mid Atlantic Apiculture Research Extension and Consortium for making the completion of this research possible. Additionally, I would like to thank John Pickering and the entire Discover Life organization for their direction and expertise in the development of the pollen identification guide. I would also like to thank Mount Cuba Center and Peter Lindtner for the constant collection and processing of samples throughout this project. I would like to thank the entire Delaney Lab at the University of Delaware for the support they have provided me throughout this entire process. I would like to thank my research advisor, Dr. Deborah Delaney for the constant guidance, encouragement, and the endless amount of knowledge she has imparted onto me since joining her research group. Furthermore I would like to thank the other members of my Senior Thesis Committee, Dr. Judith Hough-Goldstein and Dr. Nicole Donofrio for the direction and advisement they have provided me throughout the duration of this process. Finally, I would like to thank the Undergraduate Research Program and the Senior Thesis Program for the resources they have provided me, and for giving me the opportunity to conduct Undergraduate Research and a Senior Thesis at the University of Delaware.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The European honey bee (Apis mellifera) is an agriculturally significant beneficial insect that contributes an estimated $15 billion per year through the ecosystem service of pollination. Since the onset of Colony Collapse Disorder (CCD) in 2006, beekeepers have seen annual losses in overwintering colonies rise from nearly 15% to over 30% in many cases. One of the critical components to reestablishing healthy, sustainable bee colonies in the United States is ensuring that bees have suitable sources of nutrition, specifically pollen. Pollen can vary in nutritional quality depending on the % crude protein, so it is critical that bees obtain forage from floral sources that have pollen grains with high amounts of protein. In order to determine the nutritional quality of a pollen grain, it is necessary to be able to identify a pollen grain. Additionally, the type of landscape coverage surrounding bees within their foraging range may impact the amount and diversity of pollen grains that are foraged by a colony. Pollen grains were collected from four sites in the Mid-Atlantic to create a fully functional pollen library, in partnership with Discover Life. Grains were analyzed according to a variety of morphological characteristics to ensure that users would be able to identify grains to the lowest taxonomic rank possible. We examined bee-collected pollen from The University of Delaware Botanical Garden (UDBG) in Newark, Delaware and The Mount Cuba Center in Hockessin (MCC), Delaware, to determine which pollen species were most prevalent. The pollen library, while still in progress, currently contains 256 species and a fully functional identification guide that

will help users of all academic backgrounds identify pollen. While more pollen was collected at MCC, it was found that differences in foraging totals were not statistically significant. Analysis of bee-collected pollen suggested that bee foraging patterns do change temporally, as different species/genera became prevalent at different time periods. This research warrants further investigation to determine the nutritional quality of the most prevalent pollen grains, and shows the need for an extensive pollen library.

Chapter 1

INTRODUCTION

The European honey bee, *Apis Mellifera*

1.1 *Apis mellifera* as an agriculturally significant insect, and current status

The European honey bee, *Apis mellifera* is an agriculturally significant, beneficial insect of great value due primarily to its role as a pollinator of agricultural crops worldwide. Thirty-five percent of agricultural crops worldwide are dependent on pollinators, and various fruits, seeds, and nuts are dependent on honey bee pollination specifically, with some crop yields decreasing by more than 90% in the absence of honey bee pollination (Klein 2007). Because honey bees are the primary insect pollinators in agriculture, the status and population growth of honey bees is of great significance to humans. Honey bee populations have been experiencing severe losses since 2006-2007, when Colony Collapse Disorder was first recognized in the United States, as beekeepers in twenty-two states reported drastic losses in honey bee colonies (Oldroyd, 2007; Decourtye et al., 2010). Current losses in overwintering colonies have been estimated at approximately 33% each year since 2007 (Van Englesdorp et al. 2010). A decline in honey bee populations coupled with the four fold rise in agricultural production which requires animal pollination poses a serious threat to human food security on a global scale (Aizen and Harder 2009).

The decline of managed honey bee colonies is widely suggested to be correlated with many factors including (i) the use of herbicides and pesticides, (ii) the presence of pests including parasitic mite populations (*Varroa destructor*, *Varroa jacobsoni*), the small hive beetle (*Aethina tumida*), and the fungal parasite, *Nosema ceranae*, (iii) the decline in beekeeping, and (iv) inadequate nutrition (Downey and Winston 2001; Chen et al. 2004; Evans et al. 2003; Higes et al. 2006; Ingram et al. 1996; Klein et al. 2007). A likely possibility is that these elements work synergistically, with each factor making honey bee colonies more vulnerable to additional stressors.

1.2 Pollination and Floral Rewards

Bee pollination serves as an example of a classic mutualistic relationship as a product of co-evolution. Bees became reliant on floral rewards as their sole food source during the late Cretaceous period, abandoning a carnivorous diet (Michener 1974). At this time bees began to move pollen from flower to flower, aiding in pollination. This led to a period of adaptive radiation in angiosperms where diverse new forms of flowering plants began to arise. Bees have become specialized to collect these floral resources from flowers, and transfer pollen from the anther (male floral reproductive unit) to the stigma (female reproductive unit), resulting in a pollination event. This transfer of pollen enables the fertilization of gametes in floral species, and therefore the reproduction of new individuals in flowering plants. The pollen and nectar received by honey bees from these flowering plants is a product of co-evolution as well, as the characteristics of these floral products are directly related to the fitness of both plants and pollinators.

1.2.1 Nectar

The nutrition of a honey bee is directly linked to the floral resources that are available for forage. Nectar and pollen from flowering plants provides the honey bees with the carbohydrates, proteins, fats, vitamins, minerals, water, and other nutrients that are required to sustain life. Nectar is secreted from the nectary of a plant, and is considered to be the "secondary reward" available to pollinators, including honey bees. The nectary is located basally on an angiosperm, typically found on the sepals, petals, stamens, gynoecium, or nectar spurs of a plant. Nectar consists of mostly sugar (typically in the form of sucrose) and water, but also includes amino acids, lipids, and anti-oxidants which are valuable to pollinators. Proline has been designated as a critically important amino acid in honey bees, as previous studies have suggested that proline is essential in honey bee flight (Micheu, 1999). Additionally, it has been found that nectar can contain colors, odor, and tastes that may be attractive to pollinators, specifically bees. It has been found that the evolution of colored nectar has evolved independently at least 15 times in the history of angiosperms, and is directly correlated with animal pollination (Hansen et al. 2007). Furthermore, terpenoid compounds in nectar have been found to be insect attractants, and bees are able to associate the presence of terpenoids in nectar with a high sugar concentration (Nicolson and Thornburg 2007).

While factors such as nectar color and are significant and attractive to pollinators, including bees, it is ultimately the sugar in the nectar that the bees desire. Nectar typically range from 10%-75% sugar, and the sugar may vary greatly in terms of composition. Sugar concentrations typically consists of glucose, fructose, and

sucrose in varying proportions. The composition of nectar solutions has been found to be critical to the attraction of pollinators, as different groups of pollinators have specific preferences in nectar based on concentration and viscosity. The preferences in nectar concentration are due to morphological differences in tongue length among pollinating groups, as long tongued pollinators have difficulty sucking up highly concentrated, viscous nectar. Honey bees are grouped with short-tongued bees, which typically prefer nectar that is 45%-60% sugar. Honey bees have been found to have an average tongue length of 6.6 mm, about 2mm shorter than that of bumble bees (Balfour et al. 2013). Because of this morphological difference between the two groups of bees, honey bees are able to obtain nectar from more concentrated sources than bumble bees. The tongue, or proboscis, length of honey bees has a direct effect on the foraging patterns, as honey bees typically prefer radial flowers due to their higher nectar concentration. The honey bee proboscis is long and hollow, allowing them to suck nectar up from flowers.

In addition to nectar concentration, bees also to show preference for floral resources that are high in nectar volume. Both nectar and pollen radiate signals to pollinators that advertise the volume of the rewards they are offering. This phenomena is an example of "indirect signaling", where reward amount is correlated to some other feature in a flower to which pollinators respond. For example, it has been found that flower size is positively correlated with nectar and pollen amount, and bees are able to recognize this and display preference in favor of larger flowers. Furthermore, it has been suggested that specific floral scents are correlated with reward size. Evidence of this behavior was gathered by conducting dual-choice

biological assays to determine if bumblebees view a specific olfactory compound, phenylacetaldehyde, as an attractant and an honest indicator of reward size. First, phenylacetaldehyde was found to elicit a positive response by bumblebees via scent electroantennographic detection (EAD). After it was found that bumblebees responded positively to this EAD-active compound, different floral signals were examined to quantify how a signal's presence was correlated with reward amount. It was found that phenylacetaldehyde was associated with greater amounts of nectar and sugar, suggesting that bumblebees respond positively to this compound because it is an honest signal of a floral reward quantity (Knauer and Schiestl 2014).

1.2.2 Pollen

While nectar is a significant reward to most pollinators, including the European honey bee, *Apis mellifera*, pollen is often the primary reward sought after by pollen-eating animals. In angiosperms, pollen is critical in the reproduction of new individuals. Pollination occurs when the male reproductive units from one plant is transferred to the female reproductive structures of a flower. This can occur on the same plant, where the male and female structures occur together (monoecious), or on different plants, where male and female plants are physically separated (dioecious). There is great variation in the morphology, and nutritional quality of pollen grains. Because of this, it is necessary to document the physical characteristics of pollen grains for identification purposes, specifically in the identification of bee-collected pollen. Within angiosperms, pollen grains are located in the thecae of the anther,

which are supported by filaments within the flower. The thecae are the pair of pollen sacs within the anther. These thecae develop during the spring, where a single pollen mother cell will go through two stages of meiosis, resulting in four individual pollen grains.

An individual pollen grain is made up of three distinct layers; the pollenkitt, the exine, and the intine. The pollenkitt is the outermost layer of the pollen grain, providing an oily covering to the pollen grain. The oil is secreted by anther tissues within the angiosperm, and is found primarily on insect pollinated plants. This is due to the adhesive of the oil, which allows the grains to be picked up more easily by visitors. In terms of composition, the pollenkitt is made up mostly of lipids and pigment. After the pollenkitt, the exine is the next outermost layer. The exine is made up of sporopollenin, and functions as the main diagnostic layer within a pollen grain (Willmer 2011). The exine of the pollen grain is where the ornamentation of the spore becomes visible. Ornamentation refers to the pattern, or the organization of features, present on the exine of the pollen grain (Potonie, 1934).

There are many different types of ornamentations present in pollen grains. One of the most common forms of ornamentation seen in pollen grains is echinate ornamentation, where pronounced spikes can be seen on the exine. This form of ornamentation is seen in the family Asteraceae, where projections can be up to 3 microns in size. In addition to echinate ornamentation, striate ornamentation is another common pattern seen on pollen grains. Striate ornamentation refers to grooves on the exine of a pollen grain that are generally parallel in nature. This pattern

is commonly seen in the Rose family (Rosaceae). Another form of ornamentation commonly seen in pollen grains is reticulate ornamentation. Reticulate ornamentation features a network-like pattern where there are spaces, or lumina, larger than 1 micron within the grain (Praglowski and Punt, 1973). This pattern is seen in a wide variety of angiosperm families, and is common in the Phlox family (Polemoniaceae). Finally, there are also ornamentations that are difficult to distinguish without the aid of a scanning electron microscope. These grains display features of less than one micron in size, and may be described as perforate, granulate, or some other pattern depending on the nature of the ornamentation (Iversen and Troels-Smith, 1950). In addition to ornamentation, the tectum is another feature of a pollen grain that is seen in the exine. The tectum is the outer most layer of the exine that goes around the perimeter of the grain. The tectum of a grain can be described as either eutectate (continuous) or semitectate (discontinuous). A pollen grain with no tectum is described as atectate, and is less common in angiosperms (Faegri and Iversen, 1964). These differences in ornamentation and tectum are two of the primary features used by palynologists to describe and identify pollen grains.

Pollen grains exhibit great variation in size and shape. The size of a pollen grain ranges from less than 10 microns to over 200 microns in rare cases. The average size of a pollen grain however is about 30 microns. Within a family, there is less variability in the size of pollen grains. Because of this, size is often used as a parameter to identify a pollen grain to the taxonomic rank of family. Additional variation is seen in the shape of pollen grains. Palynologists classify the shape of a

pollen grain in two distinct ways. First, a pollen grain is often described according to the outline of the grain. The outline of a pollen grain generally refers to the equatorial outline, or the equator of a pollen grain that is visible when observing the polar face of a pollen grain. At this vantage point, the outline of a pollen grain can be described as "circular", "triangular", or "elliptic". Further analysis of shape can be conducted when looking at the relationship between the polar face and the equatorial face. By taking measurements of each face, one can obtain a "P/E" ratio that gives further insight to the shape of the pollen grain. With this ratio, pollen grains can be described by shape class as: peroblate (P/E <0.50), oblate (P/E 0.50 – 0.75), suboblate (P/E 0.75 – 0.88), oblate spheroidal (P/E 0.88 – 1.00), prolate spheroidal (P/E 1.00 - 1.14), subprolate (P/E 1.14 - 1.33), prolate (P/E 1.33-2.00), or perprolate (P/E > 2.00) (Erdtman, 1943). Shape classes obtained through this ratio are useful in the identification of pollen grains, but it is often difficult to obtain high quality images at each vantage point.

After the exine, one reaches the intine, the inner most portion of the pollen grain. The intine is composed of cellulose and pectin, and functions similar to that of a cell wall. The intine contains the apertures of the pollen grain where pollen tubes grow. The apertures of a pollen grain vary in both type and number. There are three primary types of apertures including colpate, porate, and colporate apertures. A colpate aperture, referred to as a colpus, refers to an elongated aperture where the length to breadth ratio of the aperture is greater than two. A porate aperture refers to a circular or elliptical aperture within a pollen grain. These apertures feature a length to

breadth ratio less than two. The final type of aperture present in pollen grains is a colporate aperture. A colporate aperture is a compound aperture, where two (or more) components occur together in one wall layer. A colporate aperture specifically refers to a pollen grain that features a pore within a colpus (Jackson 1928; Wodehouse 1935; Erdtman 1943; Erdtman 1945) and will open, or dehisce, upon maturity, thus releasing their pollen grains.

There are many methods of dehiscence and types of thecal slits that may occur within angiosperms. One way in which angiosperms may differ in terms of dehiscence is the directionality of the openings of their thecal slits. An introrse opening, where the thecal slit faces the center of the flower is more common in monoecious plants that favor selfing. In this scenario, the introrse thecal opening faces the female reproductive organs resulting in more successful pollination and fertilization events. Contrary to introrse dehiscence, extrorse dehiscence occurs when the thecal slits face away from the flower. This type of dehiscence is favored in cross-fertilized, dioecious plants. Plants that favor cross-fertilization often require pollinators, including the European honey bee, to carry pollen to the female structures of a conspecific.

In addition to varying methods of dehiscence, angiosperms also show disparity in the manner in which they present their pollen. There are two main types of pollen presentation present in modern angiosperms. In primary pollen presentation, the pollen is exposed directly on certain openings on the anther. Contrarily, in secondary pollen presentation, pollen is released from the anther to another structure where

pollinators can retrieve it. An example of secondary pollen presentation is seen in *Petromarula pinnata*, where the pollen is transferred from the anthers to the gynoecium, the female reproductive structures of the flower. The pollen in *P. pinnata* features a sticky pollenkitt, which aids in the transfer of pollen from the anther to the gynoecium. Generally, animal pollinators, including honey bees show a preference for flowers that exhibit primary pollen presentation where the pollen is retained on the anther (Willmer, 2011).

The amount and nutritional content of pollen can have a great influence on the fitness and survival of developing larvae. Previous research has confirmed the hypothesis that adult body size within Hymenoptera, which includes the European honey bee, is correlated to the amount of food, specifically pollen, consumed at the larval stage (Plowright and Jay, 1977; Ribiero, 1994; Strohm and Linsemair 1999). Because the European honey bee is a eusocial organism, the growth and development of larvae within a hive is largely dependent on the pollen foraging success of the adult worker bees. There are advantages in survival, fecundity, and mating success that have been associated with achieving a larger body size. Previous research has found that individuals with larger body size exhibit; easier maintenance of thermoregulation, increased winter survival rates in females, increased likelihood to become the primary egg layer for females, and increased mating opportunity for males (Kumar, 1975; Buckle, 1982; Tepedino and Torchio, 1982; Stone, 1993; Alcock 1995). Additionally, the nutritional value of pollen in terms of protein content differs across different families of angiosperms. Nutritional analysis of pollen in different families of

angiosperms has shown that pollen protein concentration varies greatly, ranging from 2.5-60% (Roulston et al., 2000), and that pollen concentration may influence larval bee survival. This has been shown in *Taraxacum*, or dandelions, a genus of flowering plants within the family *Asteraceae* that is known to have low protein concentrations. In an experiment where three different species of bees were grown on a diet consisting of strictly *Taraxacum* pollen, it was found that the bees were hindered in the completion of larval development (Levin and Haydak, 1957). Further experimentation in which honey bees were fed *Taraxacum* pollen showed that the bees were unable to produce brood when fed this diet (Loper and Berdel, 1980).

The wide range of protein content in pollen has led to further analysis of angiosperms with respect to their modes of pollination. Specifically, two categories of plants have been the focal point of further experimentation: anemophilous (wind-pollinated) and entomophilous (insect pollinated) plants. Anemophilous plants have been found to produce a much higher quantity of pollen, allowing for easier collection by humans. This has resulted in a very thorough understanding of the chemical make-up of the pollen produced by different genera of anemophilous plants such as *Pinus* and *Quercus*, whose pollen has been found to be low in protein content (Roulston and Cane 2000). Contrarily, the understanding of the chemical make-up of pollen in entomophilous plants is extremely limited. This uncertainty is due to the foraging behavior of the honey bee, the animal most regularly used to observe pollen chemistry in insect pollinated plants. Upon collection of pollen grains from a plant, honey bees

will mix the pollen with regurgitated nectar or honey, which alters the composition of the pollen. The effects of this were shown in *Pinus contorta*, a wind-pollinated angiosperm that also experiences visitation by honey bees. It was found that the sugars added by bees may account for up to 40% of the dry weight of pollen pellets in bee-collected pollen (Todd and Bretherick 1942), which could negatively skew protein measurements during analysis. While this is unlikely to alter the perceived nutritional value when comparing closely related anemophilious or entomophilious pollen grains, it does create uncertainty when comparing pollen grains of different families or genera when both anemophilious and entomophilious pollen may be present (Roulston and Cane 2000).

1.2.3 Pollen foraging in Apis mellifera

Pollen collection by honey bees occurs in a specific and consistent manner. This process may occur passively during flower visitation by bees to collect nectar, or actively in separate flower visits. In active pollen collection by honey bees, female workers have a number of specialized adaptations that aid in the collection of pollen grains. One such adaptation, which is used diagnostically to distinguish bees from wasps, is the presence of branched body hairs along the thorax. These hairs are seen on both male and female bees, despite females functioning as the primary foragers and pollen-gatherers. Methods of pollen collection then begin according to phylogeny, as most bees are equipped with some external device to aid in pollen collection, while

some may carry pollen internally, in the alimentary canal (Thorp 2000). In honey bees, pollen grains are collected by stiff hairs on the front legs that are moisturized with small doses of nectar or honey to facilitate the pollen-packing process. The bees then use the "pollen brushes" on their middle legs to collect the excess pollen on the thorax and the pollen collected on the front legs. During flight, the pollen grains are transferred to the hind legs where they are stored in the corbiculae, or pollen baskets, of the honey bee located between the tibia and the metatarsus. At this point, the accumulation of pollen grains is modified into a conveniently packaged pellet that may contain up to 1,000,000 pollen grains (Hodges 1974, Lindtner 1981). This intricate packaging process seen in honey bees allows them to acquire large pollen grains of over 100 μm, as well as smaller pollen grains.

Pollen foraging is an expensive activity for the honey bee in terms of energy expenditure, risk of predation, and a variety of additional factors. A single foraging trip is typically 10 minutes, but can take as long as 169 minutes (Stanley and Linskens, 1974). This is largely dependent on the surrounding landscape coverage, as areas with greater urban development and less floral diversity can require additional foraging time. Honey bees, in contrast to most wild bees, are floroconstant in their foraging behavior, collecting pollen from one floral species during a trip (Lindtner 1981). This is made possible by the large distance that honey bees can cover during a single foraging trip, as a honey bee's ideal foraging range covers a 5km radius, and honey bees are able to travel up to 10km if necessary.

Several factors influence foraging patterns in honey bees, dictating both the timing of collection, and the amount of pollen collected. First, the available flora is the primary factor that controls honey bee forage. It has been suggested that flowers with high nitrogen content are preferred by honey bees (Louveaux 1954), however, conflicting research by Faegri and Van Der Pijl (1979) suggests that flowers that emit phytosterole kairomones are preferred by honey bees, and that bees are unable to detect the nutritive value of pollen grains (Louveaux 1954; Faegri and Van Der Pijl 1979). Instead, honey bees often show a preference for fresh pollen (Doull 1966). Honey bee foraging is also limited by temperature, as honey bees are unable to collect floral resources at temperatures below 10º C. Under these conditions, honey bees are more focused on maintaining homeostasis, storing energy to conserve body heat. The third factor that affects the collection of pollen is the presence or absence of brood. Research by Al-Tikrity et al. (1972) suggested that there was a positive correlation between the amount of uncapped brood and the amount of pollen collected, and further experimentation by Barker (1971) advocated that brood rearing was limited when there were lower amounts of honey and pollen present in the hive. Under ideal conditions with regular floral diversity, temperature, and brood presence, an average pollen load will weigh between 5-15 mg. In a single day one colony can collect up to 250 grams of pollen, and up to 50kg of pollen in a season (Lindtner 1981).

1.3 Pollen library, Nutritional Calendar, and Nutritional Index

Due to the significance of pollen as the primary source of protein in the honey bee diet, it is necessary to catalog the pollen that is available to honey bees in the Mid-Atlantic. This requires the development of a "pollen library", where pollen species are listed and described by differing morphological features. The construction of a pollen library will allow trained experts in the field of entomology and botany, as well as hobbyist beekeepers and gardeners to identify a pollen grain to the lowest possible taxonomic rank. While there are currently many diagnostic keys in literature that can aid an individual in the identification of pollen, there is currently a need for an updated, digital reference collection.

The development of a pollen library allows an individual to track foraging patterns by monitoring and identifying bee-collected pollen grains. This gives rise to the creation of a pollen calendar, where honey bee foraging can be monitored at consistent intervals, and one can measure the amount, diversity, and evenness of pollen forage across an extended period of time. This process involves the collection of pollen grains in a pollen trap, and the identification of these pollen grains to the lowest taxonomic level. After the identification of pollen grains occurs, one can develop a nutritional index for pollen to determine the species, genera, and families that are most prevalent in the honey bee diet, and define the types of pollen that have the highest nutritional quality. This information can then aid beekeepers, horticulturists, and urban planners, as well as private individuals in planting the species that are most beneficial to honey bees.

Chapter 2

Materials and Methods

Development of the Pollen Library and Pollen Calendar

2.1 Development of the Pollen Library

2.1.1 Collection Sites

Flower samples were collected primarily from four sites across the Mid-Atlantic to cover the wide range of floral diversity that exists in this rich floral region. The four sites include:

1. University of Delaware Botanical Garden (UDBG)
2. Mt. Cuba Center (MCC)
3. Scott Arboretum of Swarthmore College (SA)
4. Longwood Gardens (LG)

Of the four sites used in sampling, two sites (UDBG, MCC) are located in New Castle County, Delaware, while the remaining two sites (SA, LG) are located in Delaware County and Chester County, Pennsylvania, respectively. Each site chosen for sampling is managed to some extent; however, the high floral diversity present at these sites is due to the unique location of these sites along the Coastal Plain of the Eastern United States. The four sites chosen exist where northern plant life and southern plant life overlap, maximizing the potential for floral diversity (Lindtner 1981).

Flower samples were collected for each site by using pruning shears to take a cutting of the plant, or by manually removing a small number of flowers. The samples were then placed in a glassine envelope where they were labeled with the date of collection and species name. These samples were then transferred to the University of Delaware apiculture lab for pollen dissection and photography.

2.1.2 Pollen Dissection

Pollen grains were obtained from each floral sample by first isolating the anther from the rest of the cutting. One flower from each sample was placed on a clean microscope slide under a Motic or Leica Aquire (Wetzlar, Germany) light microscope where they could be viewed in further detail, with the aid of a Schott-Fostec Ace (Arlington, Va.) light source. Using the coarse and fine adjustment knobs on the light microscope the anthers of each flower sample were located. This often involved the removal of the two outer whorls (sepal and calyx) of the flower samples as they often block the view of the anthers within the androecium. Once access to the anthers of the flower was established, 3-5 anthers were removed per floral sample to ensure that an adequate amount of pollen would be available. At this point, excess floral residue was removed from the slide to ensure that only the anthers (occasionally accompanied by filaments) were present on the slide. A single drop of ethanol was then applied to the anthers to separate the pollen from the anthers, and remove excess waxes and oils that may be present on the pollen grains. In the case of highly oily pollens (like those in the Asteraceae family) two drops of alcohol were applied to ensure the removal of oils. The anthers were then prodded with forceps to aid in the dispersion of pollen grains away from the anther. Excess floral parts were again removed from the slide, leaving only the pollen grains remaining. At this point, pollen

grains were observable as small grains underneath the light microscope; however it was impossible to determine the color of the grains at this level of magnification. After the ethanol had completely evaporated, one 12µm drop of fuchsia stain was applied to the pollen grains on the slide. The fuchsia stain aids in the identification of physical features of the pollen spores under microscopes with greater magnification. After the fuchsia stain was applied, a cover slip was mounted over the stained portion of the slide. The cover slip should be applied at a 45º angle to minimize the amount of air bubbles that are able to enter the slide. Nail polish was then applied around the perimeter of the cover slip as a protectant to lessen the amount of unwanted material that could enter the slide. Finally, a label was applied to the slide using a labeling stamp (Figure 1). Each label contained the following information:

1. *Genus species*
2. Date of collection (dd/mm/yy)
3. Date of preparation (dd/mm/yy)
4. Ascension # (if applicable).

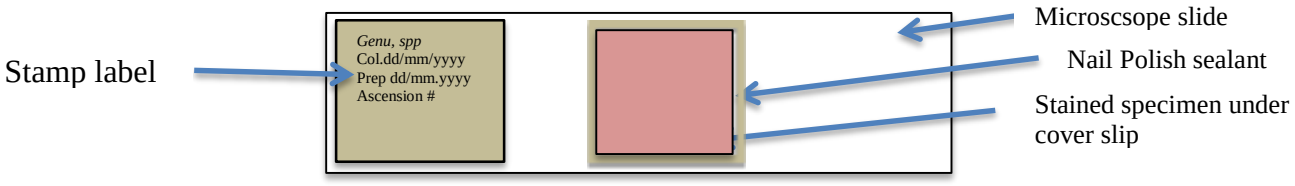


Figure 1. Sample pollen library slide containing a stained specimen under a cover slip with a nail polish sealant, adjacent to a properly labeled stamp.

2.1.3 Pollen photography

Pollen slides were photographed under a Leica Acquire ICC50 camera in conjunction with a Leica Acquire stereomicroscope. The light microscope was initially set to 100x magnification to locate the position of stained pollen grains. After the stained pollen grains were located on the slide, the magnification of the stereomicroscope was changed to 400x to view pollen grains in greater detail. At this level of magnification photographs of different groups of pollen grains were taken to determine the different groups of pollen grains that were present on a slide. Certain low detail attributes could be identified like outline and aperture type, however this level of magnification was primarily used to determine which grains were most suitable for photography at 1000x. After determining which grains were the most appropriate for photography at 1000x, the slide and the 1000x microscope lens were prepped with one droplet of immersion oil. The immersion oil is used to increase the resolution of the photograph at this high level of magnification (Abramowitz and Davidson 2002). At the 1000x level of magnification photographs were taken that captured: 1) the polar face of the pollen spore 2) the equatorial face of the pollen spore 3) the tectum of the pollen spore. These three angles of photography provided all the details needed for analysis of the morphological features of pollen grains.

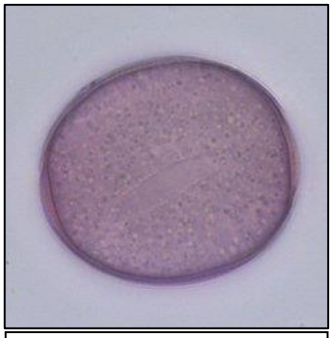
2.1.4 Pollen Analysis

Pollen images taken at 1000x magnification were used to analyze morphological features of pollen grains. In total, eight morphological features were examined in each pollen grain. They include:

1. Unit
2. Aperture number
3. Aperture type
4. Ornamentation

5. Tectum
6. Size
7. Outline
8. P/E ratio shape

The unit, or dispersal unit, of a pollen grain refers to the number of morphological components that are present when mature pollen grains are shed (Figure 2). In most cases, this occurs in one individual unit (monads), but some grains may have multiple units, or clusters. This is the case seen in the genus *Rhododendron*, where pollen grains are shed in clusters of four, called a tetrad.

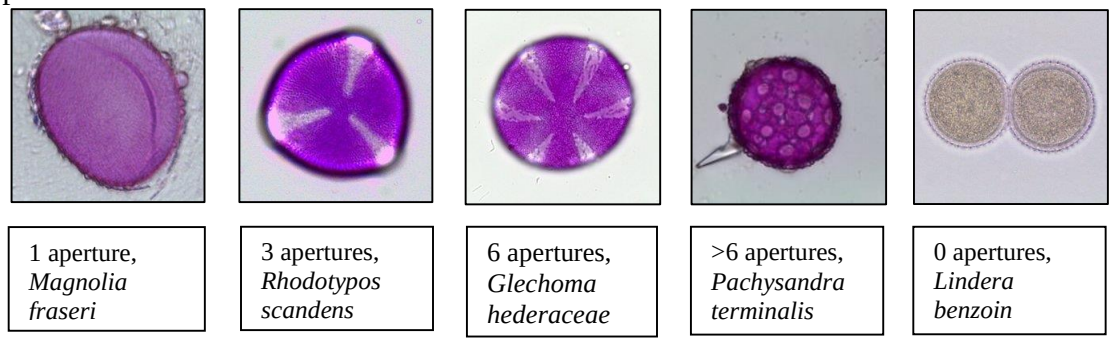


Monad pollen spore, *Uvularia grandiflora.*

Tetrad pollen spore, *Rhododendron spp.*

Figure 2. Examples of a monad pollen spore and a tetrad pollen spore.

The aperture number is simply the number of apertures that are present in a single pollen grain (Figure 3). There are typically one, three, or six apertures present in pollen grains with colpi, and three, or more than six apertures present in pollen grains with pores. Colporate pollen grains generally have three apertures in a single pollen grain. In rare cases, a pollen grain will be free of apertures, resulting in an aperture number of zero.



. 1 aperture, *Magnolia fraseri*

3 apertures, *Rhodotypos scandens*

6 apertures, *Glechoma hederaceae*

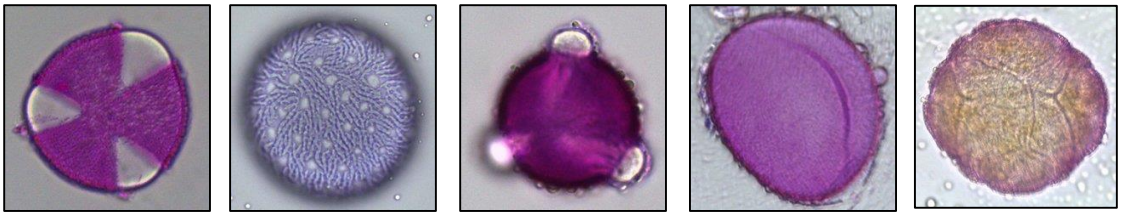
>6 apertures, *Pachysandra terminalis*

0 apertures, *Lindera benzoin*

Figure 3. Aperture numbers in various pollen grains.

There are various types of apertures that can exist within a pollen grain. A colpate pollen grain contains a colpus, or an elongated aperture with a length to breadth ratio greater than 2:1 (Erdtman 1943). This type of aperture typically looks like a triangle with a rounded base. These apertures typically come in three's or sixes in a pollen grain. A porate pollen grain contains pores, or rounded-elliptic apertures with a length to breadth ratio less than 2:1 (Jackson 1928). These apertures typically come in groups of three or more in a pollen grain. A colporate pollen grain is a compound aperture with a pore and a colpus occurring within the same aperture (Erdtman 1945). These apertures typically occur in threes within a pollen grain. A sulcate pollen grain has a sunken aperture that is similar to a colpus, diagnostically. The main difference between a sulcus and a colpus is that the former runs latitudinally

within a pollen grain, whereas the latter runs longitudinally. Furthermore, sulci typically occur as one single aperture within a pollen grain (Erdtman 1952). In rare cases, a pollen grain may not have any apertures. A pollen grain that is free of apertures is an inaperturate pollen grain (Figure 4).

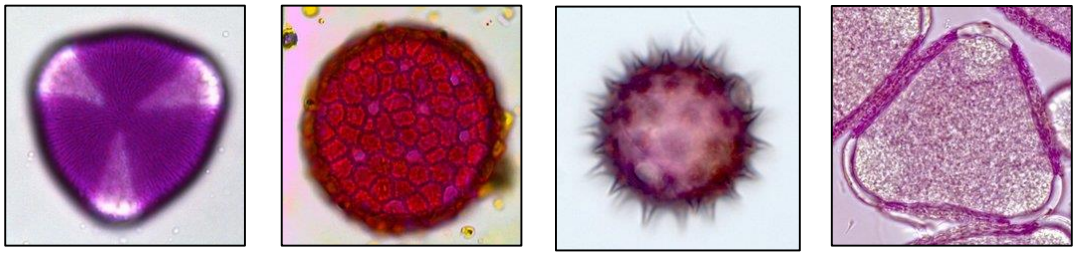


Colpate, *Corylopsis pauciflora*	Porate, *Polemonium reptans*	Colporate, *Acer japonicum*	Sulcate, *Magnolia fraseri*	Inaperturate, *Catalpa bignonoides*

Figure 4. Types of apertures in various pollen grains.

The ornamentation of a pollen grain refers to the general pattern or sculpturing seen on the face of the pollen grain. Striate pollen grains have a pattern consisting of relatively parallel grooves (Iversen and Troels-Smith 1950). Reticulate pollen grains have a network-like pattern formed by muri, or ridges. In reticulate pollen grains muri enclose spaces, called lumina that are greater than 1µm in width (Praglowski and Punt 1973). Echinate pollen grains contain spines that are greater than 1µm in length. This type of ornamentation is extremely common in the Aster family, *Asteraceae*. Additional types of ornamentations exist that are less than 1µm in size. Some examples of this are perforate pollen grains, pollen grains with small punctures on their surface, and granulate pollen grains, pollen grains with small protruding structures on their surface. Pollen grains with patterns that are less than 1µm in size

are often indiscernible under a compound microscope, and are grouped together for this reason (Figure 5).



Striate, *Prunus incisa*

Reticulate, *Phlox nivalis*

Echinate, *Coreopsis tripteris*

<1µm, *Spigelia mavilandicula*

Figure 5. Differing types of ornamentation in select pollen grains.

The tectum refers to the outer layer of the sexine. This feature can be viewed as the perimeter of the pollen grain when viewed under a microscope. Two types of tectum are common in pollen grains, eutectate tectum and semitectate tectum. A eutectate pollen grain has a tectum that is continuous all the way around, while a semitectate tectum is partially discontinuous (Faegri and Iversen 1964) (Figure 6).

Eutectate, *Waldsteinia fragaroides*

Semitectate, Corylopsis pauciflora

Figure 6. Tectum of select pollen grains.

The size, measured in terms of length, of pollen grains was measured using ImageJ software in conjunction with a Leica Acquire ICC50 HD Compound microscope and built in camera. It was determined that 1248 pixels was equal to 1µm at 400x magnification, and that 1569 pixels was equal to 1µm at 1000x magnification. A wide range of sizes was seen in pollen grains, with pollen grains as small as 10µm, and some exceeding 100µm in size. Pollen grains were measured from end to end along the longest face of the pollen grain (polar or equatorial) to determine the size of the grain.

The outline of a pollen grain refers to the general shape of a pollen grain, simply based upon the outline of its exine. This feature is categorized into three general categories: circular, triangular, and elliptic (Figure 7).

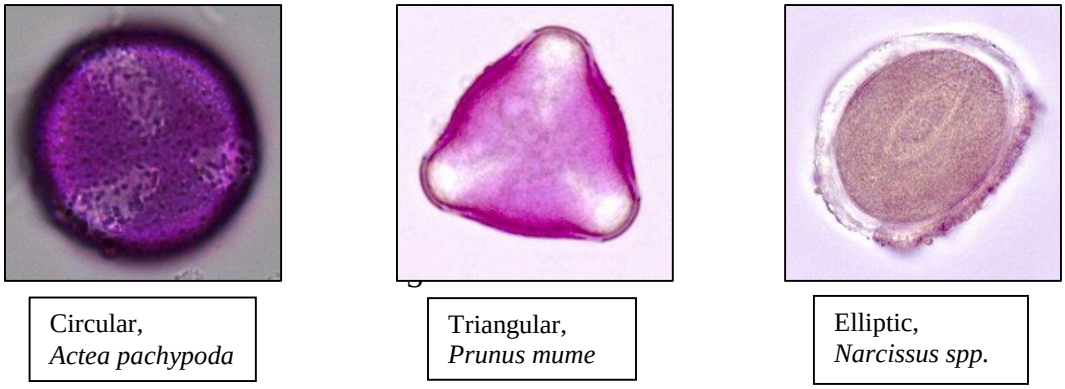


Figure 7. Outline of select pollen grains.

The P/E ratio gives further insight into the shape of a pollen grain. The P/E ratio of a pollen grain compares the length of the polar face of a pollen grain to the length of the equatorial face of the pollen grain. The specific P/E ratios result in shape classes that have been suggested by Erdtman (1943) (Table 1). In this analysis of

morphology, grains with a P/E ratio between 0.95 and 1.05 have been classified as spheroidal (Table 1).

Table 1. Shape classes of pollen grains suggested by Erdtman, 1943

SHAPE CLASSES	P . E
Perprolate	> 2 (> 8 4)
Prolate	2–1 33 (8 4–8 6)
Subprolate	1 33–1 14 (8 6–8 7)
Spheroidal	1 14–0.88 (8 7–7 8)
prolate spheroidal	1 14–1 00 (8 7–8 8)
oblate spheroidal	1 00–0.88 (8 8–7 8)
Suboblate	0 88–0 75 (7 8–6 8)
Oblate	0 75–0 50 (6 8–4 8)
Peroblate	< 0 50 (< 4 8)

2.1.5 Discover Life

The Pollen Library was constructed on Discoverlife.com in conjunction with Dr. John Pickering, founder of the site and Professor of Ecology at the University of Georgia. Discover Life functioned as a platform for the development of an ID guide and photo album for the pollen of the Mid-Atlantic. The goal of this site was to develop an identification guide for use both by professionals and hobbyists, where one can identify a flower to the lowest taxonomic rank possible based on the time of collection of the pollen grain, and the morphological features of the pollen grain. To ensure that this medium could be used and navigated easily by hobbyists, palynology jargon was avoided whenever possible. In the case that palynology jargon was used, it was explained in a user friendly manner.

Discover Life also provided users with a photo album of pollen grains, where additional photographs were contained to further illustrate the different morphological features seen in pollen grains. Each photograph was labeled with further details on the

collection site, including GPS coordinates that were overlaid onto a map to show the exact point of collection. Additionally, the method/camera used to take the photograph and the level of magnification was recorded on the label of each picture. In some cases, scanning electron microscope (SEM) photos were taken to show morphological features that could not be seen under the compound microscope. These features were generally less than 1µm in size (http://www.discoverlife.org/mp/20q?guide=Pollen).

.

2.2 Development of the Pollen Calendar

2.2.1 Collection Sites

Two selection sites were used for the development of the pollen calendar: the University of Delaware Botanical Garden (UDBG) in Newark, Delaware (Figure 8), and the Mount Cuba Center (MCC) in Hockessin, Delaware (Figure 9). Each of these sites is located within New Castle County, approximately 13 miles, or 21 kilometers (km), away from one another. This separation ensures that there is no crossover in foraging range between the colonies at the UDBG and MCC, as the maximum foraging range of the honey bee is 10 km. Even if we were to assume that the colonies were foraging at their maximum range, as close to one another as possible, there would still be a 1.6km buffer between foraging ranges. The UDBG is located within the heart of the University of Delaware's College of Agriculture and Natural Resources. While there is great floral diversity within this garden, much of the surrounding area has seen significant urban development in the form of academic buildings and businesses within the campus, and the burgeoning residential suburbs

surrounding the University. Comparatively, the MCC has seen far less urban development. MCC and surrounding lands have seen strict restrictions related to land use, and have only recently been open to human manipulation. Initially, pollen was to be collected from ten hives total, with five hives at each site. The death of one hive at the UDBG resulted in the use of just four hives at this site for the duration of this experiment.

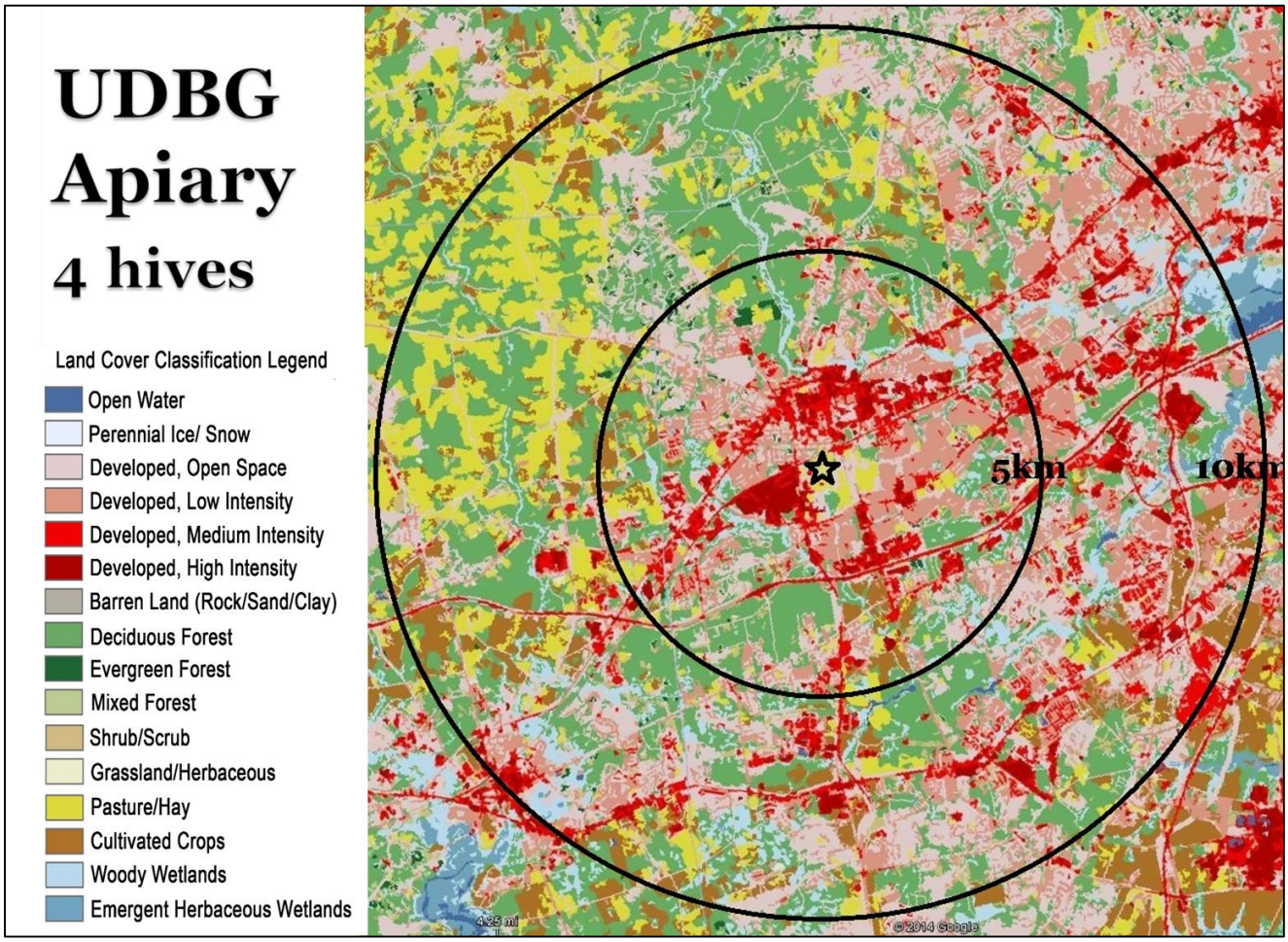


Figure 8. Landscape coverage at the University of Delaware Botanical Garden.

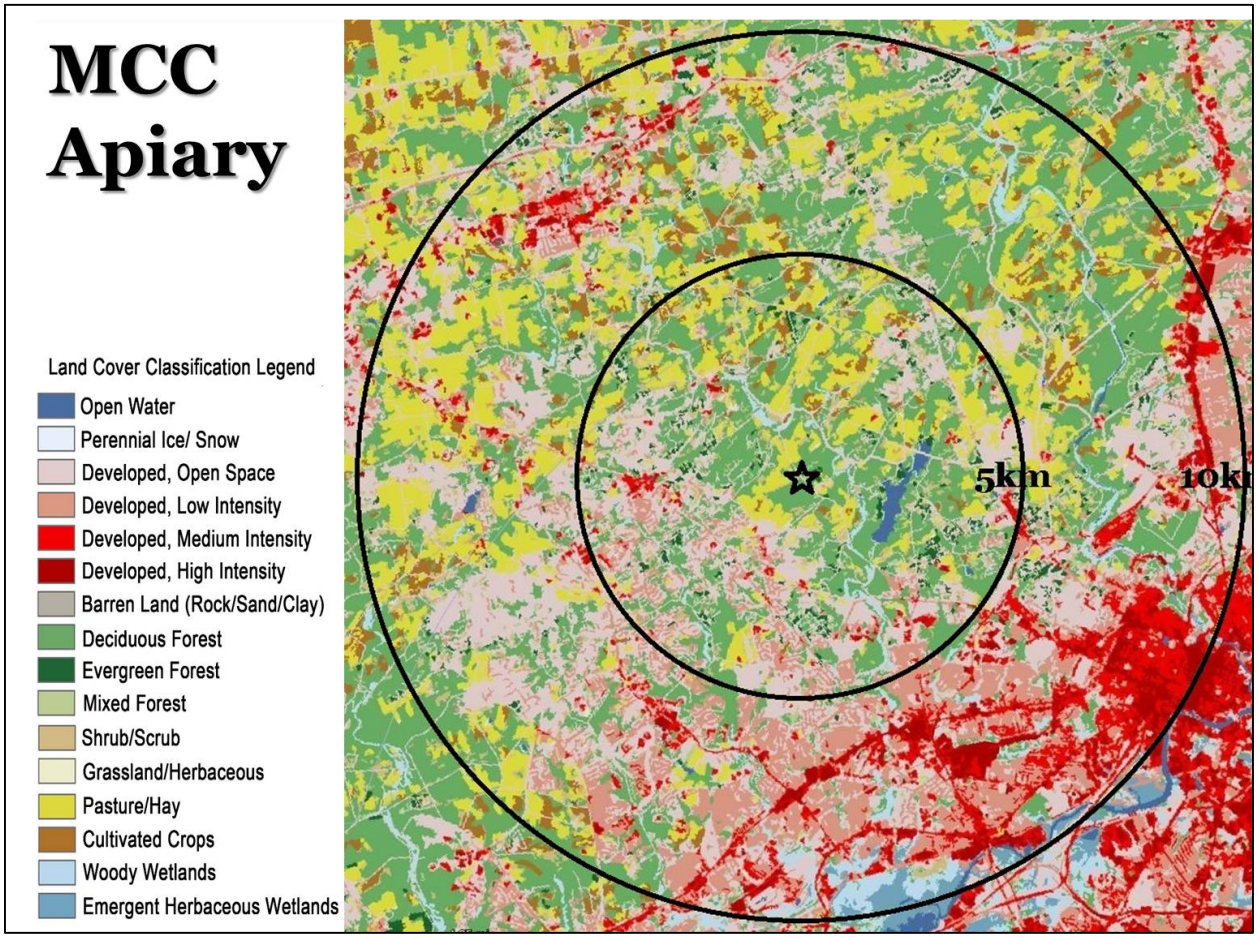


Figure 9. Landscape coverage at the Mount Cuba Center.

2.2.2 Pollen collection

Pollen was collected once a week at each site from March 20th through September 20th using pollen traps placed on the front of the hive. These pollen traps were left on for a 24 hour period, collecting about half of the pollen that would have been brought into the hive during this time period. This is made possible due to the creative design of pollen traps, whose openings are built such that half the pollen on a honey bee's corbiculae is scraped off upon entering the hive. Pollen was collected from each hive and placed in a Ziploc bag which was labeled with the collection site, hive number, and date and time of collection. These bags were then brought back to the apiculture lab at the University of Delaware for future manipulation.

In the lab, pollen from each hive was weighed on a scale to the nearest hundredth of a gram. First, the wet weight of the pollen was obtained by first obtaining the weight of an empty weighing dish, and then pouring the pollen from one hive into the weighing dish and placing this load on the scale again. The weight of the scale was then subtracted from the weight of the loaded pollen dish to determine the weight of the pollen.

After the weight of pollen loads were obtained, pollen loads were sorted by color. Many different methods were used in order to identify a method that was both accurate and efficient. First, a Pantone color wheel was used to match pollen grains to a color. After this a more extensive RAL color wheel was used to match pollen grains in an attempt to find closer color matches. It was determined that this method was not time-effective, so the methodology of using a color wheel was abandoned in favor of using a known color chart containing just 24 colors in conjunction with a scanner. Pollen grains were placed on the scanner with the color chart, and scanned in a manner that light was even and consistent. This allowed for a more consistent analysis of

pollen grains with respect to color. The RGB values for each of these colors was found and cataloged on Microsoft Excel for bookkeeping purposes. After the grains were sorted by color, samples of each color were placed in vials, and any excess pollen grains were placed back in their respective Ziploc bags, and then into a -80ºC freezer. Once in vials, distilled water was added to the pollen samples to create an aqueous solution. This aqueous solution was then vortexed for 15-30 seconds to ensure that a homogenous mixture was created.

2.2.3 Pollen Slide Preparation, Photography, and Identification

Three separate 15µm dots of fuchsia stain were placed on a clean microscope slide, side by side. The tip of a toothpick was used to obtain a small sample of pollen from each vial, and this pollen sample was placed in a fuchsia dye sample on the microscope slide. The stain-pollen solution was then covered with a cover slip, placed down at a 45º angle to ensure the minimum amount of air penetration into the slide. Nail polish was then placed around the perimeter of the cover slip to ensure that contaminants were unable to enter the slide. This process was repeated until three distinct pollen-stain samples were prepared on a single slide. The hive number, pollen color, and date of slide preparation were recorded for each sample.

Prepared pollen slides were then placed under the Leica Acquire ICC50HD compound microscope where they were photographed primarily at 400x magnification and 1000x magnification, in accordance with the photography protocol discussed earlier. Once photographed, pollen grains were analyzed based on the eight morphological features that were previously discussed. These features were then entered into the University of Delaware's Pollen Library on Discovery Life to identify these pollen grains to the lowest taxonomic rank possible. A variety of dichotomous

keys were used in conjunction with the Discover Life ID guide to ensure proper identification.

Chapter 3

Results

3.1 Pollen Identification Guide and Discover Life

Pollen samples collected from UDBG, MCC, SA, and LG in the year 2013 and 2014 were uploaded to the Discover Life identification guide. In total, 254 species from 109 genera and 56 families were uploaded onto the Discover Life identification guide. The Discover Life pollen identification guide is broken up into "characters" and "states". Characters refer to the different types of morphological traits that were described. Examples of this include aperture number, ornamentation, and aperture type. States refer to the different types of physical features within a character, but in many cases palynological terms were replaced by common terms. An example of this would be "echinate", "reticulate", "striate", and "granulate/perforate being replaced by "spiky", "network", "grooves", and "dots" within the texture (ornamentation) character. Terms describing apertures and ornamentations and other morphological features were accompanied with an explanation tool that further detailed the characteristics for users. Additionally, bloom time for each species was used as a descriptive characteristic on the Discover Life identification guide to allow for further resolve when identifying different grains. In total, 324 photographs were uploaded to the pollen photo album, including 42 SEM photographs. The SEM photographs, taken by were taken of different species within the genus *Coreopsis.*

3.2 Total forage, and Comparison of UDBG Forage and MCC Forage

In the 2014 foraging season, 4594.7 grams of pollen was collected in pollen traps between March 24th and September 20th, which included twenty-one collection dates (Table 2). Of these twenty-one collection dates, there were fourteen dates where pollen was collected from both the UDBG and MCC. There were two collection dates where trapped pollen exceeded of 500 grams total, between the two sites. This was seen on May 19th and September 15th, where 624.98 and 576.41 grams of pollen were collected, respectively (Figure 10; Figure 11; Figure 12). In total, 1415.005 grams of pollen was collected from the UDBG, and 2179.176 grams of pollen was collected from the MCC. Furthermore, when viewing the weight of foraging totals by the date of collection, it was found that total forage at MCC exceeded that at the UDBG on ten of the fourteen collection dates. Thus far, identification of pollen loads has primarily been performed on the pollen collected from the UDBG. 78.14% of the bee-collected pollen from this site has been identified to the family level or further, including the identification of 24 distinct species of pollen (Figure 13; Figure 14).

3.2.1 UDBG pollen collection and identification

Between March 24th and April 27th, honey bees collected pollen from 15 different species of flower over 6 collection dates. Throughout this time period, 858.62 grams of pollen were collected in total, including 591.80 grams of pollen from the genus *Acer*. Peaks in the collection of *Acer* pollen were seen on the first three collection dates: March 24th, April 1st, and April 6th. On these three dates, 567.93 grams of *Acer* pollen was collected by bees. During this time span, no other species accounted for more than 20 grams of pollen. During the next three collection dates, April 14th, 21st, and 27th, pollen from the genus *Acer* was still collected, but in very

low quantities. Instead, pollen from the genus *Prunus* was the primary type of pollen collected by honey bees, as 135.30 grams of *Prunus* pollen was collected on these three collection dates. Pollen from this genus was seen in collection samples until May 5th. Pollen from the species *Taraxacum officinale*, dandelion, accounted for 31.01% of all pollen grains collected on the three collection dates between April 21st and May 5th. Between May 5th and June 14th, only 9.7% (79.88 grams) of the pollen collected was identified.

Between June 23rd and July 23rd 71.76% (279.83 grams) of the pollen collected by honey bees was identified to some extent. During this time period, 5 different species of pollen were documented, including 262.07 grams of pollen from the genus *Trifolium* (clover). No other species of pollen was seen in excess of 10 grams during this time period, and *Trifolium* pollen collection continued until September 15th. Between August 4th and September 20th only 10.21% of pollen grains were identified.

3.3 MCC pollen collection and identification

Although pollen collection began at MCC on May 5th, pollen identification has primarily taken place on samples collected between July 1st and August 15th. During this time span, 74.00% of pollen grains were identified. The primary pollen type seen in samples from MCC was from the genus *Trifolium*, which was documented in four of the six collection dates. In total, 216.02 grams of *Trifolium* was collected by honey bees from the MCC colony over this time span. The second most prevalent pollen type seen during this time period was from the species *Zea mays*, corn. In total, pollen from seven different species was observed during this limited time frame.

Table 2. Pollen collected by honey bees sorted by location (apiary) and collection date, separated into sub-tables. Sub-tables contain four collection dates each, with the exception of the final sub-table which includes five collection dates. Pollen grains are listed according to their lowest taxonomic level of identification.

Sum of Weight	24-Mar-14	1-Apr-14	6-Apr-14	14-Apr-14	Grand Total
UD	213.3	241.4	177.01	13.79581	645.50581
Acer spp.	197.3025	223.7845	146.84645	2.15174	570.08519
Amaryllidaceae spp.				0.7042	0.7042
Caryophyllaceae spp.		0.709	3.1815		3.8905
Crocus spp.	15.9975	15.345			31.3425
Hamamelis spp.		0.3545			0.3545
Liliaceae spp.				0.05718	0.05718
Magnolia spp.			0.909		0.909
Pachysandra spp.		0.3545	2.727		3.0815
Platanus spp.			0.4545		0.4545
Prunus spp.				6.90318	6.90318
Rosaceae spp.			0.8611	0.08577	0.94687
Salix spp.			19.39925	3.86515	23.2644
Taraxacum officinale				0.02859	0.02859
Ulmus americana		0.8525			0.8525
(blank)			2.6312		2.6312
Grand Total	213.3	241.4	177.01	13.79581	645.50581

Sum of Weight	21-Apr-14	27-Apr-14	5-May-14	12-May-14	Grand Total
CUBA			52.93	26.94	79.87
(blank)			52.93	26.94	79.87
UD	52.74612	159.7309	60.9669	36.715	310.15892
Acer spp.	17.82564	3.891			21.71664
Aesculus spp.		3.891			3.891
Amaryllidaceae spp.	0.21448				0.21448
Baptisia spp.				1.044	1.044
Caryophyllaceae spp.	0.31718				0.31718
Fabaceae spp.			1.2532		1.2532

Prunus spp.	24.12102	104.2713	16.5516		144.94392
Rosaceae spp.	0.42896				0.42896
Salix spp.		1.1673	6.366	22.272	29.8053
Taraxacum officinale	9.83884	44.9091	15.79		70.53794
(blank)		1.6012	21.0061	13.399	36.0063
Grand Total	52.74612	159.7309	113.8969	63.655	390.02892

Sum of Weight	19-May-14	26-May-14	2-Jun-14	14-Jun-14	Grand Total
CUBA	403.189		264.36093	236.0632	903.61313
(blank)	403.189		264.36093	236.0632	903.61313
UD	221.7888	141.94702	133.50676	223.517	720.75958
(blank)	221.7888	141.94702	133.50676	223.517	720.75958
Grand Total	624.9778	141.94702	397.86769	459.5802	1624.37271

Sum of Weight	23-Jun-14	1-Jul-14	8-Jul-14	16-Jul-14	Grand Total
CUBA	117.695	123.8281	124.127	49.4142	415.0643
Allium spp.			30.81305	5.39804	36.21109
Centaurea cyanus			10.84109	27.46857	38.30966
Trifolium spp.		84.955	79.32556	14.01921	178.29977
(blank)	117.695	38.8731	3.1473	2.52838	162.24378
UD	58.518	127.76	85.64	64.959	336.877
Allium spp.		1.7649		0.78615	2.55105
Asteraceae spp.	0.12954			0.26764	0.39718
Centaurea cyanus			1.0239	0.78615	1.81005
Trifolium spp.		107.0499	60.918	52.44668	220.41458
Zea mays			1.2914	4.33612	5.62752
(blank)	58.38846	18.9452	22.4067	6.33626	106.07662
Grand Total	176.213	251.5881	209.767	114.3732	751.9413

Sum of Weight	Column Labels					
Row Labels	**23-Jul-14**	**4-Aug-14**	**15-Aug-14**	**15-Sep-14**	**20-Sep-14**	**Grand Total**
CUBA	**86.93994**	**139.0275**	**46.07716**	**404.134**	**104.45**	**780.6286**
Allium spp.		2.7918				2.7918
Asteraceae spp.		5.0465	0.809	30.4284		36.2839
Centaurea cyanus		32.1574	2.35029			34.50769
Rhus copallina			19.57721			19.57721
Taraxacum officinale		2.13				2.13
Trifolium spp.		37.7268		9.7895		47.5163
Zea mays	65.39834	0.5325				65.93084
(blank)	21.5416	58.6425	23.34066	363.9161	104.45	571.89086
UD	**53.08**	**60.56**	**12.44461**	**172.2734**	**101.0827**	**399.44071**
Asteraceae spp.			0.14025	1.3638		1.50405
Centaurea cyanus	3.699		7.9241	1.0192		12.6423
Rhus copallina		1.016	1.04838			2.06438
Trifolium spp.	41.6569	8.144	0.15876	9.2824		59.24206
Zea mays	3.6738	3.556	1.7136			8.9434
(blank)	4.0503	47.844	1.45952	160.608	101.0827	315.04452
Grand Total	**140.01994**	**199.5875**	**58.52177**	**576.4074**	**205.5327**	**1180.06931**

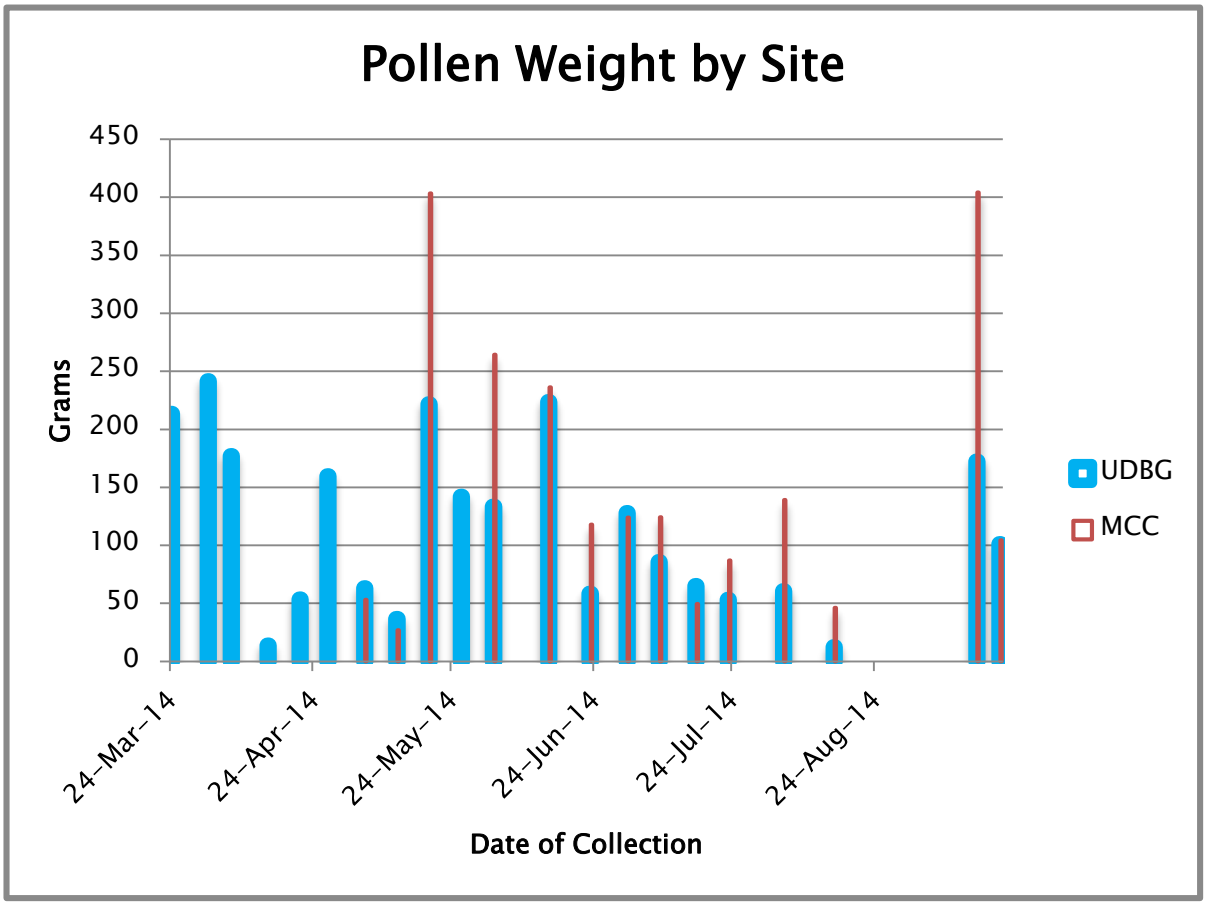


Figure 10. Pollen weight at two collection sites, sorted by date of collection. Blue bars represent pollen collected at the UDBG apiary, while red bars represent pollen collected at UDBG. Note, pollen was not collected at the MCC until May 12th, when colonies at this site were approved for establishment.

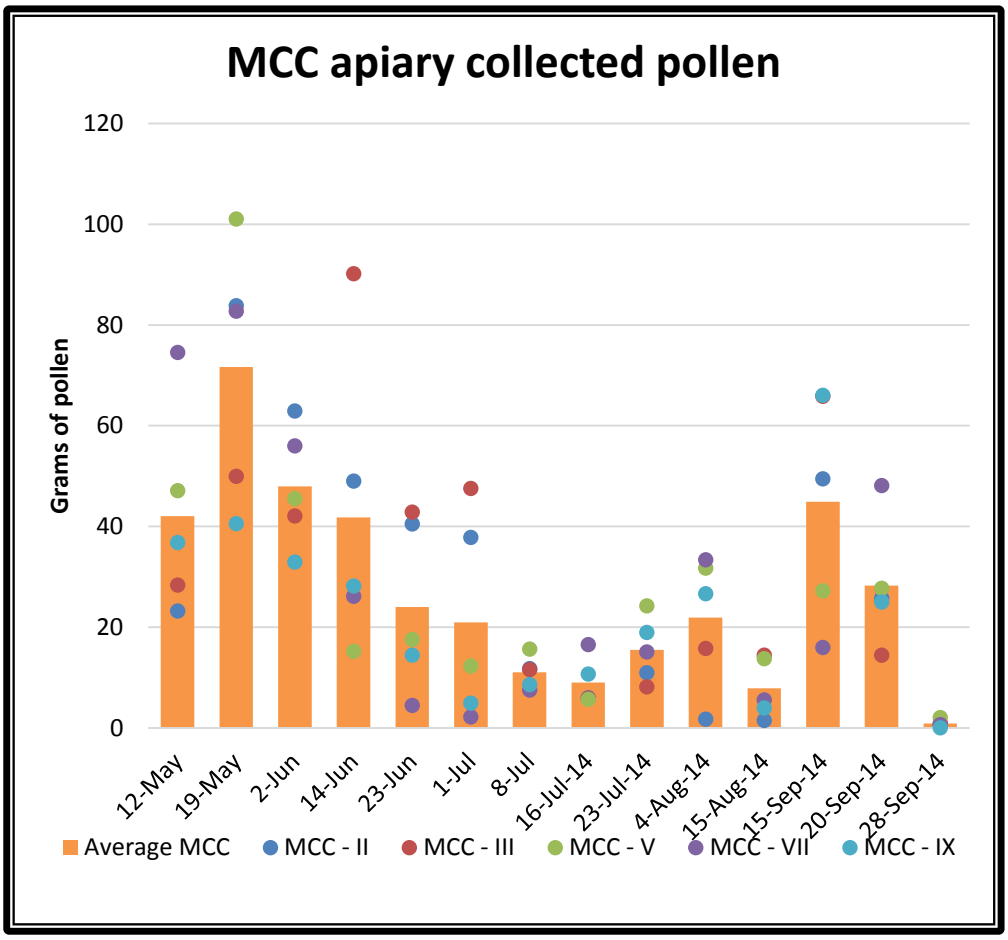


Figure 11. Pollen foraging at MCC, with an emphasis on pollen collection by hive. Bars represent the average wet weight of pollen grains from the five hives, while dots represent the wet weight of pollen at a single hive.

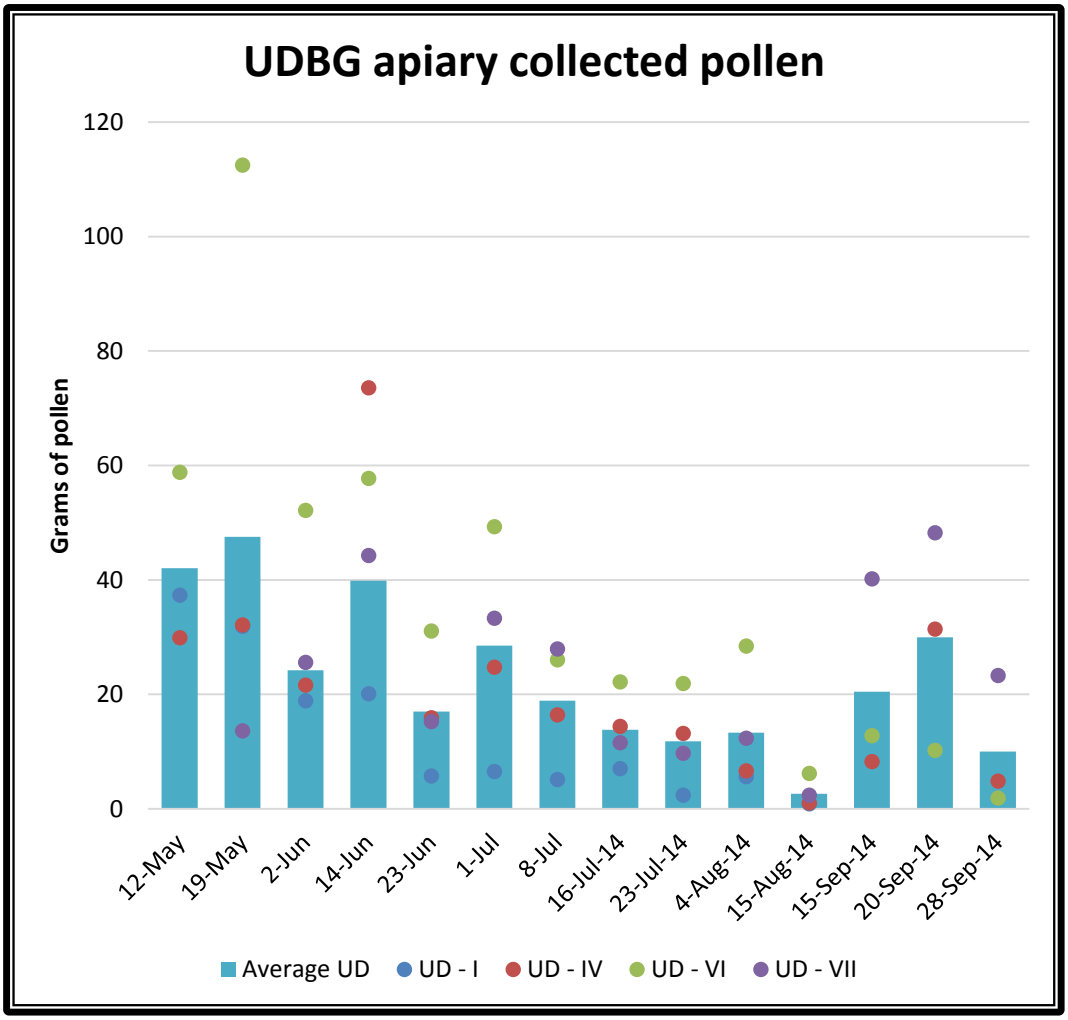


Figure 12. Pollen foraging at UDBG, with an emphasis on pollen collection by hive. Bars represent the average wet weight of pollen grains from the five hives, while dots represent the wet weight of pollen at a single hive.

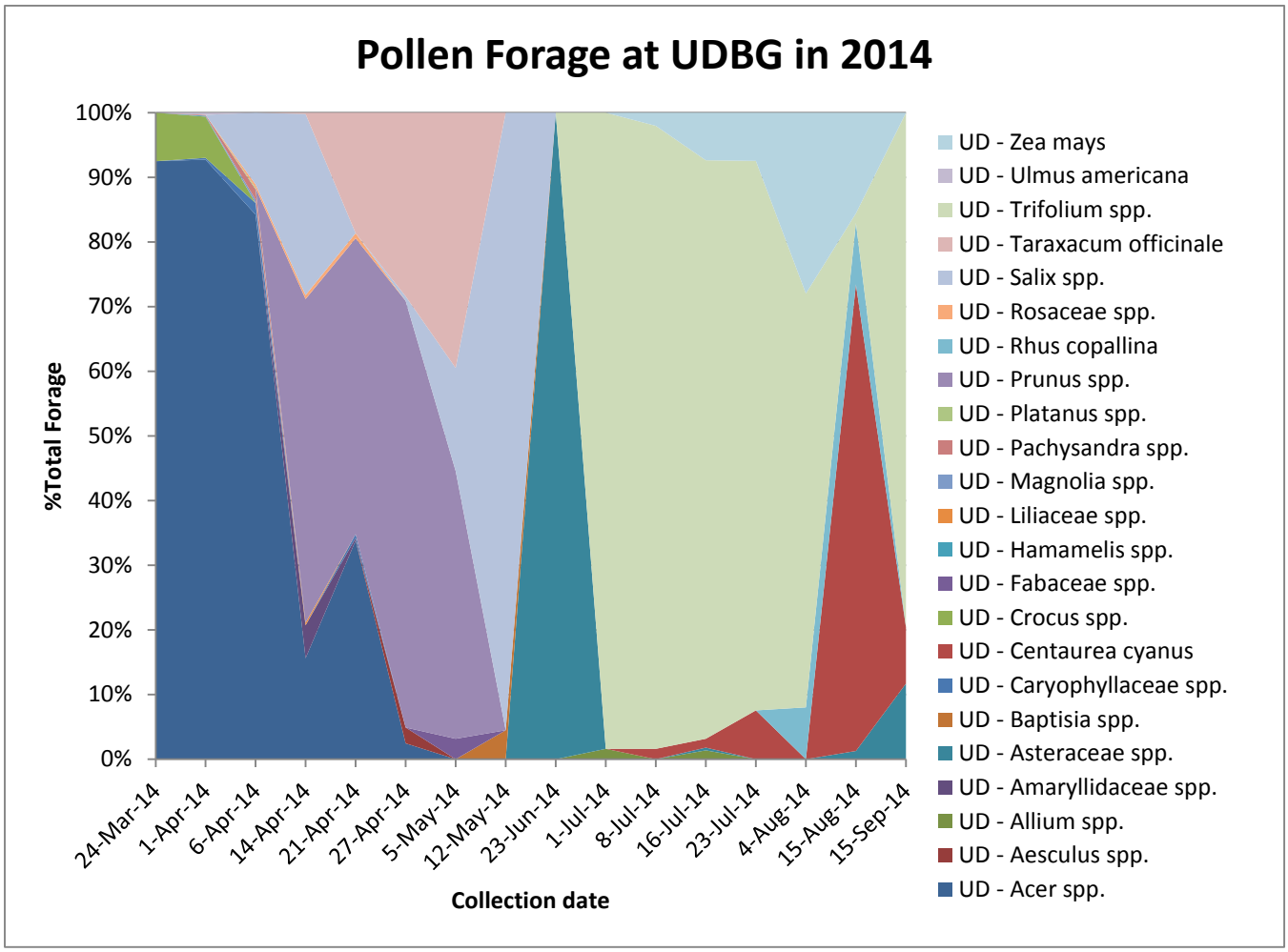


Figure 13. Pollen forage at UDBG throughout the 2014 forage season identified to the lowest taxonomic rank. Color regions represent the percent of the weight that a particular species accounted for on a collection date.

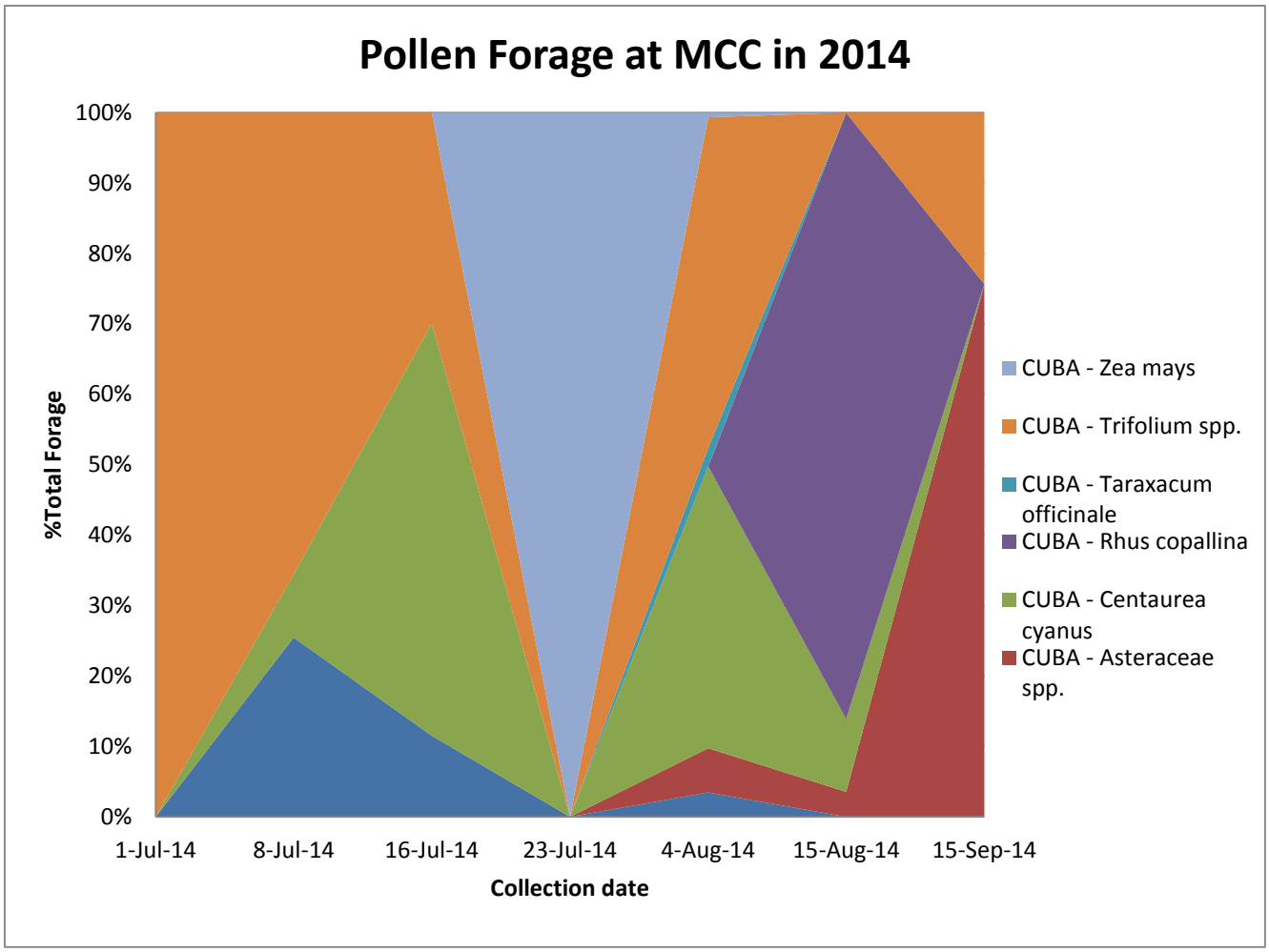


Figure 14. Pollen forage at MCC throughout the 2014 forage season identified to the lowest taxonomic rank. Color regions represent the percent of the weight that a particular species accounted for on a collection date.

Chapter 4

Discussion

4.1 Pollen Library

The pollen library serves as an identification tool in the processing of pollen samples of the Mid-Atlantic. The current sample size of 254 species from 109 genera and 56 families makes this identification guide best suited towards classifying pollen grains to a genus or family rather than to a species. Identification to species requires a much more extensive pollen library, as the 254 species in the current collection are just a small sample size of what exists across the state of Delaware, even in New Castle County alone. In order to identify pollen grains to the taxonomic rank of species, it will be necessary to collect voucher specimen for as many flowers as possible. Additionally, for proper identification to this taxonomic rank it may be necessary to take pictures of samples with the SEM. This is due to the great similarity that exists within many species of the same genus. These species will often overlap in many morphological features such as aperture number, aperture type, size, outline, and P/E shape. While there may also be some overlap in the type of ornamentation seen across different species of the same genus, pictures with a scanning electron microscope allow one to detect minute differences in pollen morphology that would otherwise go unnoticed.

Although this pollen library is still in its infancy, it is still one of the more thorough pollen identification databases available to the public, especially in the Mid-Atlantic region. This is supported by the attention the library has received from other

nearby Universities, specifically the University of Maryland which will likely collaborate in future development of the database. This collaboration will extend the geographic range and allow for accelerated growth of the pollen library. Additional interest has been expressed by Universities across the nation, including leading agriculture schools like Texas A&M University and Ohio State University. To better understand the extent that this tool is helping others in the identification of pollen grains, it may be useful to track visitation in future years. Additionally, the addition of more specific "states" that take into account more subtle differences in pollen grain morphology may increase the functionality of the database. Furthermore, adding more photographs per species could be useful in the identification process, as this will allow for pictures at varying degrees of focus, that in turn stress different features. Specifically, it is imperative to have pictures of the polar and equatorial face of a pollen grain as different features are visible on each face.

4.2 Comparison of forage at MCC and UDBG

Pollen foraging totals at MCC exceeded the foraging totals at UDBG during the 2014 foraging season, however these differences were not statistically significant. It will likely take a larger sample size and more repetitions of this experiment to determine the effect that landscape coverage has on pollen foraging totals and colony health. Increased foraging numbers can effect health on the colony level because it allows for a greater number of developing larvae at any given moment, as well as an increased stored food supply during overwintering months. Future experimentation looking at the effects of pollen collection on honey bee overwintering success will be required to validate this claim.

Looking at pollen from the two collection sites together, it is interesting to note that in both locations there is a bimodal peak in pollen foraging. Pollen forage seemed to peak first between late May and early June, then again in the end of the foraging season in September. Further experimentation looking for additional evidence to support this trend is necessary; however I suspect that these peaks are due to high amounts of developing brood in the end of May into early June, and the preparation of a winter food supply in September. If this foraging pattern is consistent, it could have significant implications on colony level nutrition. This would allow individuals to determine the time periods that honey bees are most active in terms of foraging, and ensure that high quality forage is available at this time. Alternatively, this peak in pollen foraging may be due to the high amount of flowers blooming at this time.

At the UDBG, 78.14% of pollen grains were identified to some extent. The identification of pollen grains is a time consuming process that requires intense examination of grains, often looking at very subtle details of a pollen grain. As a result of this, time is a limiting factor in the identification of pollen grains, and often the features seen in a light microscope photograph do not provide enough information for proper identification to occur. Despite the difficulties in identification, some patterns were seen in the foraging behavior of honey bees. During the first few collection dates, specifically the first three on March 24th, April 1st, and April 6th, honey bees showed a clear preference towards pollen from the genus *Acer*. This is likely due to the availability of forage during these early spring months, as very few floral species are blooming at this time. In future experiments it would be beneficial to observe the different types of flowers that are blooming during this time period in order to determine if the preference for *Acer* pollen is due to active floral constancy or

passive floral constancy. Before discussing any implications this may have, it is important to determine the nutritional quality of *Acer* pollen. If it is found that *Acer* pollen is a nutritionally valuable food source with high crude protein content, it would be beneficial to promote further planting of plants within this genus. Theoretically, this would result in increased collection of *Acer* pollen, and in turn improve the development and survival of honey bee larvae in early spring. Conversely, if it is found that *Acer* pollen has low protein levels, it would be beneficial to discourage the planting of *Acer* species, in favor of a floral species with a similar bloom time and higher protein content within pollen grains.

During the next three collection dates, April 14th, 21st, and 27th, honey bees shifted away from *Acer* pollen, and began to collect pollen primarily from the genus *Prunus.* While *Acer* pollen and *Prunus* pollen arecollected during a similar time frame and are similar in terms of ornamentation, and size, they can be differentiated from one another based on the aperture type that is present. *Acer* pollen grains have colpate apertures while *Prunus* pollen grains have colporate apertures, allowing for easy distinction between the two genera. Future research comparing the nutritional value of these two genera would be beneficial due to their overlapping bloom times.

Between May 5th and June 14th, pollen foraging patterns became much more complex. This was due to the increase in the number of floral species that bloom during this time period, and the subsequent diverse samples collected by bees. With so many species in bloom, it is very difficult to identify a pollen grain to family or genus based on morphological features alone. Additional difficulty arose due to the presence of mixed samples in bee-collected pollen, where color groups contained several different species of pollen. A mixed pollen sample is difficult to identify due

to the lack of consistency between colors and species. The same constraints apply to pollen grains collected between August 4th and September 20th, however time was also a limiting factor when identifying grains collected within this time frame. In future work, it may be necessary to take pictures of pollen grains with a scanning electron microscope during these months, as this will allow for a more thorough examination of pollen grains.

Between June 23rd and July 23rd an increase in pollen identification was seen, as pollen from the genus *Trifolium* became increasingly prevalent, specifically during a three week time period including collection dates on July 1st, 8th, and 16th. During this time frame, *Trifolium* pollen accounted for 79.18% of all pollen grains collected by honey bees at UDBG. Due to the high abundance of *Trifolium* pollen collected during this time frame, pollen from this genus is an ideal candidate to analyze further in terms of nutritional content. As with *Acer* and *Prunus*, the nutritional content of pollen within this genus will help to determine if this species is beneficial to honey bee health.

At MCC, pollen identification was limited due to the high diversity seen in collected samples, and due to the limited time frame available to analyze pictures. The majority of successful pollen identification took place on the six collection dates between July 1st and September 15th. During the first three weeks of this time period, (July 1st, 8th, and 16th), pollen from the genus *Trifolium* was prevalent. This is consistent with the findings at UDBG, and supports the idea that this genus should be analyzed in terms of nutritional content. During this three week time period, pollen from the genus *Allium*, and pollen from the species *Centaurea cyanus* were also prevalent, making these species potential candidates for nutritional analysis. On July

23rd, pollen forage at MCC was focused on *Zea mays*, corn. On this date, *Zea mays* accounted for 75.22% of all pollen grains collected. In total, honey bees collected pollen from this species on five consecutive collection dates between July 8th, and August 15th, which should justify further nutritional analysis of this species. On September 15th pollen foraging was greatest in the family *Asteraceae*, as pollen from this family accounted for 75.66% of all pollen grains. At this time, far fewer flowers are in bloom as compared to the spring and summer months between April and August, so this behavior is likely due to passive floral constancy. Despite this, pollen species from this genus may hold extraordinary value, due to the timing of its collection. Because *Asteraceae* pollen is collected late in the foraging season, much of it is likely to be stored by bees for overwintering months in the form of bee bread. With this in mind, *Asteraceae* pollen may be critical to overwinter survival if it is the main source of protein for overwintering honey bees.

REFERENCES

Alcock, J. 1995. Persistent size variation in the anthophorine bee *Centris pallida* (Apidae) despite a large male mating advantage.

Al-Tikrity, W.S., Benton, A.W., Hillman, R.C., Clarke, W.W Jr. 1972. The relationship between the amount of unsealed brood in honeybee colonies; and their pollen collection. J Apic Res. 11:9-12.

Aizen, M.A., Harder, L.D. 2009. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr Biol 2009. 9:19(11):915-8.

Balfour, N.J., Garbuzov, M., Ratnieks, F. 2013. Longer tongues and swifter handling: why do more bumble bees (*Bombus spp.)* than honey bees (*Apis mellifera)* forage on lavender (*Lavandula spp.*)?

Barker, R.J., 1971. The influence of food inside the hive on pollen collection by a honey bee colony. J. Apic. Res. 10:23-26.

Buckle, G.R. 1982. Differentiation of queens and nesmate (sic) in newly established colonies of *Lasioglossum zephyrum*. Sociobiol. 7:8-20.

Chen Y, Pettis J.S, Evans J.D, Kramer M, Feldlaufer M.F. Transmission of Kashmir bee virus by the ectoparasitic mite *Varroa destructor*. Apidologie. 2004;35:441–448.

Decourtye, A., Mader, E., Desneux, N. 2010. Landscape enhancement of floral resources for honey bees in agro-ecosystems. Apidologie. 41:3:264-277.

Doull, K.M. 1966. The relative attractiveness to pollen-collecting honeybees of some different pollens. J Apic Res 5:9-14.

Downey D.L, Winston M.L. Honey bee colony mortality and productivity with single and dual infestations of parasitic mite species. Apidologie. 2001;32:567–575.

vanEngelsdorp, D., J. Hayes Jr, R. M. Underwood, and J. S. Pettis. 2010. A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. J APICULT RES. 49:7-14.

Erdtman, G., 1943. An Introduction to Pollen Analysis. Waltham Mass., 239 pp.

Erdtman, G., 1945. Pollen morphology and plant taxonomy. III. Morina L. Svensk Bot. Tidskr., 39: 187-191.

Erdtman, G., 1952. Pollen Morphology and Plant Taxonomy. Angiosperms. Almqvist and Wiksell, Stockholm, 539 pp.

Evans J.D, Pettis J.S, Hood W.M, Shimanuki H. Tracking an invasive honey bee pest: mitochondrial DNA variation in North American small hive beetles. Apidologie. 2003;34:103–109.

Fægri, K. and Iversen, J. 1964. Textbook of Pollen Analysis. Munksgaard, Copenhagen, 2nd ed., 237 pp.

Faegri, K., van der Pijl, L. 1979. The principles of pollination ecology, 3rd revised edition. Oxford, Pergamon Press.

Hansen, D.M., Olesen, J.M., Mione, T., Johnson, S.D., Muller, C.B. 2007. Coloured nectar: distribution, ecology, and evolution of an enigmatic floral trait. 82:1:83-111.

Higes M, Martin R, Meana A. *Nosema ceranae*, a new microsporidian parasite in honeybees in Euope.J. Inv. Pathol. 2006;92:93–95.

Hodges, D. 1974. The Pollen Loads of the Honey Bee. London, England. Bee Research Association.

Ingram M, Nabhan G.C, Buchmann S.L. Impending pollination crisis threatens biodiversity and agriculture. Tropinet. 1996;7:1.

Iversen, J. and Troels-Smith, J., 1950. Pollenmorphologische Definitonen und Typen. Danm. Geol. Unders., ser. 4.3(8): 1-54.

Jackson, D.D., 1928. A Glossary of Botanic Terms, 4th ed., Duckworth, 481 pp.

Klein A.M, Steffan-Dewenter I, Tscharntke T. 2003. Fruit set of highland coffee increases with the diversity of pollinating bees. Proc. R. Soc. B. 270:955–961.

Klein, A.M., Vaissiére, B.E., Cane, J.H., Ingolf, S., Cunningham, S.A., Kremen, C., Tscharntke, T. 2007. Importance of pollinators in changing landscapes for world crops. P Roy Soc B-BIOL SCI. 274:1608.

Knauer, A.C., Schiestl, F.P. 2014. Bees use honest floral signals as indicators of reward when visiting flowers. 18:2:135-143.

Kumar, S. 1975. Relations among bee size, cell size, and caste in *Lasioglossum zephyrum* (Hymenoptera, Halictidae). J. Kansas ent. Soc. 48: 374-380

Levin, M.D., Haydak, M.H. 1957. Comparative value of different pollens in the nutrition of *Osmia lignaria*. Bee World. 38:221-226.

Lindtner, P. 1981. Identification of pollen loads of honey bees in Hagley Yard, Wilmington, Delaware (Master's thesis).

Loper, G.M., Berdel, R.L. 1980. The effects of nine pollen diets on broodrearing in honey bees. Apidolog. 11:351-359.

Louveaux, J. 1954. Études sur la récolte du pollen par les abeilles. Apiculteur 98(12):43-50.

Michener, C.D. 1974. The Social Behavior of the Bees: A Comparative Study. Harvard University Press.

Micheu, S., Crailsheim, K., Leonhard B. Importance of proline and other amino acids druing honeybee flight—Apis mellifera carnica POLLMANN. Amino Acids 18(2):157-75.

Nicholson, S.W. and Thornburg, R.W. (2007) Nectar chemistry. In Nectaries and Nectar (Nicolson, S.W., Nepi, M. and Pacini, E., eds). Berlin: Springer, pp. 215–399.

Oldroyd, B.P. 2007. What's Killing American Honey Bees? PLoS Biol 5(6): e168.

Plowright, R.C., Jay, S.C. 1977. On the size determination of bumble bee castes.

Potonié, R., 1934. I. Zur Morphologie der fossilen Pollen und Sporen. Arb. Inst. Paläobotanik Petrographie Brennsteine, 4: 5-24.

Praglowski, J., Punt, W., 1973. An elucidation of the micro-reticulate structure of the exine. Grana, 13: 45-50.

Ribeiro, M.F. 1994. Growth in bumble bee larvae: Relation between development time, mass, and amount of pollen ingested. Can. J. Zool. 72:1978-85.

Roulston, T.H., Cane, J.H. 2000. Pollen nutritional content and digestibility for animals. Plant Syst. Evol. 222: 187-209.

Roulston, T.H. Cane, J.H., Buchmann, S.L. 2000. What Governs Protein Content of Pollen: Pollinator Preferences, Pollen-Pistil Interactions, or Phylogeny. Ecological Monographs: 70(4):617-643.

Stanley, R.G., Linskens, H.F. 1974. Pollen biology, biochemistry, management. Springer, Berlin/Heidenberg/New York.

Stone, D.N. 1993. Thermoregulation in four species of tropical solitary bees: the roles of size, sex and altitude. J. Comp. Physiol. B. 163:317-326.

Strohm, E. and Linsenmair, K. E. (1999). Measurement of parental investment and sex allocation in the European beewolf Philanthus triangulum F. (Hymenoptera: Sphecidae), Behavioral Ecology and Sociobiology 47, 76-88.

Teoedino, V.J., Torchio, P.F. 1982. Temporal variability in the sex ratio of a non-social bee. *Osmia lignaria propingua*: Cresson: Extrinsic determination or the tracking of an optimum? Oikos. 38:177-182.

Thorp, R.W. 200. The collection of pollen by bees. Pollen and Pollination:211-223. Springer Vienna.

Todd, F.E., Bretherick, O. 1942. The composition of pollens. J. Econ. Entomol. 35:312-317.

Willmer, P. 2011. Pollination and Floral Ecology. St. Andrews, Scotland. Princeton University Press.

Wodehouse, R.P., 1935. Pollen grains. Their structure, identification and significance in science and medicine. McGraw-Hill, New York, 574 pp.