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An Evaluation of Hibiscus moscheutos ssp. lasiocarpos and Ipomoea pandurata as host plants of the specialist bee, Ptilothrix bombiformis (Apoidea: Emphorini) and the role of floral scent chemistry in host-selection.

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AN EVALUATION OF *Hibiscus moscheutos* ssp. *lasiocarpos* AND *Ipomoea pandurata* AS HOST PLANTS OF THE SPECIALIST BEE, *Ptilothrix bombiformis* (APOIDEA: EMPHORINI) AND THE ROLE OF FLORAL SCENT CHEMISTRY IN HOST-SELECTION.

By

Melissa Simpson

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Fulfillment of the Requirements for the
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THESIS APPROVAL

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AN ABSTRACT FOR THE THESIS OF

MELISSA SIMPSON, for the Master of Science degree in Plant Biology, presented on October 15, 2009 at Southern Illinois University Carbondale.

TITLE: An Evaluation of *Hibiscus moscheutos* ssp. *lasiocarpos* and *Ipomoea pandurata* as host plants of the specialist bee, *Ptilothrix bombiformis* (Apoidea: Emphorini) and the role of floral scent chemistry in host-selection.

MAJOR PROFESSOR: Dr. Sedonia Sipes

Ptilothrix bombiformis (Hymenoptera: Apoidea) is a specialist bee belonging to the tribe Emphorini. The emphorine phylogeny suggests that Convolvulacea is the ancestral plant family and independent evolutionary host-switches to several unrelated plant families have occurred. The role of floral scent has been well-characterized in pollination systems involving moths, butterflies, bumblebees, and honeybees, but little is known about how specialist bees mediate host selection, or how host-choice evolved in specialist bees. This research investigates the role of floral scent in host selection by *P. bombiformis*. *Ptilothrix bombiformis* has traditionally been classified as a *Hibiscus* (Malvaceae) oligolege. My research shows that it can now be placed into a more detailed dietary classification as an eclectic oligolege because it also collects pure pollen loads from a distantly-related plant, *Ipomoea pandurata* (Convolvulaceae). Using dynamic headspace sampling and gas chromatography-mass spectrometry, I obtained floral chemical profiles for *Hibiscus moscheutos* ssp. *lasiocarpos* and *Ipomoea pandurata*. Both flowers contain aliphatics, aromatic compounds, monoterpenes, and sesquiterpenes.

The host flowers have 14 shared compounds in their floral scent, which may be responsible for the bees' ability to recognize and utilize *I. pandurata,* a member or the emphorine ancestral host-plant family. Some of these shared compounds are also found in other emphorine host plants and may be responsible for their constraint in host-use.

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DEDICATION

I dedicate this work to my daughter, Haley Scarlet. May you always find beauty in the small things in life.

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CHAPTER 1

INTRODUCTION

Phytophagous Insects and Plants

Plants and insects have affected each other's evolution. Ehrlich and Raven's (1964) classical model of coevolution, generated from their detailed examination of butterfly host affinities, suggests a firm evolutionary tie between herbivorous insects and plants. Their theory declares that there is natural selection for mutations in plants that produce more or superior toxic chemicals, which moves the plants into a "new adaptive zone" and allows them to escape from herbivory. There is also natural selection for mutations in insects conferring resistance to toxins, which would free them from competition and allow diversification and radiation. With coevolution *sensu* Ehrlich and Raven, the plant changes, the insect changes in response to the plant, the plant then changes in response to the insect.

Insect-plant associations are one of the most-studied species relationships in biological research (Janz et al., 2001). One of the most interesting aspects to insect-plant interactions is the variation in diet breadth displayed by the insects. Herbivorous insects range from generalist feeders to specialist feeders (Favret and Voegtlin, 2004), with host-plant specializations dominating over generalized feeding (Schoonover et al., 2007). The majority of phytophagous insects are either oligophagous, meaning they feed on a few closely related plants, for example within a single family (Favret and Voegtlin, 2004), or monophagous meaning they feed on one host species. More than 70% of phytophagous insect species specialize on one or a few chemically similar plant genera (Labandeira et

al., 2002). In contrast, polyphagous insects feed on many distantly-related host taxa. Research has shown that polyphagous insects evaluate the nutritional quality of a host plant and can make choices of which plants are most suitable for feeding or oviposition based on nutritional quality (Bernays, 2001). Insect herbivores may show variation in diet breadth to increase foraging efficiency to ensure they choose high-quality hosts in a minimal amount of time (Bernays, 2001; Strickler, 1979).

The ability of an insect to specialize is constrained by a number of factors, including insect morphology, physiology, and ecology. Specialists must have morphological features that allow them the ability to utilize the particular botanical resource they are specializing on. Specialized insects are often more efficient feeders than generalists (Bernays and Funk, 1999). Specialized insects must possess the neural ability to detect their host plant, either chemically, visually, or by tactile means. These factors are important factors underlying host selection, but do not address the more difficult question of what evolutionary reasons underlie the specialist strategy.

Why Insects Specialize

The preponderance of specialist to generalist insects creates an interesting pattern that impels scientists to examine why and how insects specialize on a particular host plant, and how these particular affinities arose through plant and insect evolution. There are several proposed and well-studied reasons why insects are specialized feeders. Some studies have shown that predator avoidance and competition may also influence host choice (Larkin et al., 2008). Host specialization may function as niche partitioning by reducing competition by feeding on different plant structures, feeding at different times of day, feeding at different times in the season, and feeding on particular plant species at

a certain life-stage in a holometabolous insect's life cycle. The host plant serves as a reliable meeting place when males are searching for mates, so specialization may increase fitness by making mates easier to find (Praz et al., 2008).

Finally, it should be noted that specialization may not be adaptive at all, but may represent an ancestral condition in some insect taxa. The ability to exploit a new host plant may be constrained by olfaction, vision, behavior, or morphology of the insect, and by the chemical, visual, physical, and other ecological attributes of potential host plants plant (Lopez-Vaamonde et al., 2003).

Evolutionary Investigations of Insect Host-Use

Molecular systematics has opened the door to a whole new approach to investigating plant-insect associations, allowing researchers to evaluate and understand the underlying mechanisms of host selection by herbivorous insects within a phylogenetic framework. Robust phylogenies are now feasible to obtain, and can be used to track insect host-use through evolutionary time, and elucidate causes of speciation, host-switching, and character evolution in insect lineages.

Species-level insect phylogenies have become an important tool for scientists to evaluate insect host use and insect speciation over evolutionary time (Jordal and Hewitt, 2004; Futuyma and Mitter, 1996; Sipes and Tepedino, 2005). Evaluating evolutionary patterns of host choice in insects requires that the following information is available: 1) host-plant associations of the insect, 2) phylogeny of the insect, and 3) phylogeny of the plants (Patiny et al., 2008).

As phylogenies have become a key component in understanding plant-insect associations, most phylogenetic examinations of phytophagous insects show that closely

related insects have a propensity to utilize the same or closely-related host plants (Futuyma and Mitter, 1996), but that in contrast to Ehrlich and Raven's (1964) model, cospeciation is rare, and insects often can switch hosts to distantly related plants (e.g. Jermy 1984; Favret and Voegtlin, 2004; Sipes and Tepedino, 2005).

Bees: Generalists vs. Specialists

Bees (superfamily Apoidea) are herbivores because their larvae are reared on pollen and other floral resources (Michener, 2000). Bees vary greatly in their biology, lifecycle, and ecological interactions. Highly eusocial bees, such as the well-known honeybee, live in perennial colonies that contain thousands of bees at any given time. A colony consists of an egg-laying queen and many sterile female workers that are responsible for foraging, brood care, and guarding the colony (Michener, 2000). Bumblebees also live in colonies with a division in labor between the queen and workers, but their colonies are annual, starting over when a new generation emerges.

In contrast to the social lifestyles of honeybees and bumblebees, most bees are solitary, meaning a single female excavates a nest, lays her egg(s), and collects pollen and nectar provisions for her larvae without any cooperation from other bees (Michener, 2000). Solitary bee nests are often found in aggregations, but each nest is occupied by a single female.

All bees depend on pollen because it provides them with all the necessary proteins, lipids, and other nutrients that are critical for their growth and survival (Dobson and Peng, 1995). In solitary bees, pollen is the principal food source for the developing larvae while adults consume it only to some extent (Dobson and Peng, 1995).

Like other phytophagous insects, the 18,000 known bee species show great variation in their diet breadth. Generalist bees, including the well-studied honeybees and bumblebees, are termed polylectic, meaning they collect pollen from a wide range of unrelated host plants, while specialist bees that feed on a few closely-related plants are termed oligolectic (Linsley, 1958). Monolecty (using just one species for pollen) is rare, and generally represents cases in which a specialist bee uses a host plant that has no sympatric relatives (Linsley, 1958); therefore, the term specialist is functionally synonymous with oligolege with respect to bees. All of the social taxa of bees are generalists, whereas solitary bees can be generalists or specialists,

The behavior of oligolectic bees differs from that of honeybees in that they only collect pollen from a subset of the pollen hosts available to them in the community. Presumably, oligolecty represents a genetically "hard-wired" trait, perhaps arising from limited neural or olfactory abilities (Praz et al., 2008). The only experience a newly emerged oligolectic bee has with the appropriate host-plant pollen source is from the pollen that was provisioned for it during larval development (Linsley, 1958). Therefore, it is also possible that conditioning during larval development may play a role in host recognition in adult specialist bees, if specific pollen chemicals present in larval food affect the adult's behavior and/or olfaction (Linsley, 1958; Dobson, 1987).

Host-Plant Detection by Bees and Other Pollinators

Bees and other insect pollinators recognize their host through visual or olfactory cues or a combination of the two (Hill, 1977), making flower color, size and shape, as well as the strength of volatile emissions important to the perception of their pollinators (Kevan, 2005). At any stage in host plant selection, visual, tactile, olfactory, and

gustatory stimuli may act in cooperation, with the relative significance of each depending on each specific plant-insect interaction (Dobson and Bergstrom, 1999). Wright et al. (2004) found that honeybees were able to discriminate among the scents of different cultivars of snapdragons. Research shows that honeybees and lepidopterans use floral scent to recognize food-rewarding flowers during foraging (Anderson and Dobson, 2003; Wright et al., 2004). For bees, color is the main stimulus used at a distance, but olfactory stimuli become increasingly important at closer range, allowing discrimination between plant species (Dobson et al., 1996; Majetic et al., 2007). In naïve honeybees and bumblebees, odors are learned more rapidly and with greater retention than colors (Dobson and Bergstrom, 1999), but experienced honeybees seem to rely less on floral aroma and more on visual cues during foraging (Dobson, 1987).

Recent studies have shown that visual and olfactory cues function synergistically to attract insect pollinators (Majetic et al., 2007). Floral signals can include the size and shape of the flower, spectral (including UV) reflectance, or a suite of chemicals given off by particular plant taxa (Bernays, 2001). Learning of visual cues during foraging increases the bees' discrimination between plant species. Although visual cues are less species-specific, they tend to be more consistent than floral odors, which tend to fluctuate due to environmental influences and the aging of the flower (Dobson, 1987). Alternatively, some flowers may appear similar at long distances, but can be differentiated at close range by their unique floral scent (Roy and Raguso, 1996). Specifically, odors from the pollen can influence bee foraging by providing guidance to the pollen source, discriminating flowers with different amounts of pollen, and host-plant recognition by specialist bees (Dobson and Bergstrom, 1999).

The Role of Floral Scent in Host Detection

Studies of floral scents are important to better understand the chemical basis of plant-animal interactions and pollination ecology (Flamini et al., 2003). The ability of insects to discriminate among scents may depend on both, the intensity and the ratios of specific volatiles, as well as the unique mixture of all the volatiles together (Wright et al., 2004; Dudareva and Pichersky, 2000). Floral fragrances vary greatly among species in terms of the number and amount of volatile compounds present (Dudareva and Pichersky, 2000). Investigations using dynamic headspace sampling, coupled with GC-MS, have revealed that most floral scents are a complex mixture of small (100-250 D) volatile molecules containing fatty-acid derivatives, terpenoids, nitrogen-containing compounds and sulfur-containing compounds, that are dominated by sesquiterpenoid, phenylpropanoid, and benzoid compounds (Dudareva and Pichersky, 2000; Dobson et al., 2005). All parts of a flower produce volatiles, especially the petals, but in some species, the stamens or carpels make significant contributions to the overall floral scent (Dobson et al., 2005). Pollen volatiles have been implicated in the host-plant selection of several pollen-feeding insects, with most evidence coming from the studies of honeybees and bumblebees (Dobson and Bergstrom, 2000; Linsley, 1958). In contrast, Dobson (1988) found that plants that rely on animals seeking nectar, rather than pollen, as is the case with hummingbirds and Lepidopterans, tend to have a pollenkitt (the oily coating on pollen grains) with relatively few lipids, therefore less conspicuous chemical cues than bee-pollinated flowers. This may possibly be because bees collect both nectar and pollen, so it is imperative that both floral resources have the ability to attract their pollinators. Dynamic headspace sampling of 17 different hummingbird-pollinated

species revealed the flowers to be truly scentless, supporting the common opinion that odor plays no role in bird-pollinated flowers (Knudsen et al., 2004).

Evolutionary History of Host Choice in Bees

Phylogenetic studies have shown that polylecty in bees can arise from oligolecty (Müller et al., 1996; Larkin et al., 2008). In fact, a robust family-level phylogeny of all bees shows there is a basal assemblage of mostly-oligolectic taxa, suggesting that oligolecty may be the ancestral condition for the bees (Danforth et al., 2006). Oligolectic bees may be neurologically or chemically constrained to the hosts they can use, thus sometimes preventing them from evolving to a generalist diet (Sedivy et al., 2008). These neurological constraints may explain why, when host-switching does occur, it is often to related plants that may be similar in morphology, color, and/or chemistry (Sedivy et al., 2008). Oligolectic species may have relatives that feed on distantly-related plant families (Sipes and Wolf, 2000; Sipes and Tepedino, 2005), but any given species may be restricted in how it can exploit the resource (Wcislo and Cane, 1996). In order to evaluate how and why host-switching may occur, it is important to understand and identify what chemical and visual signals are used to attract a pollinator to potential host-plants.

Host Selection and Host-Use of *Ptilothrix bombiformis*

Although the role of floral scent and visual cues has been well-characterized in pollination systems involving moths, butterflies, bumblebees, and honeybees, surprisingly little is known about how specialist bees mediate host selection, or how host-choice evolved in specialist bees.

Ptilothrix bombiformis (Hymenoptera: Apoidea) is a specialist bee belonging to the tribe Emphorini (Michener, 2000). It is a solitary ground-nesting bee that is considered oligolectic, meaning it only collects pollen from a few closely-related plants (Linsley, 1958; Sipes and Tepedino, 2005). Phylogenetic studies indicate that 1) Emphorini is monophyletic and 2) Convolvulaceae is their ancestral host plant family (Sipes and Wolf, 2001; Sipes and Tepedino, 2005). Through evolutionary time, some *Ptilothrix* species radiated onto other host plants, including hosts in Malvaceae (Sipes and Tepedino, 2005). The tribe Emphorini has undergone independent evolutionary host switching to taxa in the same 5 or so plant families (Figure 1), but no research has been done to determine the proximate factors involved in host choice of these bees. A comparative analysis of floral volatiles in this bee's host plant may improve our understanding of how host choice has evolved in these bees.

Ptilothrix bombiformis and its primary host plant, *Hibiscus moscheutos*, are both native to southern Illinois. Here and elsewhere in its range, *P. bombiformis* has been observed also visiting flowers of *Ipomoea pandurata*, a taxon that is used by other emphorines (e.g. *Melitoma* spp.) and that is in the same plant family as the proposed ancestral emphorine host. Moreover, the resemblance of these particular species of *Hibiscus* and *Ipomoea* flowers to one another was so striking that it intrigued me to investigate why the specialist bees would deviate from *Hibiscus* and also examine the *Ipomoea* flowers.

The objectives of my project are to: 1) quantify pollen host preferences of *P. bombiformis* using behavioral data and scopal pollen analysis 2) obtain and compare chemical profiles for the floral scents of *Hibiscus moscheutos* ssp. *lasiocarpos* and

Ipomoea pandurata in order to evaluate the potential role of scent in *P. bombiformis* host recognition. My hypotheses are: 1) *P. bombiformis* uses both plants for floral resources and 2) *Hibiscus moscheutos* ssp. *lasiocarpos* and *Ipomoea pandurata* share some sesquiterpene compounds in their floral scent.

I hypothesize that both plants may be used as pollen and nectar hosts and that these two plants may have a similar chemical composition for several reasons. Emphorini has a convolvulous ancestral host plant. *Ptilothrix* and at least three other emphorine bee taxa have switched independently to malvaceous hosts. If specialist bees have narrowly-limited abilities to recognize host plants, then evolutionary host-switching events may be constrained by host chemistry. Therefore, these plants may share chemical attributes that facilitated the evolutionary host switches. Sesquiterpenes dominate the floral scent of bee-pollinated flowers, therefore making it likely that these compounds may be responsible for attracting this specialist pollinator.

CHAPTER 2

MATERIALS AND METHODS

Study System

The host-pollinator relationship of interest for this study is *Hibiscus moscheutos* ssp. *lasiocarpos* (Malvaceae) and its specialist bee *Ptilothrix bombiformis*. I have observed *P. bombiformis* utilizing floral resources from *Ipomoea pandurata* (Convolvulaceae), which is not the preferred host plant for *P. bombiformis*, but is closely related to hosts of other emphorine bees. I examined one potentially important aspect of host selection in this system: floral chemistry.

Pollination ecology literature documents that flower size, shape, scent, color, motion, and pattern correspond to the sensory capabilities of pollinators (Guldberg and Atsatt, 1975). Both *H. moscheutos* and *I. pandurata* look similar to the human eye in terms of their size, shape, color, and pattern, which leads me to believe that they are perceived as visually similar by the bees. However, the role of floral scent in host selection by *P. bombiformis* is unknown. A chemical profile is needed for both plants to determine what floral scent compounds could potentially attract this *Hibiscus* specialist to both flowers. Qualitative comparison of the plants' floral odors, together with information from the chemical ecology literature, may highlight specific compounds or combinations of compounds that may be important in bees' host plant selection.

The Host Plants

Hibiscus moscheutos is a self-compatible herbaceous perennial native to fresh and brackish marshes of the eastern United States (Snow and Spira, 1993). Individual plants produce many shoots that grow 1-2 meters tall that emerge from a large, woody

rootstock each spring (Snow and Spira, 1993; 1995). The plants are in bloom July through September (Mohlenbrock, 2002) and individual flowers are only open for a single day (Snow and Spira, 1991). Anthesis occurs in early morning and flowers generally close by late afternoon or evening (Spira, 1989). Flowers are l0-15 cm in diameter with a corolla that is usually white or pale pink with a conspicuous red nectar guide at their base (Spira, 1989).

Ipomoea pandurata is a herbaceous perennial vine commonly found along roadsides, fence rows, fields thickets, and disturbed areas (Mohlenbrock, 2002; Stucky and Beckmann, 1982) and blooms June through October (Mohlenbrock, 2002). According to the USDA NRCS Plants Database, this plant's range includes southern Ontario south to the Gulf Coast and the Atlantic Ocean west to Nebraska. The USDA lists it as a state threatened plant in Michigan and a state endangered plant in New York. Its flowers are erect, funnelform corollas up to 8cm long and 10 cm broad at the apex, supported on stout, ascending peduncles that exceed the petioles. The corolla is white with a maroon center (Stucky and Beckman, 1982). Stucky and Beckman (1982) found that anthesis occurred between 2:00 AM and 4:00 AM and the flowers begin to wilt and collapse 6-8 hours later.

The Specialist Bee

Ptilothrix bombiformis (Cresson) is a solitary, specialist bee belonging to the tribe Emphorini. All emphorine species for which host-plant affinities have been studied, including *Ptilothrix* species, are specialists, typically collecting pollen from a single host genus or several related genera. Solitary bees are most diverse in warm, arid climates and usually nest in the ground (Linsley, 1958). Female *Ptilothrix* collect water to

moisten the hard-packed soil while excavating burrows (Linsley et al., 1956; Michener, 2000). Emphorine bees place their egg on the ventral surface of a dry, convex pollen mass that is used to nourish the larvae during development (Rust, 1980). The emphorine larva is unusually elongate and curls around the pollen mass, eating its way around. When feeding is complete, the larva deposits a layer of fecal material that covers the entire interior of the cell, and then covers itself in a cocoon. The layer of feces appears as a layer of pollen exines without recognizable fecal pellets, which is a unique emphorine characteristic (Michener, 2000).

Ptilothrix (Smith) is an amphitropical genus (Michener, 2000). Its North American range extends from New Jersey to Kansas, south to Florida, Texas, and Arizona, USA and to Oaxaca, Mexico (Michener, 2000). Its South American range extends from Para, Brazil, south to Bolivia, Paraguay, and Cordoba and Entre Rios provinces, Argentina (Michener, 2000). The only species of eastern North America, *Ptilothrix bombiformis* (Cresson), is an oligolectic visitor of *Hibiscus* (Michener, 2000). It nests in hard-packed roadways and levees in close proximity to water sources (Rust, 1980). Females alight on the water's surface to collect water that is used to soften the soil while excavating her nest (Linsley et al., 1980; Rust, 1980; Michener, 2000). Nests are vertical and usually one or two-celled and each cell contains an egg and a pollen provision (Rust, 1980). The rapid-developing larvae continually move around the pollen mass while feeding and over-winters as a post-defecating larvae (Linsley et al, 1956; Rust, 1980). Female *Ptilothrix* construct and provision several nests during one season (Rust, 1980). Mating behavior involves male bees resting in *Hibiscus* flowers, waiting

for foraging females, and males will also fly from blossom to blossom in search of a female.

Study Sites

My primary study sites were in the Shawnee National Forest in Oakwood Bottoms Greentree Reservoir and Gorham Tract in Jackson County, southwest of Carbondale, Illinois (Table 1). I collected additional females for scopal pollen analysis at three additional sites along Crab Orchard Lake where *H. moscheutos* grew along the shoreline.

Populations of *H. moscheutos* and *I. pandurata* occur along roadsides and flooded ditches in Gorham Tract and the Big Muddy levee in Oakwood Bottoms. *H. moscheutos ssp. lasiocarpos* is prevalent throughout Oakwood Bottoms along ditches and also in annually flooded levied parcels. Both sites are part of the Mississippi River and Big Muddy River floodplains and are predominantly bottomland hardwood forests due to their close proximity to both rivers and the presence of a high water table. These site conditions provide little drainage relief and there is often standing water present throughout much of the growing season. Oakwood Bottoms is an oak-hickory dominated ecosystem, with willow (*Salix* spp.) and eastern cottonwood (*Populus deltoides*) established along the Big Muddy River. Tall fescue (*Schedonorus phoenix*) and Johnson grass (*Sorghum halepense*) are the dominant grasses along the levees and I have identified 33 different forbs in my study sites (Table 2). I collected and pressed all the flowering species that were present and blooming in my field sites and a voucher of each species is catalogued in the Southern Illinois University Carbondale Herbarium in the Department of Plant Biology.

These bottomland forests were drained by a series of channels in the early 1900s and were in intensive agriculture until it was bought by the federal government as part of the National Forest System. Oakwood Bottoms was acquired by the U.S. Forest Service in the 1930s and has been managed since 1964 as a public waterfowl hunting area (Phillipe, 1995). The reservoir site is flooded during the fall to provide waterfowl habitat and is drained before the onset of the growing season. Because the Big Muddy River levee prevents natural flooding of this site, flooding is accomplished by pumping water from the Big Muddy River into the managed units.

Quantifying Host Preference of *P. bombiformis*

I quantified host preference of *P. bombiformis* in two ways: 1) by observing foraging choices of both females and males at a site where both host plants were present and 2) by identifying pollen collected by foraging females. Most observation and collection efforts were focused on the morning and early afternoon, as host flowers of both species are closed by mid-afternoon (Spira, 1989; Stucky and Beckman, 1982). I observed and collected floral visitors from July 19, 2008 through September 25, 2008. *P. bombiformis* was easily distinguishable in the field from other large robust bees visiting *H. mosheutos* (*Bombus* spp. and *Xylocopa virginica*). Males and females of *P. bombiformis* were distinguishable in the field because males are less robust than females and lack the easily-visible scopal hairs of females.

Visitation rates were observed at the Big Muddy Levee site because there were large populations of both plants growing intermingled with each other, therefore this site provided the potential for *Ptilothrix* to choose either plant species for floral rewards. Foraging bees were observed for 30 minute periods by one to three observers. A single

bee was followed for as long as possible and the number and species of flowers it visited was recorded. Because *Ptilothrix* was very abundant at this site and distinguishing individual bees with certainty was not feasible, each bee encountered was assumed to be a new individual. Observations were recorded for 55 individuals.

I also experimented with *Hibiscus syriacus* (Rose of Sharon) to see if *Ptilothrix* reacted differently to a cultivated host when it was presented along with its natural host, *H. moscheutos*. *H. syriacus* flowers are light pink with a red center or white with a red center (both colors occurring on one bush) and have a slightly smaller diameter than *H. moscheutos*. I placed picked *H. moscheutos* flowers alongside *H. syriacus* flowers in a *H. syriacus* bush. I also picked and placed *H. syriacus* flowers to serve as a control. I recorded visits to all the flowers within my field of view for a total of 4 man-hours. I also placed picked *H. syriacus* flowers in with naturally-occuring *H. moscheutos* (Figure 3). I also picked and placed *H. moscheutos* flowers to serve as a control. I recorded visits to all flowers in my field of view for 1 man-hour.

I opportunistically collected foraging bees using insect nets and euthanized them in cyanide kill jars. I collected a total of 56 pollen-bearing female *P. bombiformis* that were foraging both *H. moscheutos* ssp. *lasiocarpos* and *I. pandurata*. Insect specimens were databased and deposited in the Southern Illinois Pollinator Collection, Department of Plant Biology, Sipes Laboratory, at Southern Illinois University Carbondale in Carbondale, Illinois 62901.

Using a clean insect pin, I removed pollen from within the tibial scopal hairs of one leg, leaving the other scopal load intact for future research needs. I scraped the pollen onto a clean microscope slide and placed one drop of 70% ethanol onto the slide to

remove some of the oily pollenkit, making the exine morphology more distinguishable. I mounted the pollen in glycerine jelly containing light basic fuchsin stain (Beattie, 1971). I melted the jelly using low heat and covered it with a cover slip. I analyzed pollen using an Olympus BX40 light microscope at 100x magnification (10 x 10). I characterized pollen by type, based on exine morphology and size, and compared them to reference slides of pollen from both study species and also other plants present in my study site. The *Hibiscus* pollen grains were 172.5 ± 7.9 µm in diameter, whereas *Ipomoea* pollen grains were 99.0 ± 7.2 µm in diameter, making them easily distinguishable under a light microscope. Also, all other pollen grains present in the samples were much smaller and shaped differently. I categorized the pollen as *Hibiscus*, *Ipomoea*, or other and identified all of the grains on the slide. The loads were classified as pure if they contained ≥ 90% a single species. A species was considered contamination if it was present in ≤ 10% of the sample.

Whole Floral Scent Collection

I collected floral volatiles using dynamic headspace sampling technique (Figure 4). Dynamic headspace sampling was ideal because it allowed me to collect scent from intact inflorescences as it was emitted (Ashman et al., 2005). This method causes no damage to intact flowers, which is important because injury to a plant can cause a change in the emission profile due to the release of defensive volatiles at the site of injury (Dudareva and Pichersky, 2000). I followed methods for dynamic headspace sampling described by Raguso and Pellmyr (1998), who proposed a standardized method of floral scent analysis based on comparisons of different trap sorbents, elution solvents, and flow rates. The results of their experiments revealed that Porapak Q and hexane outperformed

other sorbents and solvents, respectively, in quantitative aspects of floral scent trapping and elution.

I collected floral scent in the mornings and early afternoon before the flowers closed in mid-afternoon, from July 28- August 29, 2008. I collected scent directly from living flowers at 6 locations within Oakwood Bottoms and Gorham Tract. I collected floral scent from 10 *H. moscheutos* ssp. *lasiocarpos* and 10 *I. pandurata.* I took control samples from an empty polyvinylacetate bag that I placed in my collection sites. I also collected scent from a single leaf of each plant species, which I used as controls to identify any vegetative volatiles. For floral scent collection, I covered a single flower with a polyvinylacetate bag (Reynolds, Alcoa Consumer Products) and secured the bag tightly with a plastic tie. These bags were determined by Raguso (1998) to produce the fewest artificial volatiles while, at the same time, being very economical. To reduce the amount of plastic volatiles released from the bags, the bags were oven-baked at 300°C for ten minutes. During sampling, no foliage was included in the bag. I cut a small slit in the bag to create an opening for the scent collection trap. The scent trap was constructed from a glass Pasteur pipette packed with 10 mg of Super Q adsorbent (80/100 mesh size, Alltech Associates, Deerfiled, Illinois, USA) between 2 plugs of glass wool. I inserted the pointed end of the pipette into the plastic tubing connected to the vacuum pump (PAS-500 Micro Air Sampler, 40-200 cc/min, Supelco, Bellefonte, Pennsylvania, USA), then inserted the other end into the slit in the plastic bag and secured the pipette with a plastic tie. I attached the pump to a tripod to adjust it to the height of the flower, if needed.

I collected scent for a range of times (1.5 hrs, 1.75 hrs, 2 hrs, 2.5 hrs, 3 hrs, 4 hrs, 4.5 hrs, and 6 hrs). If the collection time was too brief, compounds in small amounts may not show up in analysis. If collection time was too long, the scent trap may saturate and bleed off compounds that are produced in copious amounts. Although this collection method has few replicates per collection time, the range of collection times may overall allow for more compounds to be collected from the floral headspace. This method likely increases among-sample variation, but minimizes the chances of missing floral scent components across all samples. When collection was complete, I stored the scent traps individually in polyvinylacetate bags and kept them in a small cooler on icepacks while I transported them back to the laboratory. In the laboratory, I eluted the samples with 3 mL of hexane and stored them in glass vials at -80° C until they could be analyzed by gas chromatography-mass spectrometry.

Floral Scent Analysis

Gas chromatography-mass spectrometry (GC-MS) is the premier analytical technique used for the separation and identification of volatile compounds (McNair and Miller, 1997). GC-MS provides both qualitative and quantitative identification of unknown compounds (McNair and Miller, 1997). The technique for identification of floral volatiles using gas chromatography-mass spectrometry follows that described by Adams (2007).

To prepare the samples for GC-MS analysis, I concentrated my scent samples to approximately 200 µL using a flow of nitrogen gas to evaporate the hexane. Once the samples were concentrated, I pipetted the 200 µL sample into silanized polyspring inserts within the glass GC-MS vials and securely sealed the caps.

I obtained mass spectra on a Saturn 2100T mass spectrometer, coupled directly to a Varian 3900 gas chromatograph, fitted with a J&W DB-5, 30 m x 0.25 mm, 0.25 µm coating thickness, fused silica capillary column. The GC-MS was operated using Adam's Method (Adams, 2007): injection temperature of 220°C, transfer line of 240°C, the oven temperature at 60-246°C at 3°C/min, using Helium as a carrier gas (34.96 cm/sec or 1.02 mL/min at 210°C). The single injection contained 2 µL of sample using a split ratio of 1:20.

Volatiles from 10 individuals of each host species were identified using published databases of mass spectra and retention times of known chemicals. The software used to analyze the GC-MS output was the Varian MS Workstation with the NIST Spectral Database and Adams Library of Flavors and Oils Retention Times. I searched each individual chromatogram by extraction of fragment ions that are characteristic of monoterpenes (m/z 77, 79, 93, 121, 136) and sesquiterpenes (m/z 161, 204). These ion searches allowed for clearer examination of the chromatogram by removing common contamination peaks and showing possible floral compound peaks. I used the peaks found in the ion searches to determine peak area of each compound. In my analysis, I excluded compounds that were present at similar abundance in the ambient and vegetative controls and considered them to be contaminants from the plastic bag, the collection apparatus, other floral parts, or the surrounding vegetation. To determine the identity of a compound, I 1) compared the retention time of my compound to known retention times and 2) compared the mass spectra to mass spectra of compounds whose retention times were close to the retention time of my given chemical. Using retention

time and mass spectra, I determined the identity of all the compounds detected in my floral scent samples.

Data Analyses

In order to assess host plant preferences, I calculated the proportion of each plant species, *H. moscheutos ssp. lasiocarpos* and *I. pandurata*, present in the scopal pollen loads from 56 individual *P. bombiformis* collected at my study sites. Each pollen load was characterized as "pure" *H. moscheutos*, "pure" *I. pandurata*, or mixed loads. Following the designations of Sipes and Tepedino (2006), scopal loads were considered pure if they were 90% or more one taxon; this cutoff allows for various sources of contamination (unintentional incorporation of pollen from nectar hosts, contamination from common kill vials, etc). Bees were collected while foraging from both plant species. In order to establish whether or not *Ipomoea* is a pollen host for *P. bombiformis* (as opposed to only a nectar host) I compared the loads of bees collected from *H. moscheutos* and bees collected from *I. pandurata*. If *I. pandurata* is only being used as a nectar source, then both groups of bees would be expected to have predominantly *H. moscheutos* pollen. Using SAS Institute Inc. © version 9.1 statistical software, I ran a one-way ANOVA (analysis of variance) to determine if there was a difference in the proportion of the scopal load that was *Hibiscus* pollen between bees collected from *Hibiscus* versus bees collected from *Ipomoea*. I did not include the "other" pollen category in statistical analysis because it is considered contamination in most cases. The proportions of pollen were arcsin transformed to improve normality and $\alpha=0.05$.

Behavioral observations for host plant visitation were recorded for 14 females and 41 males. The number of visits to *H. moscheutos ssp. lasiocarpos* and *I. pandurata* were

recorded for each bee observed. I used a Student's t-test to determine visitation rates for both males and females to see if they preferred *Hibiscus* over *Ipomoea*. The number of visits to each species was square root transformed to improve normality and $\alpha=0.05$.

I compared the floral scents of both species two ways: I used Chi-Square tests to test for presence/absence differences for individual chemicals, and I also used a MANOVA (multivariate analysis of variance) to examine quantitative differences among chemical groups. The individual chemicals present in both host flowers were tested as present or absent in all of the 10 flowers of each species. This presence/absence data was analyzed in SAS 9.1 using a Chi-Square test to determine if any individual chemicals were found in significantly higher amounts in one flower species versus the other. Due to the high number of tests performed (41), I used a Bonferroni adjustment, making $\alpha=0.001$ ($\alpha=0.05/41$).

To perform the multivariate analysis, I grouped the chemicals into their chemical classes: aliphatics, aromatics, monterpenes, and sesquiterpenes. This grouping was necessary because the high variation in the individual chemicals. For each individual flower, I calculated the percentage each chemical class represented in the total floral scent. I then changed the percentages into proportions, and then proportions to log ratios. The log ratios take care of two problems: 1) the lack of normality in proportions and 2) the fact that proportions sum to one and are therefore a highly dependent set of variables. Using SAS 9.1, I ran MANOVA (multivariate analysis of variance) to determine if there was a difference in the proportion of chemical classes present in the floral scent of the two host species. The chemical class proportions were log transformed to improve normality and $\alpha=0.05$.

CHAPTER 3

RESULTS

Floral Visitors

All of the following field observations were taken in my 2008 field season. *I. pandurata* began blooming 2 weeks before *H. moscheutos*, but both species bloomed concurrently in late July. Male *Ptilothrix* were first observed on July 19 and females on August 9. These observations are typical of solitary bees; male bees emerge from nests before female bees because male eggs are laid last, nearest the entrance to the nest cell.

During the period without females, male *Ptilothrix* were observed actively patrolling *Hibiscus* flowers in search of females. The patrolling flights were distinguished from nectaring visits (Figure 5) because the male bees do not stop at the flower, they only pause their flight a few centimeters from the flower (Praz et al., 2008). Male *Ptilothrix* would patrol mixed patches of *Hibiscus* and *Ipomoea* and stop to drink nectar in both flowers. Males would patrol the same group of *Hibiscus* and fly by each open flower in the same pattern every time it made a round-trip. Males displayed aggressive behavior towards other males who would fly near or land on *Hibiscus* flowers they were repeatedly patrolling. This aggressive behavior included chasing the other away or physically fighting on the flower's petals until they both fell off. This aggressive, territorial behavior was common among the males in *Hibiscus* patches, but they seemed amicable when they were drinking nectar from *Ipomoea*. Often, when the temperature was high and the skies were clear and sunny, males would rest in the *Hibiscus* flowers. Bumblebees were also very abundant on *Ipomoea* and didn't seem to cause conflict with *Ptilothrix* males.

Female *Ptilothrix* would drink and collect pollen from *Hibiscus* and I observed them entering *Ipomoea* to drink nectar and possibly collect pollen, although I never saw them actively collecting *Ipomoea* pollen. When a female landed on a *Hibiscus* that was already occupied by another female, it would fly away to another unoccupied flower without showing any aggressive behavior.

Other floral visitors were found on both species of flowers. *Xylocopa, Bombus,* and *Melissodes* spp. were observed visiting both flowers, but were found much more often on *Ipomoea* than *Hibiscus*. Other minor floral visitors included other solitary bees, honey bees, flies, wasps, katydids, Japanese beetles, weevils, and butterflies. All of the floral visitors that were captured are deposited in the Southern Illinois Pollinator Collection, Department of Plant Biology, Sipes Laboratory, at Southern Illinois University Carbondale in Carbondale, Illinois 62901.

Nesting Behavior

Females were initially observed displaying nesting behavior on August 16. Females at the nesting site would fly very low to the ground and fly in a zig-zag or circling pattern in search of their nest or a site to build a nest. They had no scopal pollen loads, indicating that they were constructing their nests and had not yet begun provisioning their nests. Nests were found in aggregations with a 2-3 cm turret built around the entrance. The only nesting site I found contained approximately 20 nests and was located in a dirt and gravel parking lot that was approximately 5-10 meters from a small pond. The nests were built in hard-packed soil with sparse vegetation (Figure 6). Heavy rainfall on August 23 destroyed any evidence of nests I previously flagged and one small aggregation was completely inundated. On August 28, I saw 7 nests reappear

once the ground had dried. The nesting site experienced heavy rainfall on September 4 and 5, causing some turrets to collapse into the nest entrance. I then counted 17 nests. Nests were still present on September 11, but no females were seen flying and there were only a few *Hibiscus* still blooming at the nesting site. On September 18, all *Hibiscus* had dehiscent fruits and no flowers remained. I observed no bee activity and only saw 3 nests.

I observed one female land on the ground, moisten the soil with water from her scopal hairs, and begin to dig a small divot out of the soil with her front legs. She dug for 3 minutes before flying away. I watched the same spot for 30 minutes hoping she would return to continue the excavation, but she never returned.

Host Preference

I observed that when *Hibiscus moscheutos* flowers were picked and placed among the flowers of *Hibiscus syriacus* (Rose of Sharon), *Ptilothrix* would visit the picked *H. moscheutos* flowers just as often as they would intact *H. syriacus* and they ignored the picked *H. syricacus*. When the picked *H. syriacus* flowers were placed next to the intact and picked *H. moscheutos* flowers (Figure 3), they largely ignored the *H. syriacus* flowers and visited *H. moscheutos*, whether it was picked or intact. I also place large, red *Hibiscus* cultivars in with native *H. moscheutos* and the red flowers were completely ignored.

Using a paired *t*-test, there was a highly significant difference in female *Ptilothrix* preference for *Hibiscus* over *Ipomoea* ($P < 0.001$). There was no difference in male preference for either flower ($P = 0.118$) (Table 3).

There was a highly significant difference in the amount of *Hibiscus* pollen present in the scopal loads of female bees collected from *Hibiscus* versus bees collected from *Ipomoea* (F= 112.95, df=1,54 and $P < 0.0001$). The mean proportion of *Hibiscus* pollen present in the scopal load of the bees collected on *Hibiscus* was 0.959 ± .086 and the mean proportion of *Hibiscus* pollen present in the scopal load of the bees collected on *Ipomoea* was 0.363 ± 0.335 (Figure 7).

Floral Scent Composition

There were a total of 38 chemicals detected from the floral headspace of *H. moscheutos ssp. lasiocarpos* (Table 4) and 26 from *I. pandurata* (Table 5). The chemicals represent four chemical classes: aliphatics, aromatics, monterpenes, and sesquiterpenes. *H. moscheutos* and *I. pandurata* floral scents have 14 chemicals in common (Table 6, Figure 8), most of which were sesquiterpenes. *Para*-cymene, which was found in *Ipomoea*, is a monoterpene-derivative, but I listed it in the monoterpene group so it was comparable to the compound classes in *Hibiscus*, thus fitting into the chemical classes used for statistical analysis.

For all of the individual chemicals present in both flowers, only 6 were found in significantly different amounts (Table 7) at α= .05. After a Bonferroni correction where α= .001, only two chemicals, β-ocimene from *Hibiscus* and 4-ethyl-benzaldehyde from *Ipomoea* were found in significantly different amounts.

There was a highly significant difference in the proportion of chemical classes present in the whole-flower scent of both species (MANOVA *Wilks' Lambda* F= 26.49, df= 3,16, $P < 0.0001$) (Figure 9). The *Hibiscus* floral scent was dominated by sesquiterpenes, with monoterpenes, aliphatics, and aromatics found in nearly equal

amounts. The floral scent of *Ipomoea* had sesquiterpenes and aromatics in nearly equal amounts, followed closely by aliphatics comprising most of the remaining scent. Monoterpenes only accounted for 1% of the total floral scent in *Ipomoea*, where it is the second most common chemical constituent group in *Hibiscus*.

CHAPTER 4

DISCUSSION

Host-Use of *Ptilothrix bombiformis*

Like other emphorines, *Ptilothrix* species are restricted in their pollen host use. Of the species of *Ptilothrix* for which host-use has been documented, *P. plumata* is narrowly oligolectic on a few species in Malvaceae (Schlindwein et al.2009), *P. relata* is narrowly polylectic (Tellería, 2003), *P. tricolor* is oligolectic on Cactaceae (Díaz and Cocucci, 2003), and *P. fructifera* is oligolectic on *Opuntia* (Schlindwein and Wittmann, 1997).

Ptilothrix bombiformis is an eclectic oligolege because it collects pollen from a few fixed genera of plants belonging to different families (Cane and Sipes, 2006). Previously, *Ptilothrix bombiformis* has been classified as oligolectic on *Hibiscus* (Malvaceae) (Michener, 2000), but evidence from my study shows that *P. bombiformis* also utilizes *Ipomoea pandurata* as an alternate pollen source. This evidence reveals that *P. bombiformis* has a broader diet breadth than previously known, lending itself to be placed under a more specific dietary classification as an eclectic oligolege.

P. bombiformis has been documented as a rare visitor to *Ipomoea purpurea*, a smaller, purple convolvulous flower, but it was not documented whether the visit was for nectaring or gathering pollen, or which sex visited the flowers (Galetto and Bernardello, 2004). My study documents male *P. bombiformis* nectaring and females both nectaring and collecting pollen from *I. pandurata*. Male bees nectar for themselves but don't collect pollen provisions, so males of oligolectic species may not be as closely associated with the pollen host plant. The data show no significant difference on male preference

for *Hibiscus* vs. *Ipomoea* when nectaring, but females do prefer *Hibiscus* over *Ipomoea* as a pollen host.

Overall, female *P. bombiformis* do significantly prefer *Hibiscus* over *Ipomoea* as a pollen host, but I found some evidence that there may be individual variation in host preference within a given population. The variability in pollen host use was revealed during the scopal pollen analysis. Oligolectic bees should have pure pollen loads comprised of at least 90% a single flower species (Cane and Sipes, 2006). For the bees collected on *Hibiscus*, 69% had pure *Hibiscus* pollen loads while the remaining bees had mixed loads of *Hibiscus* and *Ipomoea*. The bees collected on *Ipomoea* had mostly mixed loads of *Ipomoea* and *Hibiscus*, but 9% had pure *Hibiscus* pollen loads while 36% had pure *Ipomoea* pollen loads. It was surprising that a bee previously described as a *Hibiscus* oligolege actually had more pure loads of *Ipomoea* than *Hibiscus* when the bee was collected from the non-preferred host flower. These bees were collected in mixed stands of *Hibiscus* and *Ipomoea* and thus had equal opportunity to forage for pollen from both plants species. Yet, some bees seemed to overlook their host plant and preferentially collect pollen from a non-host, *I. pandurata*.

There are several hypotheses as to what factors are responsible for specialization in bees. Some authors suggest that larval imprinting in responsible for adult feeding preferences, meaning adult insects will seek out the pollen they were reared on as larvae. However, this hypothesis does not readily explain why host preferences are generally a species-level characteristic in oligolectic bees. There are more recent suggestions that host-choice is genetically controlled. Praz et al.(2008) found that when the specialist bee, *Heriades truncorum* (Megachilidae) was reared on non-host pollen, adult females still

only collected pollen from their preferred host plant and adult males exclusively patrolled the host flowers. This strongly suggests a genetic basis rather than larval imprinting as controlling host recognition in this specialist bee.

In general, some pollinators may have evolved an inherent preference for a particular host, but learning during foraging allows flexibility in host-use (Shiestl and Schlüter, 2009). Adult learning during foraging is well documented for honeybees and bumbles and other pollinators, but there are only a few studies demonstrating learning in specialist bees. Dötterl et al. (2005) studied a *Salix* specialist, *Andrena vaga*. By conducting electroantennogram studies, they found that foraging-naïve bees could recognized pollen-specific odors, but experienced adults relied on the whole-flower odor blends that they learned through foraging experiences. Dobson and Bergstrom (2002) demonstrate that *Chelostoma florisomne,* a *Ranunculus* specialist, can recognize pollen odors when it emerges from its nest, but also learns the whole-flower scent of its host plant during foraging bouts and depends upon this for host recognition as an experienced adult.

As discussed above, experimental evidence suggests that there is a genetic basis to host-use in specialists (Praz et al., 2008), imprinting may play a role for host recognition in foraging-naïve bees, and there may also be a learning component. As for *Ptilothrix bombiformis*, I propose that there is a genetic component to host-recognition due to the fact that its host use of *Hibiscus* is consistent throughout its range in the eastern United States (Michener, 2000). Like some other specialist bees, *P. bombiformis* may also be able to learn whole-flower odor, which may be a possible explanation as to why it utilizes the non-host, *I. pandurata*, because most of the chemicals present in the

floral odor of *I. pandurata* are present in *H. moscheutos*. Further study is needed to speculate on imprinting in this species. I do not know what pollens the larvae were reared on, but it would be interesting to know if the bees caught on *Ipomoea* and had full *Ipomoea* loads were reared on *Ipomoea* pollen as larvae.

There is evidence that some oligolectic emphorine bees have the ability to utilize non- host pollen. Several *Diadasia* species, also oligoleges belonging to Emphorini, have collected pure pollen loads of alternate host species (Sipes and Tepedino, 2005). Tellería (2003) proposed that the Argentinian bee *Ptilothrix relata* should be classified as narrowly polylectic rather than oligolectic because it collects pollen from several unrelated species. She found that *P. relata* collected pollen from Malvaceae, Onagraceae, and Asteraceae.

It is interesting to note that *P. bombiformis*'s alternate host, *Ipomoea*, is a member of Convolvulaceae, which is suggested to be the ancestral host-plant family for Emphorini (Sipes and Tepedino 2006). *P. bombiformis*, or at least those individuals who collected *Ipomoea* pollen, may have some residual ability to recognize their ancestral plant family. It is important to note that all documented host families for *Ptilothrix* species at large are also host families to other emphorine bees, revealing that the tribe as a whole seems to be constrained to a limited number of pollen host families. Chemistry may be the key to explaining why *P. bombiformis* has the ability to recognize and utilize non-host plant and that host-chemistry may be the constraining factor in emphorine host use.

This research also adds to the understanding of host-use of the tribe Emphorini as a whole. Emphorini is constrained to using Asteraceae, Cactaceae, Convolvulaceae,

Malvaceae, Onagraceae, and a few other rare accounts of other families, for pollen sources to provision their nests (Sipes and Tepedino, 2005). Emphorine bees have large, unbranched scopal hairs that give them the ability to transport large pollen grains (Michener, 2000; Schlindwein and Martins, 2000). Most of the plant families utilized by emphorines have large or spiny pollen grains, a characteristic that has been hypothesized as a possible explanation for their constrained host-use (Schlindwein and Martins, 2000; Schlindwein et al., 2009). However, other plant families possess large, spiney pollen and may be present in the bees' range without ever serving as hosts for the bees. In addition to host-plant pollen, Tellería (2003) found large, spiny pollen grains from non-host plants in the scopal loads of *Ptilothrix relata*, but these represented <1% of the pollen load, which does provide evidence that *Ptilothrix* scopal hairs do allow for the transport of large pollen grains, but provides no evidence to suggest that they are selecting a host simply because it has large pollen.

Floral Scent Composition of *Hibiscus moscheutos* ssp. *lasiocarpos* and *Ipomoea pandurata*

The field of pollination biology opens a whole new avenue of understanding the role secondary metabolites have in plants. For the past 15 years, there has been a wealth of research conducted on floral volatiles (reviewed in Raguso, 2008; Hartman, 2009), and more recently, researchers are integrating their understanding of floral chemical ecology into pollination biology by searching for specific chemicals that are responsible for attracting pollinators to a given taxa (Plepys et al., 2002; Schlumpberger et al., 2004; Dötterl et al., 2005; Jürgens et al., 2009).

Nearly all floral scent compounds result from the major pathways in secondary metabolism in plants (Knudsen et al., 2006). There are over 1700 known volatiles that have been detected in the floral headspace of more than 990 species in over 90 families (Knudsen et al., 2006). *Hibiscus moscheutos* ssp. *lasiocarpos* and *Ipomoea pandurata* contain some of the most common floral scent compounds. The monoterpene β-ocimene, and the aromatics benzaldehyde and benzyl alcohol are found in 54-71% of the plant families that have been reported in the literature and the sesquiterpene, caryophyllene has been reported in more than half of the families (Knudsen et al., 2006).

Specific compounds that are present in *H. moscheutos* and *I.pandurata* have been shown to attract pollinators. Benzaldehyde has been proven to be an attractant for flies, butterflies, and hymenopterans (Jürgens et al., 2009). During electroantennographic studies, the monoterpene β-ocimene and the sesquiterpene α-farnesene elicited a response in *Andrena vaga*, a *Salix* oligolege (Dötterl et al., 2005) and benzaldehyde and benzyl alcohol elicited responses by honey bees (Bruce et al., 2005). Germacrene D, β-ocimene, and α-farnesene were found to be primary chemicals present in kiwi flowers that are dependent upon bees for pollination (Nieuwenhuizen et al., 2009; Hartmann 2009).

Although volatile identification has become much more sensitive and accurate with the use of gas chromatography-mass spectrometry (Hartman, 2009), there are several factors that can make chemical identification difficult. Sesquiterpenoids are the most abundant compounds and they are often difficult to identify using standard GC-MS because there are many compounds that have similar mass spectra and retention times (Dudareva and Pichersky, 2006). I tentatively identified the sesquiterpenes α-copaene and α-cubebene based on their mass spectra and retention times, but internal standards

would yield a more certain identification do to the close similarity of these chemicals' mass spectra and retention times. Geometric isomers commonly co-occur in a single species (Dudareva and Pichersky, 2006) and may often be overlooked as two forms of the chemical, leading to fewer reported compounds than may actually be present in the flower. Based on the obvious difference in retention times of (*E*)-cinnamaldehyde and (*Z*)-cinnamaldehyde, I am confident that both isomers are present in the *Hibiscus* floral scent.

Determining which compounds are restricted solely to the floral scent can be difficult because most of the chemical compounds present in floral scents have also been identified as defense chemicals to deter herbivores (Dudareva and Pichersky, 2006). Herbivory is known to change floral headspace. Muhlemann et al.(2006) found that benzaldehyde in *Cirsium* spp. was emitted at lower levels when florivores were present and increased when pollinators were present. Theis et al. (2009) found that herbivory on leaves cause an increase in floral scent emission in male flowers of *Cucurbita pepo* ssp. *texana*. There were Japanese beetles and weevils present on the *Hibiscus* flowers I sampled. The flowers from which I collected floral scent had no Japanese beetles in them when sampled, but they were in the vicinity, providing a possible release of some defense chemicals from the floral headspace. Weevils were almost always present in the flowers, but were always removed with forceps before sampling. The abundance of weevils and beetles in the flowers and in the study area may influence the emission of certain floral chemicals, making it difficult to determine if some of the floral chemicals are actually used to guide or attract pollinators.

Some bees recognize their host plant by the blend of volatiles, often in specific ratios, rather than just detection of a single compound (Bruce et al., 2005). Therefore, the bee must be able to distinguish the specific host-plant blend of volatiles from all the other volatiles emitted from surrounding plants. The 14 compounds that *H. moscheutos* and *I. pandurata* have in common may be the key factor in the bees' ability to detect and utilize *I .pandurata* as an alternate host. Most of the shared compounds (Table 6) are present in 20-45% of the plant families that have been studied and caryophyllene is present in over half of the plant families studied (Knudsen et al., 2006). The remaining shared compounds have been reported as floral scent constituents in far fewer families than the other compounds. For example, benzoic acid has only been reported in six families, acetophenones in 14 families, longifolene in four families, and β-copaene in four families. Possibly, these rarer compounds may allow *P. bombiformis* to detect the host plants against background floral fragrances and should be targeted in future research.

There is also preliminary evidence suggesting that *Hibiscus* and *Ipomoea* may be chemically similar to host plants used by other emphorine bees. Messinger and Sipes (unpublished data) have collected scent from the floral headspace of species in Cactaceae, Convolvulaceae, Malvaceae, and Onagraceae that are used by *Diadasia* species as pollen hosts. Their preliminary analysis found eight chemicals that are common to my study species (Table 8), including caryophyllene and copaene that are shared in *H. moscheutos* and *I. pandurata*. This provides evidence that emphorine bees may be constrained by floral chemistry, rather than (or in addition to) pollen size, when selecting a pollen host.

I found variation among individual flowers in scent composition with both *H. moscheutos* and *I. pandurata* (Tables 4 and 5, respectively). For example, nonanal was detected in seven samples, comprising nearly 49% of one sample, while it was completely undetected in three of the samples. Nonanal was also detected in seven *Ipomoea*, accounting for 95% of the total floral scent, while it was undetected in three of the flowers. Similar variation has been reported for other plant species. Both the qualitative and quantitative floral scent composition can vary within species for a variety of reasons (Dudareva and Pichersky, 2006; Salzmann et al., 2007). The fragrance of a living flower can show a continuous change due to several internal and external factors (Theis et al., 2007;Stashenko and Martinez, 2008). Individual variation of the volatile profile of the same species in a given population could be due to genetic factors (Stashenko and Martinez, 2008).

Environmental factors, including light availability, temperature, and soil moisture content can cause intraspecific variability in the volatile profiles (Stashenko and Martinez, 2008). Scent production can peak when the flower is most receptive for reproductive success (Muhlemann et al., 2006) and the scent profile may change once pollination has occurred (Negre et al., 2003; Muhlemann et al., 2006). It has been demonstrated that species can show geographic variation in their scent profiles (Svensson et al., 2005) and also floral scent emission can follow circadian rhythms (Muhlemann et al., 2006; Raguso et al., 2003). The diurnal patterns in floral scent emission and composition has often been attributed to the type pollinators that are attracted to the plants (Knudsen et al., 1999). The intra-specific variability in the chemical profiles of both *H. moscheutos* and *I. pandurata* may be attributed to any combination of the above

factors. Alternatively, my collection methods may have lead me to be near the threshold of detection for some compounds such that they were present but not detected in some individuals.

Future Research

In addition to floral scent, it is important to determine what visual cues are attracting these bees to both the host and non-host plant. The flowers appear similar to the human eye, but they must been analyzed to account for the visual range of bees, which includes UV wavelengths. Separate treatment of the visual and chemical cues is not realistic, and their interrelationships of the stimuli should also be considered (Kevan, 2005). These investigations are most revealing when behavioral bioassays are carried out in parallel with chemical analysis of the scents (Dobson et al., 2005).

Honeybees are model organisms for studying the complexity of visual perception in organisms with small nervous systems (Chittka and Wells, 2004). The role of color vision in honeybee and bumblebee foraging is more understood than in any other natural forager-plant system (Chittka and Wells, 2004). It is well documented in the literature that honeybees and bumblebees use color to discriminate between flowers, learn to associate flower color with reward, and to discern the flower from its background vegetation. Visual signals to pollinators result from natural light being absorbed, reflected, refracted, or possibly fluoresced from the surfaces of the flowers and floral parts (Kevan, 2005). Flowers are visible to their pollinators and other visitors because they appear to be different from the general background vegetation, ground, or sky (Kevan, 2005).

All species within Hymenoptera (except ants) are trichromates, with receptors most sensitive near 345 nm (UV-receptors), 440 nm (blue receptors) and 535 nm (green receptors) (Kevan, 2005; Briscoe and Chittka, 2001; Chittka and Wells, 2004). Many flowers have nectar guides, or visual markings, that are assumed to guide the pollinator to the nectar or pollen source in the center of the corolla (Dafni et al., 1997). UV- patterns in most flowers are formed by UV-absorbing central parts, including pollen, anthers, and nectar guides, in contrast to the outer portions of the flower that are UV-reflecting (Lunau, 1992).

Future research should target integrating the role of visual cues along with the olfactory cues to obtain a more complete analysis of the degree to which these flowers may be detected as similar by *P. bombiformis*. Visual studies should focus on obtaining the UV reflectance of both flowers to determine if there are any visual differences from the bees' perspective. Behavioral studies should be conducted to determine to what degree visual cues play a role in host selection and detection. These may include manipulating live flowers in the field by reducing petal size and also painting the flower with sunscreen to block any attractive UV patterns (Andersson and Amundsen, 1997; Johnson and Andersson, 2002).

The floral scent composition of both flowers can be investigated further by solid-phase micro-extraction (SPME) to determine the contribution of different floral structures to the whole-flower odor. Because these bees are specializing on a pollen host, I think it is most important to analyze the pollen to see if there are any chemicals present that weren't detected in the whole flower scent and also see the ratios of pollen chemicals compared to that of the whole-flower scent. Behavioral analysis for investigating which

part of the flower is emitting the biologically active scent can include observing the bees' reactions when a particular floral organ or tissue is removed, for example, the anthers. Also, *Hibiscus* pollen could replace *Ipomoea* pollen in an *Ipomoea* flower, and vice versa, and observe if the bees change their behavior.

Conclusions

My research provides a better understanding of the host-use of *Ptilothrix bombiformis* and documents this bee utilizing a new pollen host, lending its self to be classified as an eclectic oligolege. Additionally, I have provided baseline descriptive data to explore the hypothesis that floral chemical cues may be responsible for host recognition and utilization in this specialist bee. I have shown that at least two emphorine host plants share chemical compounds: *H. moscheutos* and *I. pandurata* had 14 shared chemicals in their floral headspace. These chemicals should be targeted for further investigations of their role in host selection by *P. bombiformis*

This research also adds to the understanding of host-use of the tribe Emphorini as a whole. Emphorini is constrained to using Asteraceae, Cactaceae, Convolvulaceae, Malvaceae, Onagraceae, and a few other rare accounts of other families, for pollen sources to provision their nests (Sipes and Tepedino, 2005). Possibly, Emphorini at large is constrained to a limited number of chemically-similar plant families for pollen hosts. However, further floral scent studies of more emphorine hosts, in combination with evaluations of the visual similarity of host plants, will be needed to fully understand host choice in these specialist bees.

TABLES

Table 1. Study site locations in southern Illinois.

Site Name	Latitude	Longitude (-)	Elevation (m)	Location	County
CORN	37.720429°	89.465013°	112	12.47 km SW Murphysboro	Jackson
JALC	37.744683°	89.087267°	123	1.74 km SW Carterville	Williamson
MARINA	37.744767°	89.117767°	125	3.25 km SW Carterville	Williamson
RR	37.673367°	89.466467°	115	13.90 km SW Murphysboro	Jackson
DITCH	37.729283°	89.463600°	112	11.51 km SW Murphysboro	Jackson
BML POND	37.657150°	89.437667°	116	15.10 km SW Murphysboro	Jackson
FLOODFIELD	37.673450°	89.444933°	116	15.39 km SW Murphysboro	Jackson
INTERSECT	37.673383°	89.436500°	116	13.63 km SW Murphysboro	Jackson
HAVEN	37.743702°	89.128177°	125	4.12 km SW Carterville	Williamson
LARUE	37.585266°	89.440516°	129	21.81 km SW Murphysboro	Jackson
REND	38.110133°	88.911967°	124	4.46 km SW Ina	Jefferson
BML	37.609588°	89.454641°	112	20.15 km SW Murphysboro	Jackson

Table 2. Angiosperms in bloom throughout the study sites.

Scientific Name	Common Name
Allium vineale	Wild Garlic
Ambrosia artemisiifolia	Common Ragweed
Asclepias incarnate	Swamp Milkweed
Asclepias syriaca	Common Milkweed
Bidens cernua	Nodding Beggartick
Calystegia sepium	Hedge Bindweed
Campsis radicans	Trumpet Creeper
Carduus nutans	Nodding Plumeless Thistle
Cephalanthus occidentalis	Buttonbush
Chamaecrista fasciculate	Partridge Pea
Cichorium intybus	Chicory
Commelina communis	Asiatic Dayflower
Conyza canadensis	Horseweed
Coronilla varia	Crownvetch
Daucus carota	Queen Anne's Lace
Desmanthus illinoensis	Prairie Mimosa
Desmodium sp.	Ticktrefoil
Dipsacus fullonum	Teasel
Erigeron sp.	Daisy Fleabane
Ipomoea lacunosa	Small White Morning Glory
Lycopus americanus	Cutleaf Water Horehound
Melilotus albus	White Sweet Clover
Melilotus officinalis	Yellow Sweet Clover
Phyla lanceolata	Lanceleaf Fogfruit
Physalis virginiana	Virginia Ground Cherry
Polygonum hydropiperoides	Swamp Smartweed
Polygonum pennsylvaticum	Pennsylvania Smartweed
Prunella vulgaris	Self Heal
Saponaria officinalis	Bouncingbet
Silphium perfoliatum	Cup Plant
Sium suave	Hemlock Waterparsnip
Solanum carolinense	Horse Nettle
Trifolium pretense	Red Clover
Trifolium repens	White Clover
Vernonia gigantean	Tall Ironweed
Vicia dasycarpa	Smooth Vetch

Table 3. Descriptive statistics from a paired *t*-test for male and female *Ptilothrix* visitations to *H. moscheutos ssp. lasiocarpos* and *I. pandurata*.

	Observations	Visits Per Individual		*P* two-tail
		Actual Mean	Transformed Mean	
Males	41			0.118
Hibiscus		3.39	1.45 (1.33)	
Ipomoea		2.29	0.93 (1.47)	
Females	14			<0.001
Hibiscus		1.79	1.23 (0.31)	
Ipomoea		0.21	0.21 (0.18)	

The means were square root transformed for normality and the variance is given in parenthesis.

Table 4. Relative amounts of compounds present in the floral headspace of *Hibiscus moscheutos* ssp. *lasiocarpos*.

		Sample ID Number									
		Scent Collection Time (hrs)									
Compound	**RT**	**1**	**4**	**37**	**39**	**19**	**20**	**23**	**15**	**16**	**17**
		1.25	1.25	2.5	2.5	3	3	3	4.5	4.5	4.5
Aliphatics											
Nonanal	11.47			48.50	53.68	37.38	36.45	30.56			19.96
Aromatics											
Acid											
4-ethyl-benzoic acid	19.03	5.98	5.11	0.81	0.63		0.59		0.39	1.35	
Alcohol											
Benzyl alcohol	8.75				8.09	29.47					
Aldehyde											
Benzaldehyde	7.31	4.91	3.75	14.29	0.85		1.08	22.39			0.10
(*Z*)-Cinnamaldehyde	14.86	20.82	15.98								
(*E*)-Cinnamaldehyde	15.7	8.16	5.54								
Ketone											
para-ethyl-acetophenone	18.08	1.85	1.37	0.42	0.26		0.27		0.10	0.28	
Monoterpenes											
(*E*)-β-Ocimene	9.21	35.02	24.56	7.76	4.13	29.47	25.19	19.28	21.58	33.90	41.10
Sesquiterpenes											
(+)-Cycloisosativene	22.55	10.01	15.37	16.13	7.34		14.60	10.78	32.72	32.21	
α-Copaene	22.82	2.15	5.08	3.06	2.31	1.37			9.13	8.13	3.59
α-Cubebene	22.86						3.47	2.83			0.72
Bourbonene	23.18		1.23	1.67	2.09		1.76	1.31	2.84	2.05	3.02
β-Elemene	23.41		2.79		2.28		1.74	1.18	4.83	2.49	2.60
Longifolene	24.25	2.50	1.52			0.87	0.64	0.65			0.18
(*E*)-Caryophyllene	24.67	1.66	6.64	1.05	3.80	1.03	4.25	3.06	7.56	5.91	3.79
β-copaene	25.12				0.96		0.79	0.71	1.38	1.26	
Bergamotene	25.22								0.92	1.33	
Aristolene	25.29		0.74		0.72		0.39				
α-Guaiene	25.32					0.40			2.16	0.77	1.40
α-Patchoulene	25.68								0.51		
α-Humulene	26.13		0.75		0.30				1.38	0.59	7.51
Allo-Aromadendrene	26.32				0.74		0.82	0.75	0.44	0.48	
trans-Muurola-4,(14)-5-diene	27.13		1.97	1.84	4.85		4.76	2.51	0.76	0.51	11.37
Germacrene D	27.16								3.41	1.36	
Seychellene	27.46						0.65				
β-Selinene	27.44			0.29				0.62	1.10	0.47	
α-Selinene	27.78						0.53	0.45	1.10	0.47	0.28
α-Amorphene	27.87			1.38	1.17						
α-Muurolene	27.88									1.20	
α-Farnesene	28.11								0.10		
γ-Cadinene	28.46				5.47			1.17	2.71	1.73	

Table 4 continued.

		Sample ID Number									
		Scent Collection Time (hrs)									
Compound	**RT**	**1**	**4**	**37**	**39**	**19**	**20**	**23**	**15**	**16**	**17**
		1.25	1.25	2.5	2.5	3	3	3	4.5	4.5	4.5
Zonarene	28.63			2.43			2.02	1.62			
γ-Amorphene	28.65	1.93	2.52						4.65	2.91	4.40
Longiborneol	30.65	1.30	1.66								
Globulol	31.13							0.15	0.24	0.61	
Unknowns											
40(37), 41(27), 44(17), 105(26), 121(20),161(28), 162(11), 204(12)	26.87			0.37	0.34						
41(54), 55(39), 67(23), 119(35), 161(85), 204(22)	28.45	0.64	1.38								
41(17), 80(26), 81(35), 84(12), 121(17), 123(11), 135(27), 161(39), 162(12)	28.86	1.56	1.32								
55(12), 66(18), 69(24), 85(36), 121(15), 163(17), 204(32)	29.71	1.52	0.72								

Unknowns are listed by ion fragments in ascending order of mass/unit charge, with abundance in parenthesis.

Table 5. Relative amounts of chemicals detected in the floral headspace of *Ipomoea pandurata*.

		Sample ID Number									
		Scent Collection Time (hrs)									
Compound	**RT**	**26**	**25**	**33**	**38**	**34**	**21**	**22**	**13**	**12**	**14**
		1.25	1.25	2	2.5	2.5	3	3	4.5	4.5	4.5
Aliphatics											
Nonanal	11.45			95.06	91.68	76.26	29.45	66.28			59.26
Aromatics											
Acid											
4-ethyl-benzoic acid	19.04	2.10	0.76	0.36			0.48	0.97			2.32
Alcohols											
Benzyl alcohol	8.81		77.52			19.49	8.80	10.92		52.02	
2,4-di-tert-butyl phenol	28.33			0.26			0.27	0.11	0.22		
Aldehyde											
4-ethyl-benzaldehyde	13.97	9.04			4.21	1.77	30.78	1.27	2.25	10.95	15.67
(Z)-Cinnamaldehyde	14.76								3.30	23.22	
Ketone											
para-ethyl acetophenone	18.06	2.04	0.74		1.64	0.46		0.46	0.52	5.20	
Monoterpenes											
para-Cymene	10.67							0.20	0.60	2.95	
Sesquiterpenes											
(+)-Cycloisosativene	22.81	2.46	1.46				1.47	4.05			
α- Copaene	22.84								1.84		1.71
Bourbonene	23.19	11.25	1.41				20.68	1.51	18.70		
Patchoulene	24.23								1.58		
Longifolene	24.27	6.03	4.30		1.22	0.85	4.03	8.44		2.47	0.99
(*E*)-Caryophyllene	24.65	4.04	1.78	0.38	1.24			0.76	9.16		
β-Longipinene	25.07							0.99			
β-Cubebene	25.08	5.78							8.86		5.89
β-Copaene	25.09	5.74	1.73						2.36		9.89
Aromadendrene	26.01						0.75		0.26		
α-Humulene	26.13								0.59		
cis-Muurola-3,5-diene	26.63								1.56		
Germacrene D	27.16						2.14		48.19		4.26
trans-Muurola,4,(14)5-diene	27.17	51.51	10.30	3.93				4.05		3.19	
α-Farnesene	28.07						0.11				
Cedrene	29.86					1.17					
Unknowns											
40(77), 44(38), 69(29), 95(24), 105(37), 122(51), 133(20), 147(49), 161(64), 204(22)	26.02						0.75				
50(7), 51(11), 71(9), 77(15), 119(17), 134(7), 161(25), 204(13)	28.63						0.28				

Unknowns are listed by ion fragments in ascending order of mass/unit charge, with abundance in parenthesis.

Table 6. Compounds shared by *H. moscheutos ssp. lasiocarpos* and *I. pandurata*.

Shared Compounds
Nonanal
Ethyl benzoic acid
(*Z*)-Cinnamaldehyde
Para-ethyl acetophenone
(+)-Cycloisosativene
α- Copaene
Bourbonene
Longifolene
(*E*)-Caryophyllene
β-Copaene
α-Humulene
Germacrene D
Trans-muurola-4,(14)-5-diene
α-Farnesene

Table 7. Chemicals found in significantly different amounts according to a Chi-Square test in *H. moscheutos ssp. lasiocarpos* and *I. pandurata*.

Chemical	*P* Value
α-Copaene	0.023
α-Selinene	0.0325
β-Elemene	0.0031
β-Ocimene*	<0.0001
γ-Amorphene	0.0325
4-ethyl-benzaldehyde*	<0.0001

** Indicates chemicals that were significantly different after a Bonferroni adjustment, n=10 for both plant species.*

Table 8. Chemical constituents from other emphorine host plants from Messinger's unpublished data.

Compound	Genera	Family
Caryophyllene	*Sphaeralcea*	Malvaceae
Globulol	*Opuntia*	Cactaceae
Benzaldehyde	*Sphaeralcea, Malvella*	Malvaceae
Benzyl Alcohol	*Malacothamnus, Clarkia, Malvella, Calystegia*	Malvaceae, Onagraceae, Convolvulaceae
α-Cubebene	*Calystegia*	Convolvulaceae
Copaene	*Calystegia*	Convolvulaceae
Aromadendrene	*Calystegia*	Convolvulaceae
Amorphene	*Sphaeralcea*	Malvaceae

FIGURES

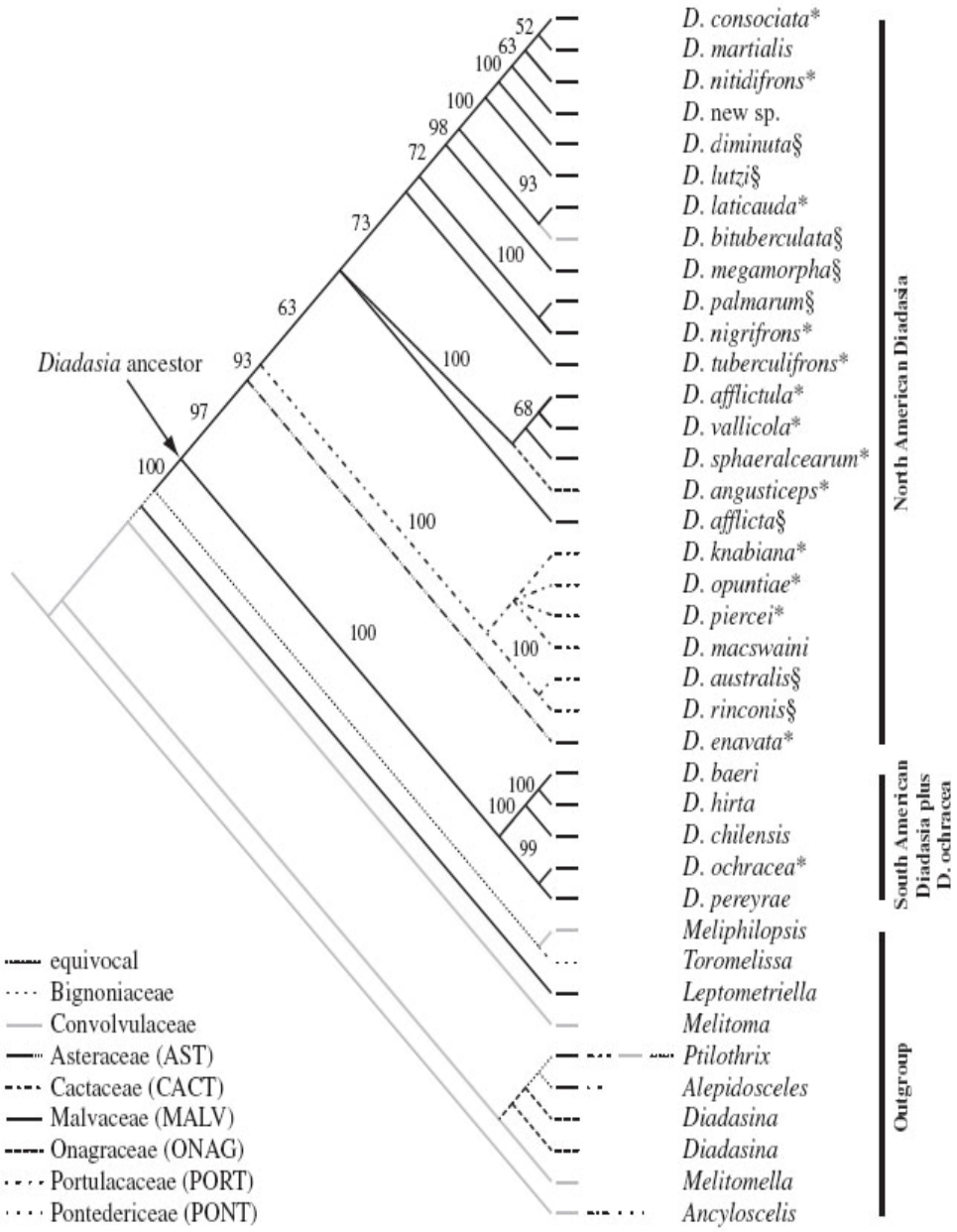


Figure 1. Phylogenetic tree for *Diadasia* and other Emphorini, taken from Sipes and Tepedino, 2005 © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*.

Figure 2. *Hibiscus moscheutos* ssp. *lasiocarpos* (top) and *Ipomoea pandurata* (bottom).

Figure 3. Flower orientation for behavioral experiment with *Hibiscus moscheutos* ssp. *lasiocarpos* and its cultivated congener *Hibiscus syriacas*. Pictured are picked *H. syriacas* flowers placed in flowering *Hibiscus moscheutos* ssp. *lasiocarpos* plants.

Figure 4. Floral scent collection apparatus for dynamic headspace sampling.

Figure 5. Male *Ptilothrix bombiformis* resting (top) and nectaring (bottom) on *Hibiscus moscheutos* ssp. *lasiocarpos*.

Figure 6. *Ptilothrix bombiformis* nesting aggregation (top) and individual nest with turret (bottom).

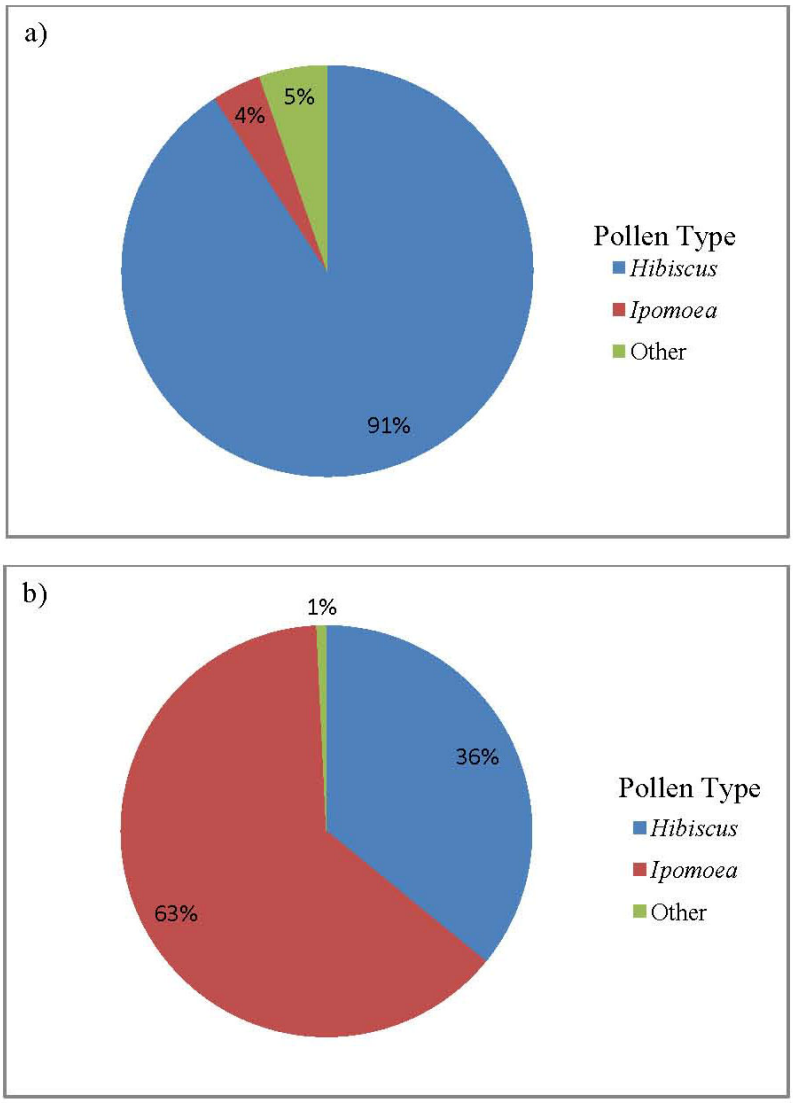
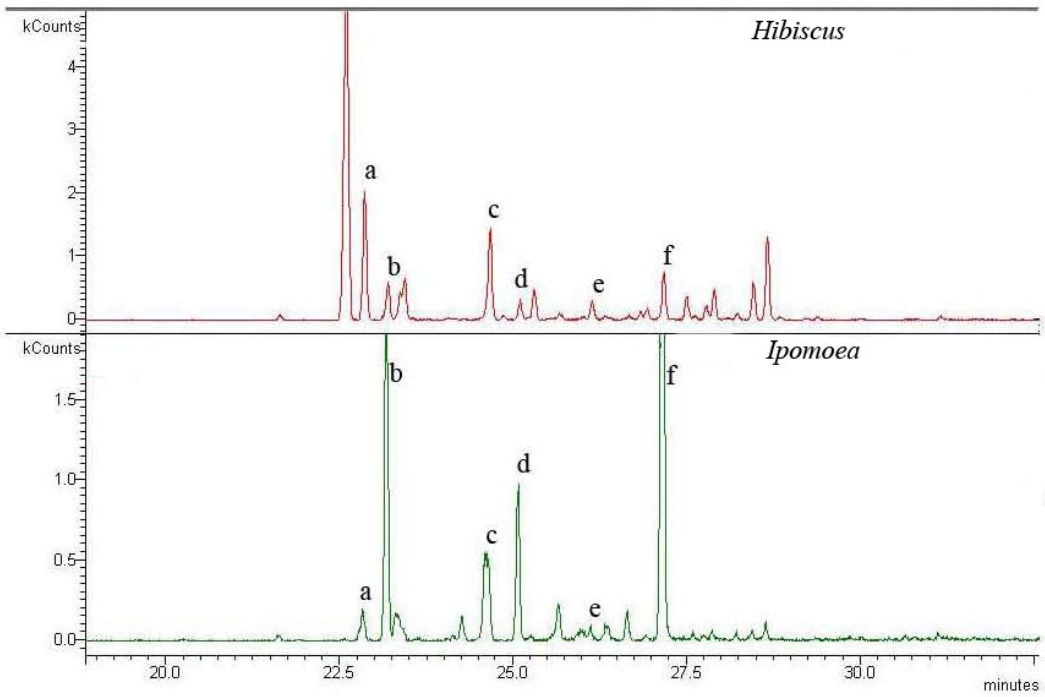


Figure 7. Scopal pollen composition from bees collected on *Hibiscus moscheutos* ssp. *lasiocarpos* (a) and *I. pandurata* (b). Note that the pie wedges represent average amounts of pollen found in scopal loads. For the bees collected on *Hibiscus* (n=45), 69% had pure *Hibiscus* pollen loads. For the bees collected on *Ipomoea* (n=11), 9% had pure *Hibiscus* pollen loads and 36% had pure *Ipomoea* pollen loads. A pure pollen load was defined as at least 90% a single flower species.



a= α-Copaene, b= β-Bourbonene, c= <*E*>-Caryophyllene, d= β-Copaene, e= α-Humulene, f= Germacrene D

Figure 8. Chromatograms showing some chemicals shared between *Hibiscus moscheutos* ssp. *lasiocarpos* and *Ipomoea pandurata*.

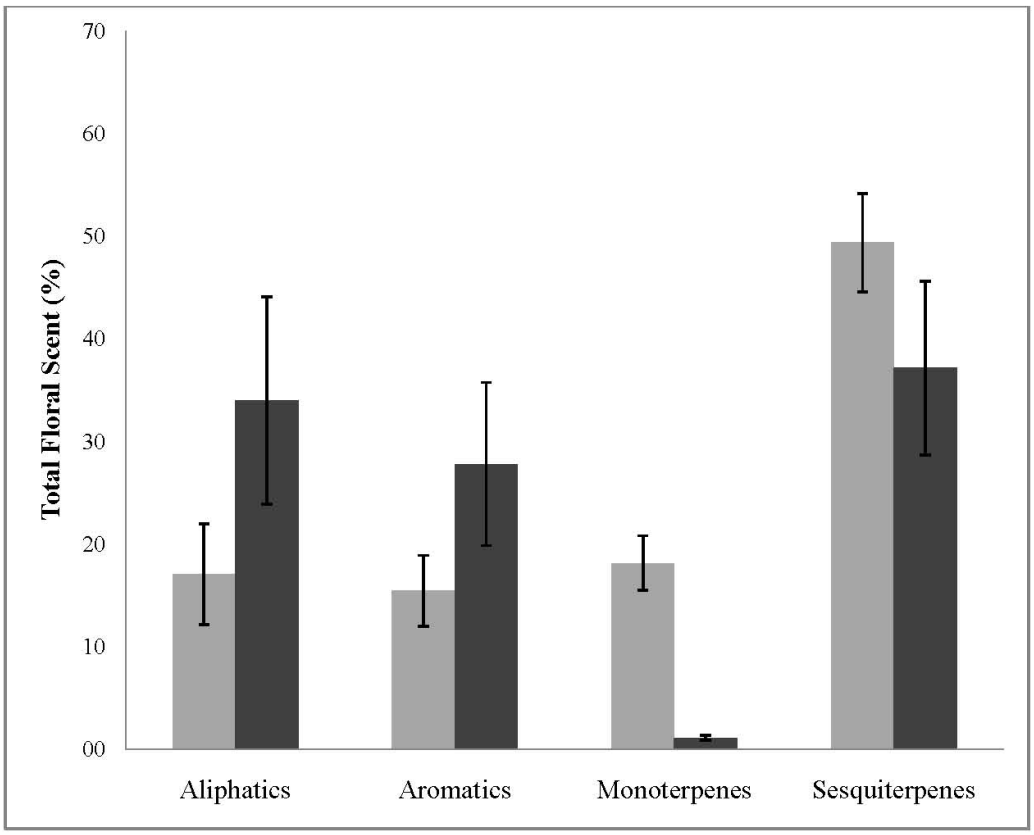


Figure 9. Chemical classes represented in the floral scent of *H. moscheutos* and *I. pandurata*. The bars, showing standard error, represent the average amount each chemical class represents of the total floral scent of *H. moscheutos* (light bars) and *I. pandurata* (dark bars) and n=10 for each species.

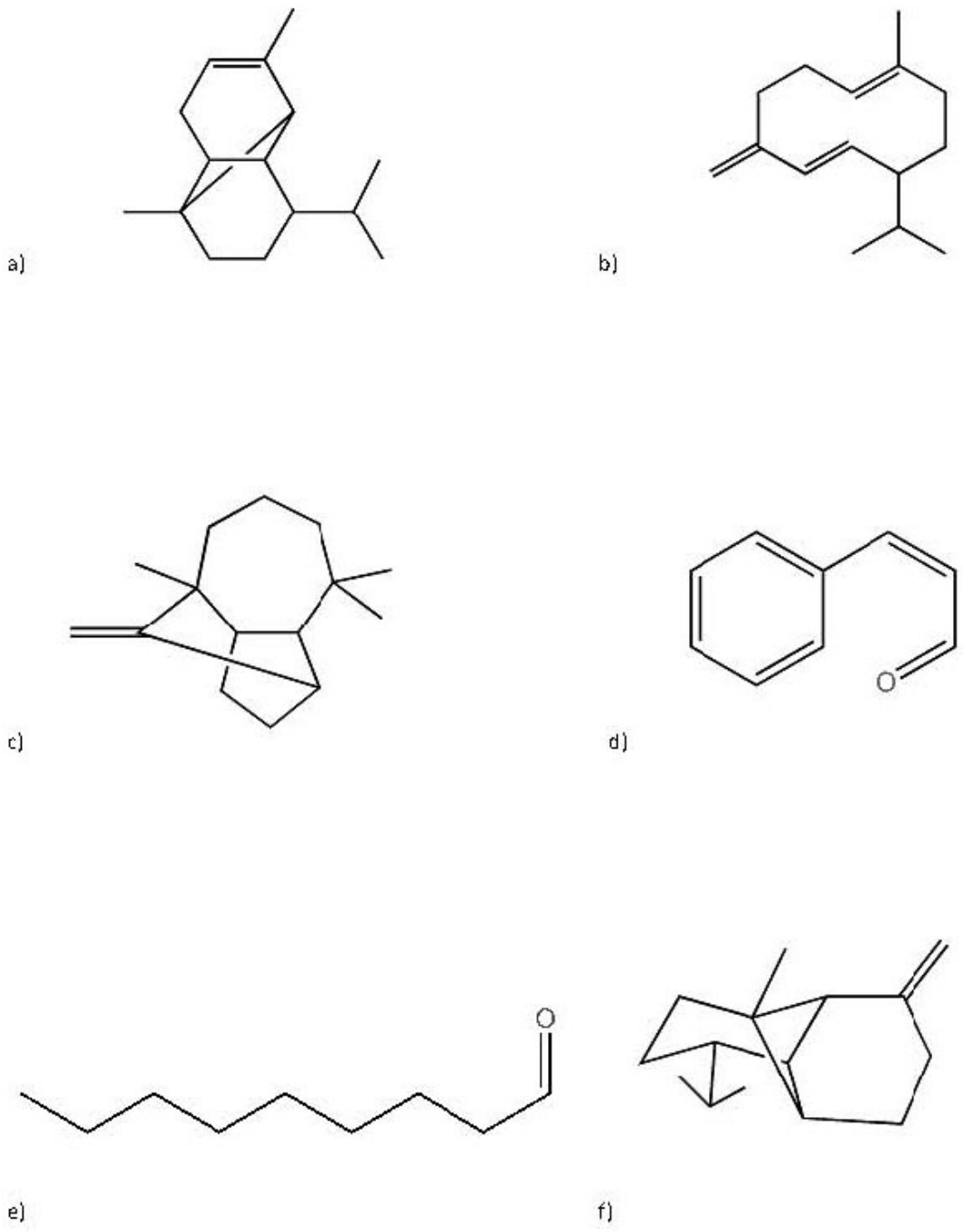


Figure 10. Chemical structures of some of the compounds shared between *H. moscheutos* ssp. *lasiocarpos* and *I. pandurata*. a) α-Copaene, b) Germacrene D, c) Longifolene, d) (*Z*)-Cinnamaldehyde, e) Nonanal, f) β-Copaene

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