ADAPTIVE RADIATION OF OIL-REWARD COMPOUNDS AMONG NEOTROPICAL ORCHID SPECIES (ONCIDIINAE)

By

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I dedicate this thesis to my husband Lou Santiago and to my parents Gaspar Silvera and Flor Silvera for their endless support and encouragement of my goals, and especially for caring about my personal growth.

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By

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Oil-secreting flowers are found throughout the angiosperms and the production of floral lipid rewards is a polyphyletic trait. In this study, I explore variation in floral-oil composition among neotropical orchids of the subtribe Oncidiinae and a malpighiaceous species (*Byrsonima crassifolia*). Many *Oncidium* species are thought to be mimics of malpighiaceous species because members of both groups flower simultaneously and have structurally and functionally similar inflorescences, thus attracting and sharing pollinators. The chemistry of floral-oil secretions in these groups is compared and insight into the radiation of oil-related compounds and their relationship with oil-collecting bees is provided. Floral oils were analyzed by gas chromatography/mass spectrometry (gc/ms). Mass spectra of significant peaks were compared among species of the same genus and among different orchid genera. I provide a major survey of oil components with over a hundred different major compounds for members of the Oncidiinae, a subtribe with the most diverse type of pollination systems found in the Orchidaceae. The results show both quantitative and qualitative differences among orchid genera, but mainly quantitative differences within genera. There is biochemical convergence between oil-reward compounds among members of the Malpighiaceae and Orchidaceae. Müllerian mimicry between these groups seems to best describe this chemical convergence. The variability in oil chemistry found among different genera of orchids can be used to understand the exploitation of oil resources by oil-collecting bees and could potentially explain the high degree of pollinator specificity for many tropical orchid species.

CHAPTER 1
INTRODUCTION

Literature Review

The discovery of pollination of orchids by oil-collecting bees opened a new area of research in pollination ecology (Vogel 1969, 1974; Buchmann 1987; Roubik 1989; Steiner 1989, 1991; Wcislo and Cane 1996). Oil-secretion by flowers among angiosperms has been proposed to be a highly successful evolutionary strategy to enhance pollination (Buchmann 1987), yet data on the evolution of oil flowers and its relationship with pollinators are still needed in order to understand radiation of the oil-pollination syndrome.

The oil-pollination system was first reported by Stefan Vogel in 1969 and first presented at the International Botanical Congress in Seattle in the same year ("Flowers Offering Fatty Oils Instead of Nectar"). He studied this system in 1964 while conducting research in Argentina, observing species of *Angelonia* (Scrophulariaceae s.l.) in the field. In the oil-pollination system, oil-collecting bees gather floral lipids from oil-secreting flowers. The majority of oil-producing species rely exclusively on oil-collecting bees for pollination. Female bees collect these oils with specialized structures on their forelegs, absorb the oils by capillary action, and carry the oils in the scopae of their hind legs. It is important to note that the term floral oils is used to describe non-volatile oils rather than volatile essential oils (mainly terpenes) used as sexual or odor attractants in many insect-visited flowers.

Vogel (1974) published the most detailed account on this syndrome, and included descriptions of the oil-secreting glands and a detailed table of all families and genera possessing this syndrome. Flower structures and pollination behavior of 24 plant species were explained in detailed for the first time. This publication became the framework for many other studies and the beginning of a new area of research in pollination ecology. Simpson, Neff and Seigler (1977), Simpson and Neff (1981), and Buchmann (1987) discussed this system in more detail. It is now known that the oil-pollination system is predominantly found in the neotropics, mainly within six families: Krameriaceae, Iridaceae, Scrophulariaceae s.s., Calceolariaceae, Malpighiaceae, and Orchidaceae. Flowers offering floral lipids are found in 8 orders, 11 families, 79 genera and 2402 species of flowering plants worldwide, but these numbers are increasing as we learn more about this system (Buchmann 1987). Gesneriaceae, Melastomataceae, Solanaceae, and Primulaceae contain oil-flower species but their pollination is unknown.

Orchids that produce oils are well represented in the neotropics. Their flowers produce floral lipids in the form of oils that (in most cases) are secreted from structures called elaiophores and are visited exclusively by bees (Vogel 1969; Buchmann 1987). These floral lipids may serve as pollinator larval supplements since the energetic value of lipids is higher than that of nectar and pollen (Simpson et al. 1977; Roubik 1989; Wcislo and Cane 1996). Adult bees are not known to ingest lipids, but specialized bees can utilize these oils as nesting material (water-resistant cell linings) or accessory pollenkit (Buchmann 1987; Roubik 1989).

Elaiophore as an Oil-Secreting Organ

Vogel (1974) was the first to describe the different types of oil-secreting organs or “elaiophores” present in angiosperms. He recognized two different types of elaiophores:

trichomal and epithelial elaiophores. Trichomal elaiophores are composed of glandular hairs or outgrowths from the epidermis, which consist of up to 50,000 glandular hairs that constantly secrete oils. There can be up to 3 mg of oil per individual flower, and oils can be secreted and accumulated directly from the trichomes, or it can be stored below the cuticle until removed by combs in the forelegs of female bees (Vogel 1974). Trichomal glandular hairs have been reported in the following families: Cucurbitaceae, Iridaceae, Primulaceae, Scrophulariaceae (s.s), Calceolariaceae, Solanaceae, and Orchidaceae (Vogel 1974; Buchmann 1987). The trichome is generally club-shaped and measures about 100 μm long and 23 μm wide [*Calceolaria* species, (Vogel 1974; Raman 1989)]. Vogel found that the trichomes were formed once the floral bud reached 2 mm in length. The floral oils start appearing one to two days after corolla opening and after eight to ten days the corolla then wilts (Vogel 1974; Molau 1988).

The other type of oil-secreting gland, an epithelial elaiophore, is characterized by small compact tufts of glandular epithelial or epidermal cells, often well circumscribed, with one or two layered patches of columnar epithelia, capable of copious secretions. This type of elaiophore is the most widespread one and has been described for many species of the families Malpighiaceae and Krameriaceae and a few orchid species (Vogel 1974; Buchmann 1987).

Even though nectar and pollen are still the primary sources of floral rewards in most plant communities, natural selection has promoted the elaboration of alternative floral rewards. Production of other sources of reward may be advantageous since it can promote strong selection towards utilizing specific suites of pollinators that can collect and use these oils in a given community. Natural selection may promote pollinator specificity.

However, there are disadvantages as rapid extinction could occur if there are highly species-specific pollination relationships. In orchids, the pollen is packed into pollinia, and the size of the pollinator determines whether the pollinia may be easily removed and transported to another orchid flower. Selection may favor relationships with a limited number of specialized pollinators that can transport the pollinia correctly. In other words, by producing a highly energetic reward, such as floral lipids, orchids may be able to attract a limited number of oil-collecting bees that can benefit by this relationship and maintain orchid diversity by increasing pollinator specificity.

Objectives of This Study

In this study, I explore the oil-pollination syndrome radiation within neotropical orchids by relating variation in floral-oil reward compounds to phylogeny and to pollinator-flower specificity among neotropical orchid species. This study comprises three parts. In the first part (Chapter 2) I discuss the chemistry of oil-reward compounds and relationships with pollinators. The second part (Chapter 3) consists of a study on pollination observations of different oil-secreting species and a comparison of fruit production among species that vary in floral oil content. The last section and conclusion (Chapter 4) explores the evolution of the oil-pollination syndrome in orchids using a well supported phylogeny to speculate on the adaptive radiation of floral-oil reward compounds in neotropical orchids. A proposed hypothesis of evolutionary relationship for the subtribe Oncidiinae (Williams et al. 2001) is used as a framework for this study, as this tribe has the most diverse types of pollination systems found in the Orchidaceae (Williams 1982). The wide variety of pollination mechanisms in orchids makes them an appropriate group for studies of speciation and pollination evolution (Steiner 1994). Orchidaceae are the largest family of flowering plants and their members occupy a wide variety of habitats (Arditti 1992; Dressler 1993b). As a consequence, orchids show a large number of morphological, anatomical, ecological, and physiological characteristics that allow them to excel in a variety of environments. Orchids and pollinators have coadapted resulting in an array of highly specialized pollination systems that have contributed to the success of orchids in specialized habitats (Pijl and Dodson 1966; Dressler 1993a).

CHAPTER 2
CHEMISTRY OF OIL-REWARD COMPOUNDS

Introduction

Literature Review

In the neotropics, most flowers offer nectar or pollen to their pollinator organisms. Other flowers offer floral lipids, an unusual type of floral reward, instead of nectar or pollen. These floral oils are released from structures called elaiophores (*sensu* Vogel 1974) and are utilized by oil-collecting bees to waterproof the cell linings or mix with pollen as a larval provision (Cane et al. 1983). The relationship between flowers and their pollinators is a mutualistic one and many visual, chemical, and sensory cues are important in this relationship. From a chemical point of view, floral lipids are energetically more costly than nectar or pollen, and therefore, have a higher energy content when compared to carbohydrates. Oil-collecting bees may benefit from such a high quality food source for their larvae.

Floral oils are a combination of saturated fatty acids, paraffins, glycerols, and esters. Other components related with elaiophore exudates are diglycerides, amino acids, glucose, carotenoids, phenolics, glycosides, nonvolatile isoprenoids, and saponins (Buchmann and Buchmann 1981; Neff and Simpson 1981; Buchmann 1987; Roubik 1989). The floral lipids are clear and usually colorless, sometimes yellow, without odor and light in viscosity (Vogel 1974; Buchmann 1987). The chemical composition of some floral oils have been identified in a few species, ranging from free fatty acids (Buchmann and Buchmann 1981; Buchmann 1987; Vinson et al. 1997) to acetyl-glycerol derivatives with β-acetoxy-fatty acids (Reis et al. 2000) to β-acetate-substituted free fatty acids or mono- di- or tri-glycerides (Vogel 1976; Siegler et al. 1978; Simpson et al. 1979; Vinson et al. 1997). A study of the floral lipids from *Calceolaria* (Calceolariaceae) and *Krameria* (Krameriaceae) revealed a C_{16}-C_{20} β-acetoxy substituted free fatty acids, and an unusual 3-hydroxy fatty acid (Siegler et al. 1978; Buchmann 1987).

Floral Mimicry in Oil-Secreting Flowers

In the neotropics, many *Oncidium, Tolumnia* (Orchidaceae: Oncidiinae) and relatives mimic the flowers of malpighiaceous species. Members of both groups flower simultaneously and have functionally similar inflorescences, consequently attracting and sharing similar pollinators (Ackerman 1986). However, it has not yet been established if the orchid oil compounds are a more specialized form of reward than the common malpighiaceous oils. In Panama, for example, *Trichocentrum stipitatum* (Lindl.) M. W. Chase & N. H. Williams mimic the flowers of the malpighiaceous species *Byrsonima crassifolia* (L.) Kunth, thus sharing a similar set of pollinators present in a given habitat. Some orchid species may produce floral lipids similar to those of malpighiaceous species, thus adding a chemical as well as a visual element to the mimicry between orchids and Malpighiaceae. Many malpighiaceous species, especially vines, are visited by *Centris* bees that gather oils from paired sepaline flower glands.

Examples of orchid species mimicking malpighiaceous species, especially in the genus *Tolumnia* (formerly included within *Oncidium*) are *T. guianensis* (Aubl.) Braem, *T. haitiensis* (Leonard & Ames ex Ames) Braem, *T. quadriloba* (C. Schweinf), *T. X osmentii* (Moir) Braem, and *T. compressicaulis* (Withner) Braem. Female bees of the genus *Centris* are known to visit these species looking for floral lipids (Cingel 2001).

Chemistry of Floral Lipids in *Oncidium*

The first report on the presence of oils in a species of *Oncidium* included a brief description of the nature of the floral lipids found in *Oncidium ornithorrhynchum* Kunth. Thin layer chromatography was used to determine the presence of oils in the flowers, but no further chemical or spectroscopic characterization was provided (Vogel 1974).

The first report on the pollination of an oil-secreting species of *Oncidium* using chemical evidence included the chemistry of floral oils in *Oncidium pubes* Lindl. (Reis et al. 2000). These oils were composed of diacylglycerols and triacylglycerols with one or two acetyl residues and one long chain fatty acid. The constitution of major compounds still requires further study. Floral lipids constitute a form of reward different from nectar, the most widespread form of reward. *Oncidium pubes* offers floral lipid rewards instead of nectar, and the pollination is by oil-collecting bees and not by an attraction–deception system found commonly in orchid species (Reis et al. 2000).

Even though the pollination of many *Oncidium* species is known to be mediated by oil-collecting bees, biologists have failed to provide evidence of the chemical relationship between the pollinators and the oil-secreting flowers. Dodson (1967) observed *Centris* bees visiting *Oncidium* species, and Vogel (1974) described the oil-pollination system in detail. Since then, biologists have added little information about the chemistry of these flowers.

Material and Methods

Study Species and Study Sites

The study was conducted at various sites throughout the Republic of Panama. Oil-orchid species were collected directly in the field at seven different sites (Fig. 2-1). The orchid species were transported to a greenhouse at the University of Florida and fresh flowers were collected directly from the plant for analysis. Table 2-1 is a list of the species used in this study, including a literature reference to the pollinator of each species where known.

Additionally, three species from Ecuador were included in this study: *Sigmatostalix portillae* Königer*, Sigmatostalix oxyceras* Königer & J. G. Weinm.*,* and *Sigmatostalix marinii* Königer. I have also included a malpighiaceous tree species from Panama, *Byrsonima crassifolia*. The flowers of *Byrsonima crassifolia* were collected directly in the field in Panama and washed in hexane for 5 minutes. The samples were transported to the University of Florida for analysis. Vouchers of all plant species included in this study are deposited at the Herbarium of the University of Florida (FLAS, see Appendix B). In total, this study included sixteen species, comprising seven different genera (Table 2-1).

Chemical Analysis

In order to analyze oil composition among orchid species, 1 to 5 flowers were washed in hexane (1 ml. of hexane/flower) and samples were stored at –20 C. The samples were analyzed using gas chromatography/mass spectrometry (gc/ms) at the University of Florida (Analytical Toxicology Core Laboratory). Samples were first methylated and then separated on a HP-5MS column. Gas chromatography parameters were: inlet temperature 280 C; 4 min hold at 40 C, ramped at 10 C/minute to 270 C (held for 5 minutes) and ramped at 25 C/minute to 300 C and held for 7 minutes. Mass spectra of significant peaks were compared to a library and identified whenever possible.

Figure 2-2 shows the alkane nonacosane as an example of a mass spectrum from this study. The mass spectra and retention times were also compared manually against all species to determine if the same compounds were encountered across species. Isomers,

Table 2-1. List of species used in this study with references to their pollinator. Species are separated by family.

Family Orchidaceae	**Pollinator**	**Reference**
Erycina cristagalli (Rchb.f.) N.H.Williams & M.W.Chase	Unknown	no reference
Erycina pusilla (L.) N.H.Williams & M.W.Chase	Unknown	no reference
Lockhartia micrantha Rchb.f.	Unknown	no reference
Lockhartia oerstedii Rchb.f.	Unknown	no reference
Oncidium cheirophorum Rchb.f.	*Paratetrapedia* sp.	Personal observation
Oncidium klotzschianum Rchb.f.	Unknown	no reference
Oncidium ornithorrhynchum Kunth.	Unknown	no reference
Ornithocephalus bicornis Lindl. exBenth.	*Paratetrapedia calcarata*	Cingel (2001) Dressler, personal communication
Ornithocephalus cochleariformis C.Schweinf.	*Paratetrapedia* spp.	Cingel (2001)
Sigmatostalix picturatissima Kränzl	Unknown	no reference
Sigmatostalix marinii Königer	Unknown	no reference
Sigmatostalix oxyceras Königer & J.G.Weinm	Unknown	no reference
Sigmatostalix portillae Königer	Unknown	no reference
Trichocentum ascendens (Lindl.) M.W.Chase & N.H.Williams	*Trigona nigra*	Parra-Tabla et al. (2000)
Trichocentrum stipitatum (Lindl.) M.W.Chase & N.H.Williams	*Centris* sp.	Cingel (2001)
Family Malpighiaceae	**Pollinator**	**Reference**
Byrsonima crassifolia Rich. ex Kunth.	*Centris* spp.	Albuquerque et al. (1989) Barros (1992)

for example, display similar fragmentation patterns and identification solely based on mass spectra may be misleading. Therefore, comparison of mass spectra and retention times is helpful for identification of compounds. The many unknowns were treated similarly to known compounds; by comparison of individual mass spectra and retention times it is possible to determine if a specific unknown is present across species.

Total ion abundance (defined as the integrated ion abundance across a peak) of individual compounds was used as an indicator of composition amounts present in each species. The mass of 10 to 45 individual flowers per species was used to calculate an average of the mass of flowers. Total ion abundance of oil compounds per gram of fresh flower mass was used to calculate the average amount of floral lipids present per flower. Total ion abundance per unit of fresh flower mass of each species was used as an indicator of quantity of floral oil present per species.

Data Analysis

The ion abundance of individual compounds and total ion abundance per species were used to calculate percentages of individual compounds in each single species and across species. The results for *Oncidium klotzschianum* Rchb. f., *Erycina cristagalli* (Rchb. f.) N. H. Williams & M. W. Chase, and Erycina *pusilla* (L.) N. H. Williams & M. W. Chase were eliminated from further analysis since the total ion abundance present was less than 0.05 total ion abundance per gram of fresh flower in each case (Table 2-2). Replicates for *Oncidium ornithorrynchum* and *Trichocentrum stipitatum* were included in the analysis to test accuracy of the gc/ms analysis and variation within samples. The data were arcsine transformed to meet requirements of normality. The transformed data for all other species were used to perform a Principal Component Analysis (PCA) in order to reduce the large number of original variables to a smaller number of principal components (SYSTAT, Wilkinson et al. 1992). The PCA was performed in SYSTAT version 9, using no rotation, same number of variables as factors, and a matrix of extraction using correlations among the variables. A Scree Plot was performed to evaluate the relationship between the Eigenvalues and number of factors that explain the variance. The total variance explained by the three most important factors, Factor 1, Factor 2, and Factor 3 were used to construct a factor-loading plot.

A hierarchical clustering analysis (cluster tree) was also performed using SYSTAT version 9. I used the same data as the PCA analysis, an average linkage method (unweighted pair group method with arithmetic mean: UPGMA), and Euclidean distances as a distance metric. Finally, the cluster tree was used to evaluate individual relationship between clusters within the tree with pollinator diversity. The pollinator of each species in the cluster tree was determined by a combination of literature review, personal communications and personal observations.

Results

Chemistry of Oil-Flowers

One hundred thirty different compounds were found by gc/ms analyses. The majority of these compounds were unknown (72%). I found 93 unknowns and 37 known compounds. Even though unknowns were the most common, ion abundance showed an opposite pattern: unknowns constituted only 44% of the total abundance, whereas known compounds constituted 56% of the total abundance. Of the known compounds, the most abundant were C_{14}-C_{44} alkanes (36%). I also found esters (15%) mostly from methylations of fatty acids, aldehydes (2%), phenols (2%), alcohols (0.8%), and alkenes (0.2%). Nonacosane alone constituted 11 % of the total abundance and was the most common compound found in this study (Fig. 2-2). Interestingly, 31 out of 97 unknown compounds (32%) display a fragment *m/z* 157 characteristic of diacyl glycerols.

Quantity of oil varied among members of the same genus (Table 2-2). *Sigmatostalix picturatissima* Kränzl, *Sigmatostalix oxyceras*, and *Ornithocephalus bicornis* Lindl. ex Benth have the largest amounts of oil-related compounds per flower (Table 2-2). Although *Ornithocephalus* and *Sigmatostalix* species have legitimate oil-reward flowers, they are not floral mimics of malpighiaceous species, in contrast with the other orchid species that possess large yellow flowers, oil-reward compounds in the flowers, and similar inflorescences to malpighiaceous species. There is a gradient of amounts of oil-related compounds among these different species. *Oncidium klotzschianum*, *Erycina cristagalli*, *Erycina pusilla* produce almost no oils (ion abundance/g fresh flower <0.005); *Trichocentrum* species produce intermediate amounts of oils (ion abundance/g fresh flower from 0.17 to 0.68); and *Sigmatostalix picturatissima* and *Ornithocephalus bicornis* produce the highest amounts of oils (ion abundance/g fresh flower of 4.69 and 2.18, respectively, Table 2-2).

Principal Component Analysis (PCA)

The entire data set for the PCA analysis consisted of 130 different compounds and 16 plant taxa (see Appendix A). A Scree Plot showed an inverse relationship between Eigenvalues and number of factors (Fig. 2-3). Using the scree factor graph, Factor 1, Factor 2, and Factor 3 were selected as the most influential factors, accounting for the majority of the variance. Factor 1, Factor 2, and Factor 3 accounted for 64.6% of the totalvariance (Factor 1=34.47%, Factor 2=16.89, and Factor 3=13.2%, Fig. 2-3). The PCA showed four main groups (Fig. 2-4).

Table 2-2. Quantity of oil-related compounds given as ion abundance per unit of fresh flower mass in various oil-secreting species.

Species	Ion abundance/g of fresh flower mass (x10^{11})
Byrsonima crassifolia	0.400
Erycina cristagalli	0.031
Erycina pusilla	0.016
Lockhartia micrantha	1.995
Lockhartia oerstedii	0.210
Oncidium cheirophorum	0.845
Oncidium klotzschianum	0.041
Oncidium ornithorrhynchum	0.453
Ornithocephalus bicornis	2.183
Ornithocephalus cochleariformis	0.578
Sigmatostalix picturatissima	4.687
Sigmatostalix marinii	0.450
Sigmatostalix oxyceras	2.158
Sigmatostalix portillae	0.788
Trichocentrum stipitatum	0.165
Trichocentrum ascendens	0.679

The first group, represented by open circles, is composed of the Ecuadorian species: *Sigmatostalix marinii*, *Sigmatostalix oxyceras*, and *Sigmatostalix portillae* (Fig. 2-4). The second group, represented by open squares, is the largest group and is comprised mostly of Panamanian orchid species and the central american species, *Oncidium ornithorrhynchum*. Other species in this group are: *Lockhartia micrantha* Rchb. f., *Lockhartia oerstedii* Rchb. f., *Ornithocephalus bicornis*, *Ornithocephalus cochleariformis* C. Schweinf., *Trichocentrum stipitatum*, *Trichocentrum ascendens* (Lindl.) M. W. Chase and N. H. Williams (formerly called *Oncidium teres*), *Sigmatostalix picturatissima*, and *Oncidium cheirophorum* Rchb. f. (Fig. 2-4). The third group, represented by an open triangle, is composed of the oils from a bee's leg (*Paratetrapedia* sp.), and the fourth group, represented by an open diamond is composed of the oils from a malpighiaceous species, *Byrsonima crassifolia* (Fig. 2-4). *Paratetrapedia* sp. is the suggested pollinator for *Oncidium cheirophorum*, an orchid species from the second group, and it was collected gathering lipids from fresh opened flowers of *Onc. cheirophorum*.

Hierarchical Cluster Analysis (Cluster Tree)

The hierarchical clustering analysis of oil-related compounds revealed slightly different results from the PCA analysis. First, the groupings found by the cluster analysis do not represent phylogenetic relationships, in fact, oils from some orchid species are more similar to those of the malpighiaceous species, *Byrsonima crassifolia*. In other words, orchid species that are close relatives (see Fig. 4-1) did not necessarily appear in the same clusters. For example, oils from *Sigmatostalix picturatissima* were more similar to the oils of *Ornithocephalus* spp. than to other *Sigmatostalix* species (Fig. 2-5). In general, I found two main phenetic clusters. The first was composed of the oils of Ecuadorian *Sigmatostalix* species, a group which was also found in the PCA analysis. The second group is larger and is composed of the oils from the malpighiaceous species *Byrsonima crassifolia* which is similar to all the rest of the orchid species (Fig. 2-5). In this second group, we also found that *Oncidium ornithorrhynchum* is more similar to a group composed of all Panamanian orchids than it is to *Byrsonima crassifolia*. Interestingly, there seems to be a geographical correlation in the pattern of oil compositions in these orchid species, since oils from the Ecuadorian species form a distinctive separate cluster. The oils from the Panamanian species form a distinct cluster as well, and the oils from the Central American species *Oncidium ornithorrhynchum* are most similar to those produced by the Panamanian species. Finally, oils from *Oncidium cheirophorum* form a group with oils from the bee's leg (*Paratetrapedia* sp.) and these are embedded in the group of all Panamanian species (Fig. 2-5). This result was expected since this bee was collected gathering lipids from *Onc. cheirophorum*; however, the PCA analysis places the oils from the bee's leg individually and outside all orchid groups, suggesting the presence of oils from taxa other than orchids (Fig. 2-4).

The geographical separation found in the cluster analysis appears to be related to pollinator diversity, and pollinator diversity may partially explain the groups found in the Cluster Analysis. Figure 2-6 shows the relationship between pollinator diversity and cluster groups. I divide the pollinator diversity into four categories: The first category is composed of pollinators for *Byrsonima crassifolia* (Malpighiaceae). It is known that many species of *Centris* bees pollinate flowers of *Byrsonima* (Albuquerque and Rego 1989; Vinson et al. 1997) mention at least 14 species of *Centris* bees responsible for collecting oils in flowers of *Byrsonima crassifolia* in Costa Rica. This first category, therefore, is represented by many species of *Centris* bees (Fig. 2-6). The second category is represented by territorial male *Centris* bees, with a smaller number of bee species than the first category. Species of rat tail *Oncidiums* (now treated as *Trichocentrum* spp.) are known to be pollinated by territorial male *Centris* bees (Nierenberg 1972; Cingel 2001).

Pollinators for *Lockhartia oerstedii* and *Lockhartia micrantha* are unknown. However, it is likely that these species are all pollinated by species of *Centris* bees because of their similarity in oil-related compounds (Fig. 2-6). *Oncidium ornithorrhynchum* is still a mystery. It is not clear in which category it should be placed, and for lack of placement, it will be included in both *Centris* categories, but cautiously since it is phenetically distinctive and could be pollinated by a different species of oil-collecting bee (Fig. 2-6). The third category is represented by the *Paratetrapedia* species.

These small bees are known to pollinate small oil-producing flowers such as those of *Ornithocephalus bicornis* and *Ornithocephalus cochleariformis*. The group formed by *Oncidium cheirophorum* and the oils from the bee's leg provide evidence that the unknown *Paratetrapedia* sp. may pollinate this species. I failed to find pollinia attached to the body of the bee. However, based on my observations, and the size of the bee, I propose that this bee is a pollinator of the orchid *Oncidium cheirophorum*. *Paratetrapedia* sp. collects the floral reward, has the appropriate size to detach the pollinia from the flower and spends enough time collecting the oils from the flower, increasing the possibilities of pollinia removal. The last category is represented by pollinators of the Ecuadorian *Sigmatostalix* species (Fig. 2-6). There is no published information on the pollination of these *Sigmatostalix*. All *Sigmatostalix* species produce oils from elaiophores, but there are no records of pollination observations (Cingel 2001). This group needs further study.

Discussion

Biochemical Convergence of Orchids and Malpighiaceous species

The biochemical convergence among members of the Malpighiaceae and certain orchid species is evidence for Müllerian mimicry in plants. Müllerian mimicry is a system in which both plant species mutually benefit because both species have similar flowers and inflorescences that can share similar pollinators (Dafni 1984). In this study, not only do the majority of the orchid flowers mimic members of Malpighiaceae, but they also produce similar chemical compounds. There seems to be convergence, first to produce lipids in the orchid flowers and second, to produce similar oil-reward compounds and flowers that are yellow and resemble those of Malpighiaceae. This is the first report of Müllerian mimicry involving orchid species.

Mimicry among malpighiaceous species and orchid species has been poorly documented in general. Most of the orchid mimicry studies to date involved Batesian mimicry, in which one of the species (the mimic) is rewardless and imitates the flowers of a reward species (the model) (Boyden 1980; Dafni and Ivri 1981a, b; Nilsson 1983; Kjellsson et al. 1985; Beardsell et al. 1986; Johnson 1994, 2000). The majority of these studies involve terrestrial mimic orchid species that do not produce rewards. Batesian mimicry in *Oncidium cosymbephorum* C. Morren (mimic) and a malpighiaceous species *Malpighia glabra* L. (model) has been studied by looking at the reproductive success of *Oncidium cosymbephorum* when *Malpighia glabra* is blooming in the same area (Carmona-Diaz 2001). This is the only study that provides substantial evidence for this mimicry and discusses the effects of population density, floral density, and reproductive success between Malpighiaceae and *Oncidium* species. The pollination mechanism of equitant species of *Oncidium* (now placed in *Tolumnia*) in the Caribbean that mimic malpighiaceous flowers but do not offer any rewards (deceit flowers) has also been described (Nierenberg 1972), but evidence for the mimicry was provided solely on the basis of both species blooming at the same time, occupying the same areas, and having similar floral UV patterns, which can attract the same pollinators.

In this study, there is only chemical evidence for Müllerian mimicry. Field investigation would be necessary to confirm the system. Floral Müllerian mimicry, in general, is less commonly accepted than Batesian mimicry (Roy and Widmer 1998) and four conditions should be established: (1) both species should have overlapping phenologies and distributions, (2) the pollinator should be necessary for fruit set, (3) both species should share same pollinator species and the pollinators should be able to move freely between individuals of both species, and (4) the similarities between species should enhance plant fitness. The last condition would determine whether the similarity is an adaptive condition (Roy and Widmer 1998).

Analysis of Oil-Related Compounds Using gc/ms and Multivariate Methods

Nonacosane is the most abundant compound found by gc/ms. Alkanes, in general, constituted the bulk of general compounds found across oil-producing species. These alkanes form fatty acids that join with glycerols to form glycerides. Additionally, the majority of the unknown compounds in this study display a fragment *m/z* of 157 (31 compounds and 18.7% of total ion abundace). This fragment is characteristic of diacyl glycerols. ß-acetoxy free fatty acids and diacylglycerols derived from them have been reported as common in oil-secreting flowers (Simpson and Neff 1983). Esters, the second most common compounds found in this study, were present as methyl esters that resulted from methylations of fatty acids with long carbon chains. For example, oleic acid, one of the three most common fatty acids present in triacylglycerols, was observed through fatty acid methylations (9-octadecenoic acid, methyl ester). Although alkanes were the most abundant compound found, the exact oil-compositions of the species used in this study were not determined because samples were methylated and no further analysis was performed. By methylating samples one can enhance volatility, predict the mode of cleavage of larger compounds and obtain characteristic peaks (Silverstein et al. 1974), but on the other hand, one compromises the integrity of the whole compound. Many fragments obtained from methylated samples can rearrange and obtaining the molecular formula of the original compound becomes difficult. The results here are preliminary findings that can be used for a more elaborate process that requires Nuclear Magnetic Resonance (NMR) procedures and repeating gc/ms with non-methylated material using a higher temperature program that can detect glycerols more accurately. Here I used Cluster Analysis and PCA analysis of the oil-related compound matrix as a way of reducing the data to clusters (or groups) utilizing the variability generated by the data. This is the first report on the chemistry of these orchid species, except for *Oncidium ornithorrhynchum* whose oil chemistry has been reported using a different method of oil analysis than the one described here (Vogel 1974).

PCA and Cluster analysis results were somewhat different. Both phenetic techniques use different algorithms and use different assumptions to calculate groupings. The hierarchical cluster analysis seems to be more effective in joining groups according to pollinator diversity. The PCA analysis showed 4 main groups (Fig. 2-4) and the Cluster analysis showed 2 main groups (Ecuadorian *Sigmatostalix* spp. and all the rest, Fig. 2-5, Fig. 2-6). These groups are different, except for the Ecuadorian *Sigmatostalix* group, which remains the same across these two phenetic techniques. Factor 1, Factor 2, and Factor 3 from the PCA analysis accounted for over 50% percentage of the variance, therefore looking at the PCA is an effective way of selecting clusters (Manly 2000). PCA makes no assumptions about group memberships but gives the fewest possible dimensions to examine the data while maximizing its variation (Manly 2000).

Both the PCA analysis and the Cluster Analysis found patterns that seem to correlate with geographical regions and pollinator diversity and do not correlate with phylogenetic relationships. Clusters that can be explained by pollinator diversity suggest that there is biochemical convergence, and that oil-collecting bees seem to be the mediators of this convergence. Further evidence supports that *Byrsonima crassifolia*, a malpighiaceous species, has oil-related compounds that are very similar to those of mimicking orchids, and that both malpighiaceous species and orchids can be pollinated by the same oil-collecting bees. In the cluster tree (Fig. 2-6), two of the categories discerned involved *Centris* bees, and the orchid species in these groups have large yellow flowers with oil-related compounds; presumably they are mimics of malpighiaceous species.

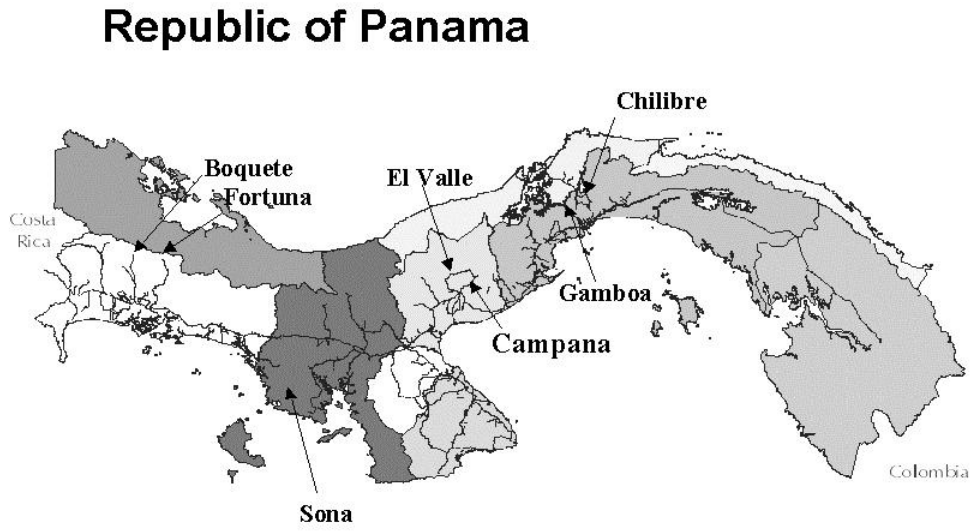

Figure 2-1. Map of the Republic of Panama with collection localities.

```
Library Searched : C:\DATABASE\WILEY275.L
Quality          : 96
ID               : Nonacosane (CAS) $$ n-Nonacosane $$ Celidoniol, deoxy-
```

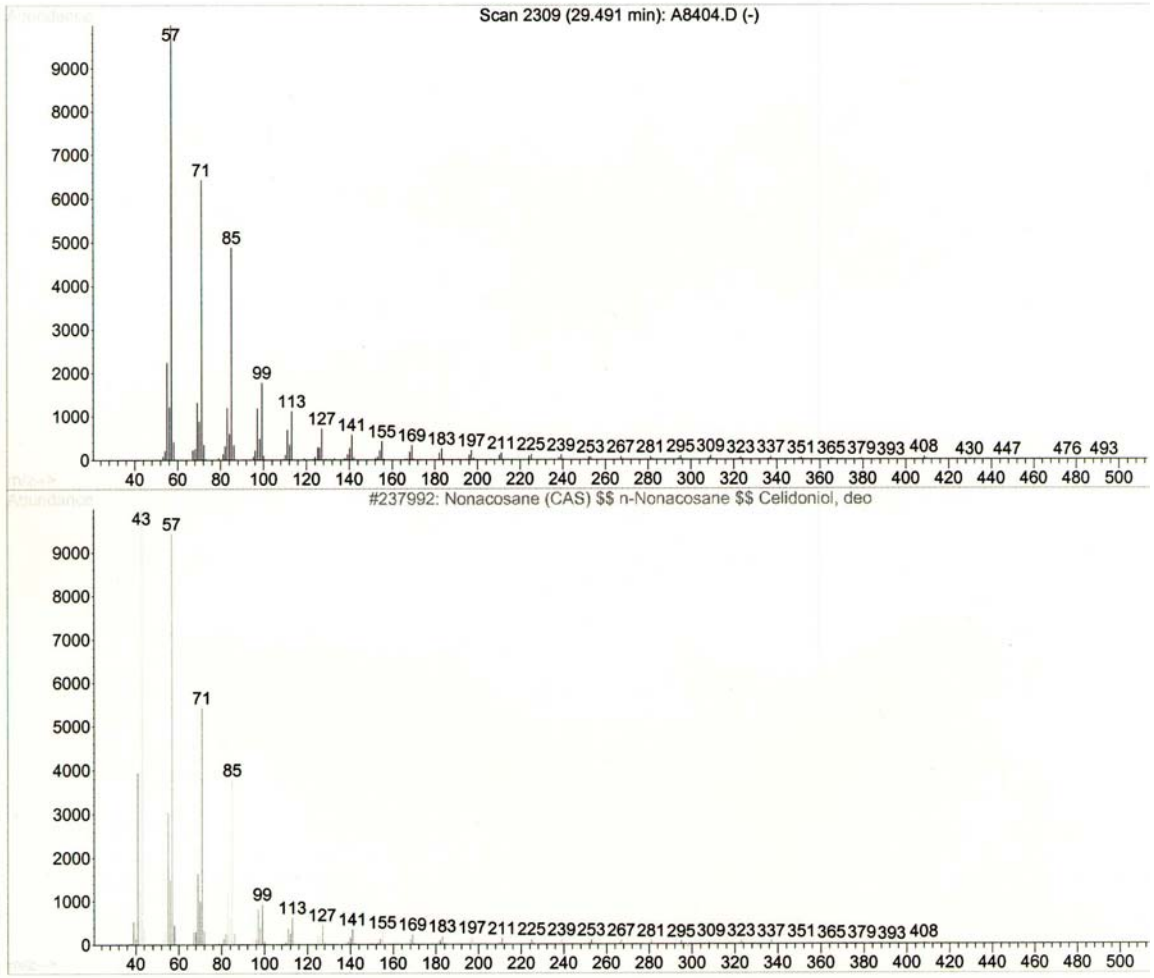

Figure 2-2. Mass spectrum of nonacosane. Top panel represents the mass spectrum found in the sample. Bottom panel represents library match.

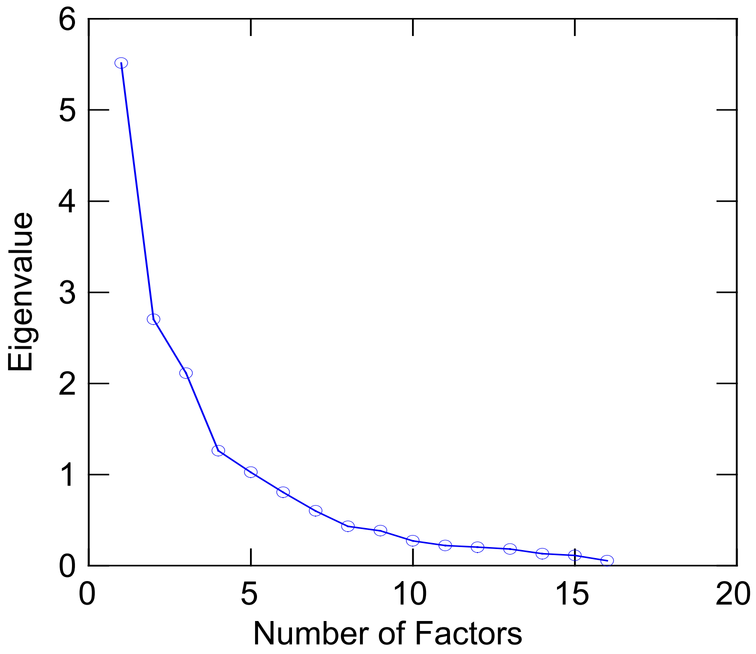

Figure 2-3. Scree plot of Eigenvalues and number of factors. Factor 1, Factor 2, and Factor 3 account for the majority of the variance.

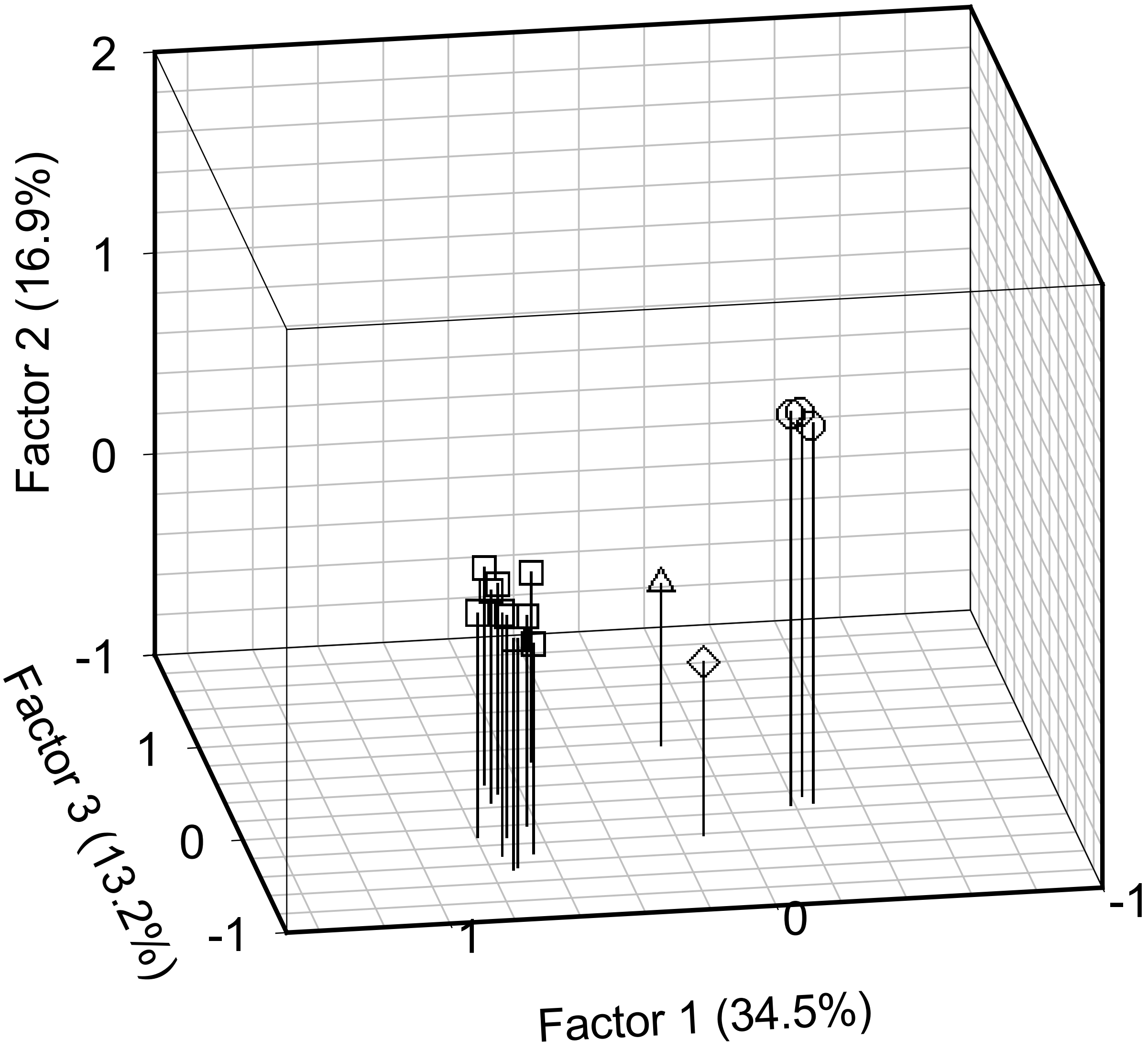

Figure 2-4. Principal Component Analysis (PCA) performed on 130 different oil-related compounds and 16 variables. Open circles represent group formed by *Sigmatostalix* spp. (Ecuadorians). Open squares represent group formed by *Onc. ornithorrhynchum*, *Onc. cheirophorum*, *Trichocentrum* spp., *Sigmatostalix picturatissima*, *Ornithocephalus* spp. and *Lockhartia* spp. Open triangle represents oils from bee's leg (*Paratetrapedia* sp.). Open diamond represents oils from *Byrsonima crassifolia* (Malpighiaceae).

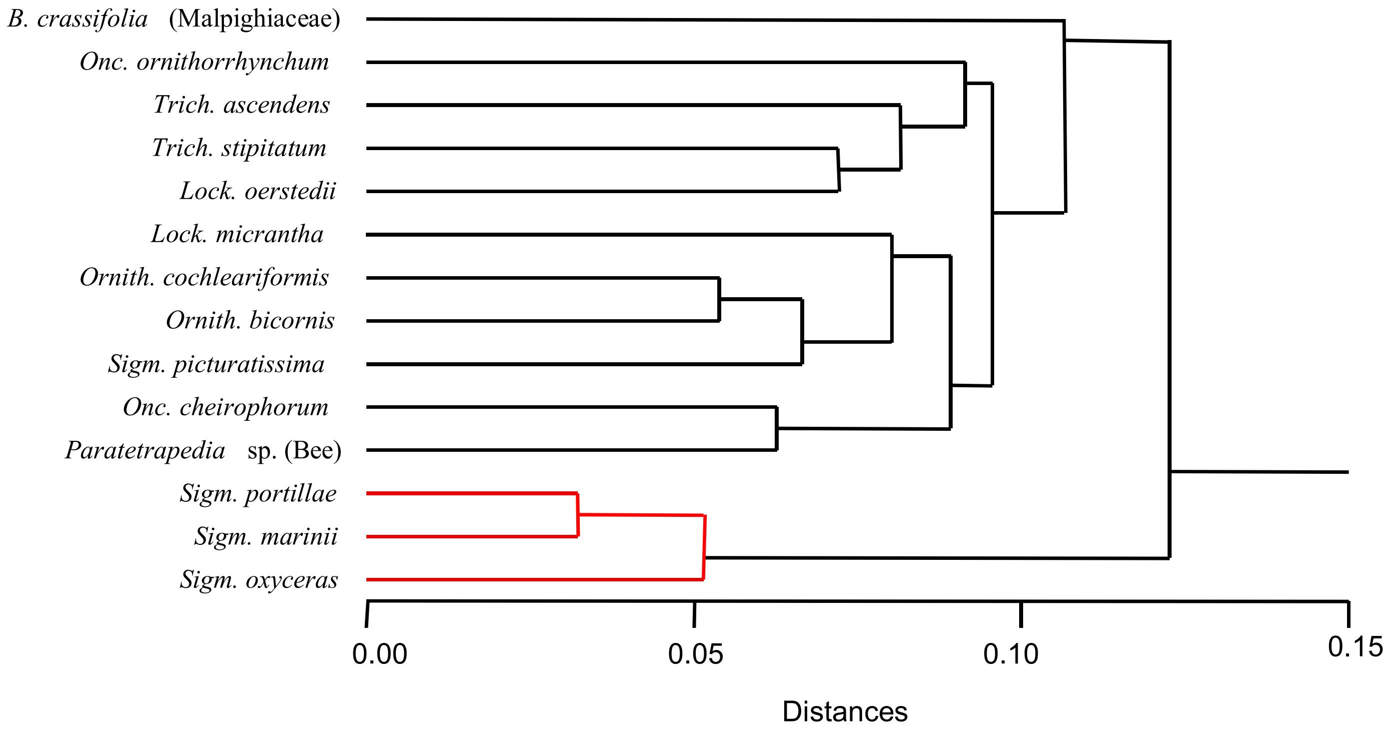

Figure 2-5. Cluster tree of oil-related compounds. Red color represents the Ecuadorian cluster. Black represents Panamanian cluster.

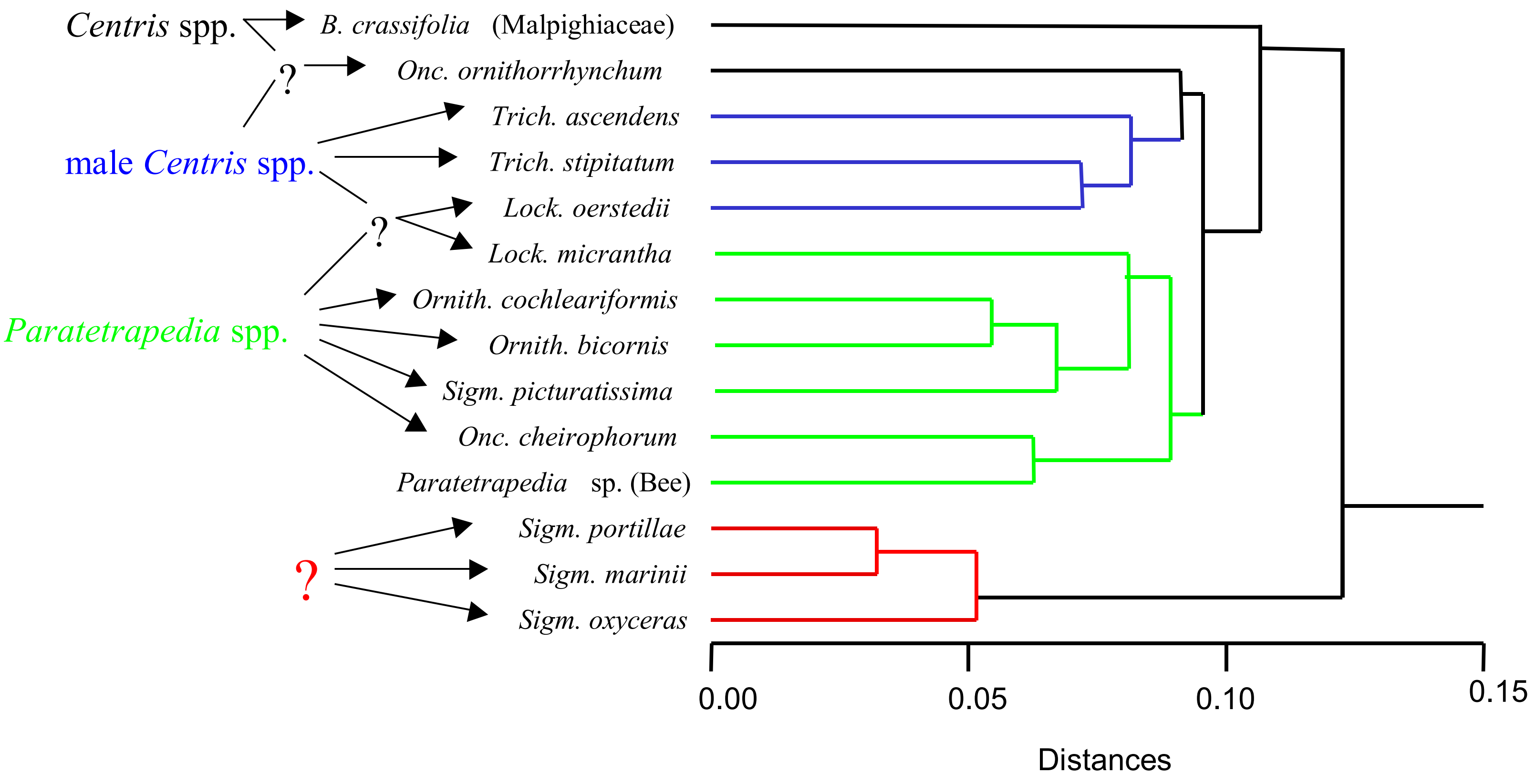

Figure 2-6. Cluster tree of oil-related compounds correlated with pollinator diversity. Colors represent different categories according to pollinator groups. Question marks represent unknowns for pollinators. References for pollinator diversity are given in Table 1-1.

CHAPTER 3
FLORAL VISITATION AND POLLINATION OF ORCHIDS BY OIL-COLLECTING BEES

Introduction

Literature Review

Bees that can collect and utilize floral lipids occur in the New and Old World, but occur most commonly in the neotropics, especially in subtropical regions of North and South America (Buchmann 1987). The ability to collect and utilize floral oils has been found primarily in five tribes and four families of bees (Roubik 1989; Steiner 1998). The majority of these bees are female, solitary bees that provision the nests with mixtures of pollen and nectar. Most of these bees belong to the families Melittidae, Ctenoplectridae, Anthophoridae, and Apidae (Buchmann 1987; Roubik 1989). The most important oil-collecting bee family in the neotropics appears to be Anthophoridae with three tribes, seven genera, and over 400 species adapted to collect floral lipids. Anthophorid tribes with oil-collecting bees are: Centridini (*Centris*, *Ptilotopus*, and *Epicharis*), Exomalopsini (*Paratetrapedia*, *Tapinotaspis*, and *Chalepogenus*) and Tetrapediini (*Tetrapedia*) (Roubik 1989). Many species (e.g. *Centris* spp.) are known to collect floral lipids from a diverse number of neotropical oil-secreting plant species.

Oil-collecting bees have specialized structures on the fore-legs (combs) used to harvest oils from floral elaiophores. These combs have been found exclusively in some members of the families of oil-collecting bees mentioned above, and are mainly modifications of basitarsal setae. These setae have been modified into rows of combs and setal pads of different size, located on the forelegs and midtarsi of the bees. They also have a unique set of combs located on the frontlegs to collect the oils directly from the flowers. These modified combs are usually flattened, curved at the tip, with additional two to five large setae that are called the "hood organ" (*sensu* Vogel 1974). The hood organ is located on the ventral surface of the anterior and middle basitarsi and is used to rupture the elaiophores so that oils may flow out of flowers.

Floral Visitation and Bee-Behavior in Oil-Secreting Flowers

Territorial behavior from male *Centris* bees in *Trichocentrum stipitatum* (formerly *Oncidium stipitatum*) has previously been observed by Robert L. Dressler in Panama (Cingel 2001). Male *Centris* bees use flower clusters as the focal point where they meet females, and guard them against other male *Centris* bees. *Trichocentrum stipitatum* has long pendent inflorescences that move gracefully with wind, and the flowers appear to imitate flying male bees in the focal point, thus arousing aggression from the territorial male *Centris* bees. When the flower moves with the wind, the bee hits the flower in an attempt to defend his territory. The bee does not land but receives pollinia on the front of its face and transports it to the next flower in the following aggression episode (Dodson and Frymire 1961; Cingel 2001). This mode of pollination has been described as "pseudotrespassing".

Female bees are known to collect the oils from *Byrsonima crassifolia* in the same region where *Trichocentrum stipitatum* grows (personal observations). I therefore argue that female bees may also be effective pollinators of this species since *Trichocentrum stipitatum* produces floral lipids. Both species grow in the same area, produce similar inflorescences, and flowers have similar temperature regimes that may be related to bee's oil-collecting activity. In this study, I discuss the pollination of *Oncidium cheirophorum*, based on personal observations of floral visitations in a cloud forest of Panama. Finally, I include the results of a brief study on fruit production of three orchid species that have large yellow flowers and are probable mimics of malpighiaceous species: *Oncidium klotzschianum*, *Trichocentrum stipitatum* (formerly *Oncidium stipitatum*) and *Oncidium cheirophorum* using a floral-oil abundance gradient.

Material and Methods

Study Site and Oil-Flower Visitation

This study was conducted in the Republic of Panama, at various sites, including lowland and cloud forests. Observations on the floral visitation of both *Byrsonima crassifolia* and *Trichocentrum stipitatum* were performed in Chilibre, at 50 m above sea level, in lowland Panama (see Fig. 2-1 for locality). I focused my observation on one emergent tree of *Byrsonima crassifolia* that had approximately 878,000 flowers when in full bloom (flowers per inflorescence = 69 ± 18; inflorescences per braches = 138.4 ± 30.8; branches per tree = 92 ± 14). Two blooming plants of *T. stipitatum* were placed on separate branches of *Byrsonima crassifolia*, so that observations could be done simultaneously. *T. stipitatum* grows naturally on trees of *Byrsonima crassifolia* in the wild (personal observations). *B. crassifolia* starts blooming in February and stays in bloom from March to June with flowers that last about 6 weeks. Floral visitations in fresh flowers were observed during nine non-consecutive days: February 17, 21, 23, 24, March 21 to 23 and April 2, 3, and 9 of 2001 for a total of 34 hours. The observations where conducted from early morning hours (7:00 a.m.) to midday. However, when pollinator activity was high, observations lasted until 4:00 p.m. When *B. crassifolia* was in full bloom (March), the peak of pollinator activity was between 9:00 a.m. and 11:00 a.m.

Vouchers for *Byrsonima crassifolia* and *Trichocentrum stipitatum* are deposited in the herbarium of the University of Florida (FLAS, see Appendix B). Insects gathering floral lipids from flowers of both species were collected using an insect net. Most bees that gather floral lipids from *B. crassifolia* are extremely fast flyers, so whenever possible, bees were collected, pinned and stored in insect boxes for identification. Vouchers of all insects collected will be deposited in the Smithsonian Tropical Research Institute (STRI) insect collection (see Appendix C). Floral visitation in *Trichocentrum stipitatum* was extremely rare, therefore observations were very limited.

Flower Temperature

Flower temperatures were measured in two flowers of two different inflorescences of both *Trichocentrum stipitatum* and *Byrsonima crassifolia* using copper-constantin thermocouples attached to a dataloger (Model LI-COR 1000, Li-Cor Inc., Linconln, NE, USA). Measurements were recorded every five minutes for three days (February 21-23, 2001). Variation in flower temperature was compared with bee's oil-collecting activities and visitation activity was compared among plant species.

Oncidium cheirophorum Flower Visitation

Observations of *Oncidium cheirophorum* flower visitations were performed in a healthy population of about 50 plants at Fortuna, 1,061 m above sea level, in a cloud forest in Panama (see Fig. 2-1 for locality). Plants had an average of seven bulbs including old bulbs from previous years. Plants start blooming in late November and early December, with peak of blooming at the end of December. Flowers produce oils from epithelial and trichomal elaiophores throughout petals, sepals and the callus. Flowers are greenish yellow with an average 12 ± 7 flowers per inflorescence.

Observations were performed from 7:00 a.m. to 1:00 p.m. on December 20-21, 2001 for a total of 12 hours. Bees collecting oils from flowers were collected with an insect net, pinned, and identified.

Fruit Production of Malpighiaceous Mimic Orchid Species

Female reproductive success (capsule formation) was measured in three orchid species that are mimics of malpighiaceous species: *Oncidium klotzschianum* growing in Fortuna at 1,061 m above sea level, *Trichocentrum stipitatum* growing in Chilibre at 50 m above sea level, and *Oncidium cheirophorum* growing in Fortuna at 1,060 m above sea level. An oil-abundance gradient among these 3 species was determined using the total ion abundance of compounds found in each species by analysis of gas chromatography and mass spectrometry. Number of flowers per plant and number of capsules produced per plant were measured in plants growing in natural populations for each species in order to calculate the mean capsule production per species. Eighteen plants for *Oncidium klotzschianum* with up to 200 flowers per plant, 25 plants for *Trichocentrum stipitatum* with up to 400 flowers per plant, and 20 plants for *Oncidium cheirophorum* with up to 85 flowers per plant, were used, for a total of 63 plants. Results were analyzed using the non-parametric Kruskal-Wallis test statistics.

Results

Byrsonima crassifolia and Trichocentrum stipitatum Floral Visitation

Flowers of *Byrsonima crassifolia* were mostly visited by members of the tribe Centridini (*Centris* and *Epicharis*), Exomalopsini (*Paratetrapedia*) and to a minor extent Tetrapediini (*Tetrapedia*) and Trigonini (*Trigona*). Table 3-1 shows a list of the species found on different days of observations. All results are based on observations of only collected individuals. There were many observations in which I was not able to collect the individual observed, and these observations were eliminated from the study since identification of bees was uncertain. *Paratetrapedia* sp. 1 was the most commonly collected oil-gathering bee for both *B. crassifolia* and *T. stipitatum* because this species tends to stay for long periods of time on the flowers collecting oils and is therefore easy to observe and collect. Large quantities of *Centris* species visited *B. crassifolia* at the end of March when the tree was in full bloom, strongly suggesting that the species of *Centris* are the primary pollinators of *Byrsonima crassifolia*.

Observation of visitation of *Trichocentrum stipitatum* was extremely rare, even when the plants were in full bloom. *Trigona corvina* Cockerell*, Trigona muzoensis* Schwarz, and *Paratetrapedia* sp. 1, were observed approaching flowers and spending from forty seconds to a minute if not disturbed, probing the flower and gathering oils with their forelegs. On only one occasion, *Paratetrapedia* sp. 1 approached the flower in hovering movements, landed on the callus of the flower, gathered oils with the forelegs, flew a few centimeters away from the flower, passed the oils from frontlegs to the hindlegs in mid air, and landed on the callus for a second round of oil gatherings. It performed this sequence of movements two more times before leaving the flower. *Paratetrapedia* spends more time in the flowers than other oil-collecting bees, but it is a very small bee, and on no occasion did I observe pollinia removal. This suggests that it may not be the pollinator of *Trichocentrum stipitatum* but merely a visitor because it is too small to remove the pollinia efficiently. On the other hand, *Centris inermis* Friese was observed approaching the flower, but it would only spend a second before moving to *Byrsonima* flowers. *Centris* bees, in general, spend just one to three seconds at or on each flower before moving to another one (personal observations), and when *Byrsonima.*

Table 3-1. List of pollinators and visitors for species of oil-secreting flowers of *Byrsonima crassifolia* and *Trichocentrum stipitatum*. Observations of *Cassia fistula* and *Plumeria* sp were included for relevant discussion.

Date (2001)	Observation time	Plant species	Insect	Activity
Feb 17	60 min	*Byrsonima crassifolia*	*Paratetrapedia* sp.1*	co-g
			Paratetrapedia moesta Cresson	co-g
Feb 21	395 min	*Byrsonima crassifolia*	*Paratetrapedia* sp.1*	co-g
		Plumeria sp.	*Trigona corvina* Cockerell*	cr
			Paratetrapedia moesta Cresson	cr
			Scaptotrigona luteipennis De la Torre	cr
Feb 23	345 min	*Byrsonima crassifolia*	*Paratetrapedia* sp. 1*	co-g
			Trigona angustula La Treille	cp
			Paratetrapedia moesta Cresson	co-g
			Eulaema nigrita Lepeletier	a
		Plumeria sp.	*Trigonia corvina* Cockerell*	cr
		Trichocentrum stipitatum	*Trigona muzoensis* Schwarz	co-c
Feb 24	182 min	*Byrsonima crassifolia*	*Paratetrapedia* sp. 1*	co-g
			Paratetrapedia moesta Cresson	co-g
		Trichocentrum stipitatum	*Trigona corvina* Cockerell	co-c
March 21	242 min	*Byrsonima crassifolia*	*Paratetrapedia* sp. 1*	co-g
			Paratetrapedia moesta Cresson	co-g
March 22	60 min	*Byrsonima crassifolia*	*Paratetrapedia* sp. 1*	co-g
			Paratetrapedia moesta Cresson	co-g
			Tetrapedia cf. *maura* Cresson	co-g
			Centris bicornuta Mocsary*	co-g
			Frieseomelitta nigra Lepeletier	cpo
			Epicharis maculata Smith	co-g
			Centris trigonoides Lepeletier*	co-g
			Cephalotrigona capitata Smith	f
			Trigona fuscipennis Friese	f
		Cassia fistula	male *Centris trigonoides* Lepeletier	t
		Trichocentrum stipitatum	*Paratetrapedia* sp. 1*	co-g
March 23	153 min	*Byrsonima crassifolia*	*Paratetrapedia* sp. 1*	co-g
			Epicharis maculata Smith*	co-g
April 2	240 min	*Byrsonima crassifolia*	*Paratetrapedia* sp.1*	co-g
			Paratetrapedia moesta Cresson	co-g
			Trigona muzoensis Schwarz	co-g
			Trigona fulviventris Guérin-Méneville	co-g
			Centris inermis Friese*	co-g
		Trichocentrum sitpitatum	*Centris longimana* Fabricius*	co-g
			Trigona mozoensis Schwarz*	co-g
			Centris inermis Friese*	co-g
April 5	120 min	*Byrsonima crassifolia*	*Epicharis rustica* Olivier*	co-g
			Paratetrapedia sp. 1*	co-g
April 9	60 min	*Byrsonima crassifolia*	*Paratetrapedia* sp. 1*	co-g
			Epicharis rustica Olivier*	co-g

* species commonly found visiting flowers at specific observation days; co-g: collecting oils from sepal glands; cr: collecting resins in stems; cp: collecting pollen; a: approaching flowers; co-c: collecting oils in callus; cpo: collecting pollen and oils; f: foraging; t: territorial behavior, striking pendent inflorescences

flowers are present, they do not seem interested in spending time at or on flowers of *Trichocentrum stipitatum.* Territorial behavior from male *Centris* bees was not observed in relationship to orchids in this study. On one occasion, I observed territorial behavior of male *Centris trigonoides* Lepeletier hitting and chasing flowers of *Cassia fistula* L., a tree that grows in the same area where all other observations were conducted. *Cassia fistula* has pendent bright yellow inflorescences that can imitate other male *Centris* bees when moved by the wind. Females of *Centris trigonoides* are known to collect oils from malpighiaceous species and it was a common species found gathering oils from *Byrsonima crassifolia*.

Flower Temperature of Oil-Flowers

Flower temperature regimes among *Byrsonima crassifolia* and *Trichocentrum stipitatum* appear to be similar (Fig. 3-1B; 3-1C). There is an increase in air temperature as the day progresses and reaches a peak at midday with temperatures of 33 C, then decreases until 0600 h of the following day when it starts increasing again (Fig. 3-1A). Interestingly, each of the peaks found above the zero line in Fig. 3-1B and 3-1C relates with pollinator activity. These peaks represent points at which the oil-flowers were slightly warmer than air temperature. For example, the peak of insect visitation for *Byrsonima crassifolia* is between 8:00 a.m. and 11:30 a.m., just before noon, the hottest time of the day. Between these hours flower visitation is at peak and flowers are slightly warmer than air temperature. Additionally, the last peaks found on Fig. 3-1C for *Trichocentrum stipitatum* relates with the observations found in February 23, 2001 for this species. I observed *Trigona muzoensis* visiting flowers of *T. stipitatum* exactly at the time of the last peak in Panel 3-1C (mean flower temperature= 30 C, 15:26 p.m.). There seems to be insect attraction at the surface of fresh oil-secreting flowers when the flower temperature is slightly warmer than air temperature.

Oncidium cheirophorum Floral Visitation

Oncidium cheirophorum produces flowers that are visited by an undescribed species of *Paratetrapedia*. Floral visitation was observed on fresh opened flowers of *Oncidium cheirophorum* at Fortuna. Activity started at 10:30 in the morning and bees were actively gathering oils for about two hours. The bee approached the flower and gathered oils from the sepals and petals for up to one minute, then left the flower and transferred the oils from the forelegs to the hindlegs in the air. The bee returned to the same flower once more and repeated the same movements. Bees also probed and visited other flowers instead of returning to the same one. One bee can visit many flowers even on different inflorescences if they are close to each other. This method of gathering lipids is similar to the one observed in pollinators of *Byrsonima crassifolia* and *Trichocentrum stipitatum*. Figure 3-2 shows a sequence of photos related to the visitation of *Oncidium cheirophorum* by *Paratetrapedia* sp. I was able to observe more floral visitation events in *Oncidium cheirophorum* than in any other species of oil-secreting orchids, which allowed time to photograph some of the visitation events.

Female Reproductive Success in Oil-Secreting Orchids

Orchids that produce oils may benefit by an increase in visitation and as a consequence, an increase in fruit production. Here I found that as oil abundance increases, there is an increase in fruit production in orchids that mimic members of the Malpighiaceae (Fig. 3-3). These differences are significant (Kruskall-Wallis non parametric test statistic = 49.105, $p<0.001$).

Discussion

Pollination of Byrsonima crassifolia and Trichocentrum stipitatum

Flowers from *B. crassifolia* were visited by many species of the family Anthophoridae, especially bees from the Tribe Centridini. It has been found that the genus *Centris* is the primary pollinator of species of *Byrsonima* (Albuquerque and Rego 1989). Additionally, other genera also visit the flowers and harvest the oils as well. It is interesting that bees that visited flowers of *Trichocentrum stipitatum* are known to pollinate *B. crassifolia*. This suggests that these species share similar visitors and pollinators. However, when *B. crassifolia* is in full bloom, visitation in *Trichocentrum stipitatum* is limited. In natural populations, *Trichocentrum stipitatum* blooms a few weeks before the beginning of the blooming season for *B. crassifolia*. I have observed *T. stipitatum* blooming in branches of *B. crassifolia* that are just developing flowering buds. This non-overlap in blooming time may be an advantage in attracting pollinators and reducing competition, which otherwise would occur if both species bloomed at the same time. Even though I did not find pollinia attached to the body of the bees collected, I suspect that a pollinator for *Trichocentrum stipitatum* is *Centris inermis*, because it has the appropriate size to detach the pollinia when gathering oils from the callus. *Trigona corvina* and *Trigona muzoensis* were also found gathering oils from *T. stipitatum*, but I

suspect these species may be only visitors because their size may be too small to detach pollinia efficiently. Also, *Paratetrapedia* sp 1. was found gathering oils from the callus of the *T. stipitatum*. This may also be a pollinator, but after spending many seconds hovering and harvesting oils, it did not approach the pollinia. There is a possibility that these bees may remove the pollinia by chance alone, if frequent visits are done to different flowers. More observations are needed to confirm whether or not these species of bees are pollinators for *T. stipitatum*.

Female bees of *Centris versicolor* Fabricius are known to visit flowers of *Tolumnia lucayana* (Nash ex Britton & Millsp.) Braem in Grand Bahama (Nierenberg 1972). The bees hover in front of the flower and land in the center of the flower looking for food or reward and leave a few seconds after finding out that the flower offers no reward. If the bee is positioned correctly, it will detach the pollinia from the orchid and carry it on the body. The similarity between orchid flowers and those of malpighiaceous species in the area could allow the bee to make another mistake confusing an orchid flower with a malpighiaceous species and transport the pollinia into the stigma of the orchid flower. The mimicry of malpighiaceous species by orchid species would apply to female bees, since they are looking for oils and can be deceived. Also, the similarity between these flowers, as shown by ultraviolet absorption patterns, appears to be key in the attraction-deception of female *Centris* bees (Nierenberg 1972).

Other species of *Oncidium* are pollinated by Anthophorid bees (mostly Tetrapediini) of the genera *Paratetrapedia* and *Tetrapedia*. For example, in Southern Brazil, Paraná, *Oncidium paranaense* Kraenzl. has been shown to be pollinated by *Tetrapedia diversipes* Klug, gathering oil from two elaiophores on the lip (Cingel 2001). Buchmann (1987) mentioned that *Tetrapedia* species are perhaps the probable pollinator of most oil-secreting orchids in the neotropics. *Tetrapedia* was not observed visiting flowers of *T. stipitatum*, however, it did visit flowers of *Byrsonima crassifolia*.

Trichocentrum ascendens in Yucatán, Mexico is pollinated by *Trigona nigra* Cress, a native bee that searches for resin (Parra-Tabla et al. 2000). In Panama, two species of

Trigona (*T. corvina* and *T. muzoensis*) were observed gathering lipids from both *B. crassifolia* and *T. stipitatum*. *Trigona corvina* was commonly found collecting resins from stems of *Plumeria* sp. in the same area as *B. crassifolia*, suggesting that this species specializes in oil and resin collecting. The fact that *Trichocentrum stipitatum* produces pendent inflorescences, blooms adjacent to malpighiaceous species, and shares similar visitors and pollinators suggest that this group may constitute a mimetic system, in which *Byrsonima crassifolia* is the model and *Trichocentrum stipitatum* the mimic. There are two additional conditions that were not tested but are necessary to conclude that there is Müllerian mimicry between these species: (1) the pollinator should be necessary for fruit set, (2) the similarities between species should enhance plant fitness. Similarities would determine whether there is an adaptive condition. Pollinators and visitors seem to move freely between species and phenology overlaps enough to exploit local pollinators while minimizing competition.

Physiologically, both species are capable of attracting oil-gathering bees. It is interesting that as flowers become slightly warmer than air temperature, bees are attracted to the flowers. This may be due to the oils being volatilized and available for insects at this specific point. There seems to be a relationship between bee visitation and the point in which flowers are slightly warmer than air temperature. It is possible that bees detect changes in flower temperature and this difference plays an important role in oil-secreting flower pollination.

Evidence suggests that the pollination of *Trichocentrum stipitatum* is truly based on a reward system mediated by oil-collecting bees, and does not constitute a deceit system as is found in the majority of neotropical orchid species. *Trichocentrum stipitatum* flowers produce rewards for oil-gathering bees and the mechanisms of attraction are similar to those of the model species *Byrsonima crassifolia.*

Capsule Production and Reproductive Success in Oil-Secreting Flowers

Orchids that mimic members of the Malpighiaceae can also produce an oil-reward. I found that oil production is positively correlated with fruit production. As mimic orchids produce more oil-reward compounds, there is an increase in capsule production, which may be related to an increase in pollinator visitation. This gradient goes from mimic species that produce little to no oil (e.g., *Oncidium klotzschianum*) to species that produce large amounts of oil-reward compounds (e.g., *Oncidium cheirophorum*). Interestingly, there seems to also be a gradient of mimicry systems. Orchid species can go from Batesian mimicry, producing no reward and exploiting the pollinators of malpighiaceous species (e.g., proposed mechanism for *Onc. klotzschianum*), to Müllerian mimicry, producing reward compounds that are utilized by oil-collecting bees that also pollinate malpighiaceous species. This mimicry gradient suggests that pollinator availability may be acting as a selective force in determining which mimicry system may be favored. There seems to be one critical question related to this mimicry gradient. Why would orchid species develop deceit systems (Batesian mimicry) if it negatively influences plant reproduction by decreasing chances of pollinator visitation and pollination events? Deception mechanisms can be seen as a two way process: (1) long distance attraction has to occur and (2) pollinator behavior needs to be stimulated. Long distance attraction can be gained by flower coloration alone (Johnson 1994). For example, mimic orchids may produce flowers that resemble in color and shape the flowers of malpighiaceous species. Eliciting collecting behavior in bees is the second process, and color alone will not provoke oil-gathering behavior (Johnson 1994). One explanation is that it is energetically advantageous to not produce a costly reward at the expense of reduced pollination success (Johnson 1994). Orchid flowers may produce areas in the callus of the flower that resembles food, some non-oil producing species may also look shiny, deceiving the pollinator into thinking there is oil. Orchids have the advantage of having pollen packed into pollinia that can be attached to the body of the bees and by fooling the pollinator a few times, orchids can get the pollinia transferred among different flowers. The real task is to determine if reproductive success is being favored by deceit mechanisms in the long term. The majority of orchids seem to have evolved deceit mechanisms. The loss of nectar or rewards in general, combined with an effective mimicry system, could result in increase in plant fitness.

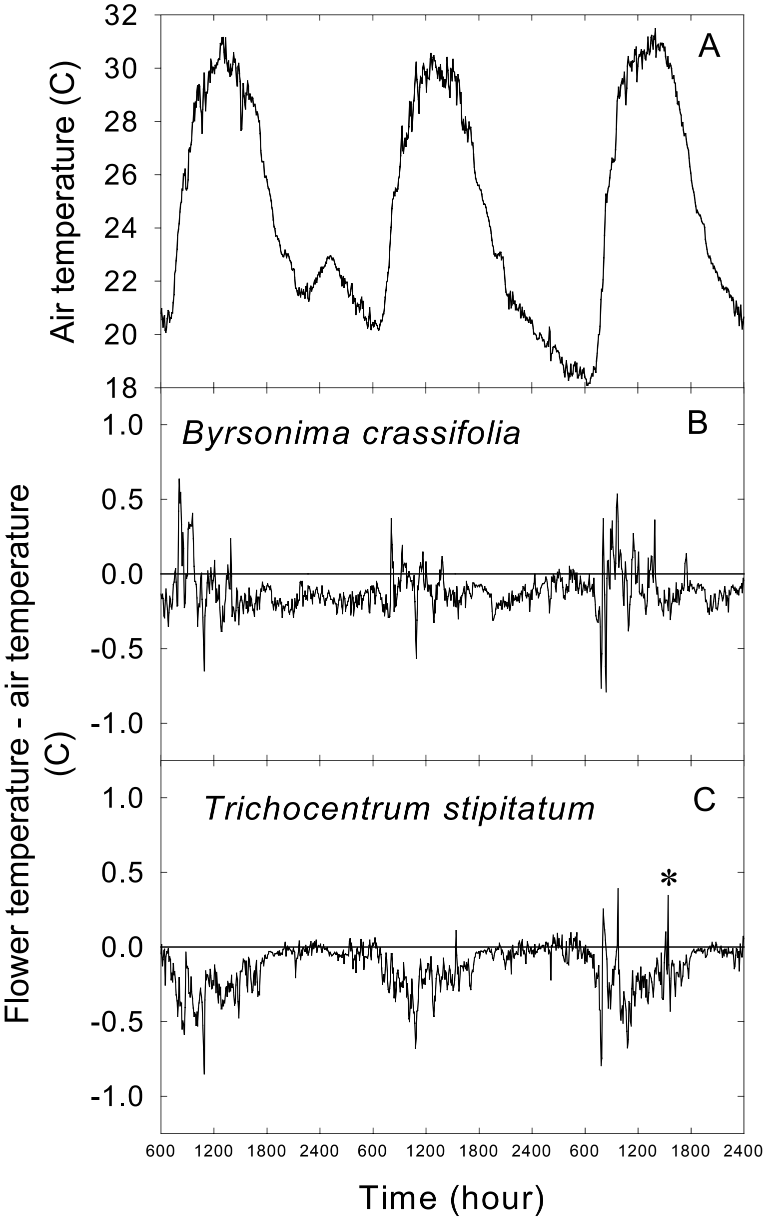

Figure 3-1. Flower temperature of two species of oil-secreting flowers measured during three consecutive days. Panel A represents air temperature measurements. Panel B and C represent the difference between air temperature and flower temperature for *B. crassifolia* and *T. stipitatum* respectively. * Represents temperature peak that matches pollination observation.

Figure 3-2. *Paratetrapedia* sp. gathering floral lipids from flowers of *Oncidium cheirophorum* in Fortuna, Panama.

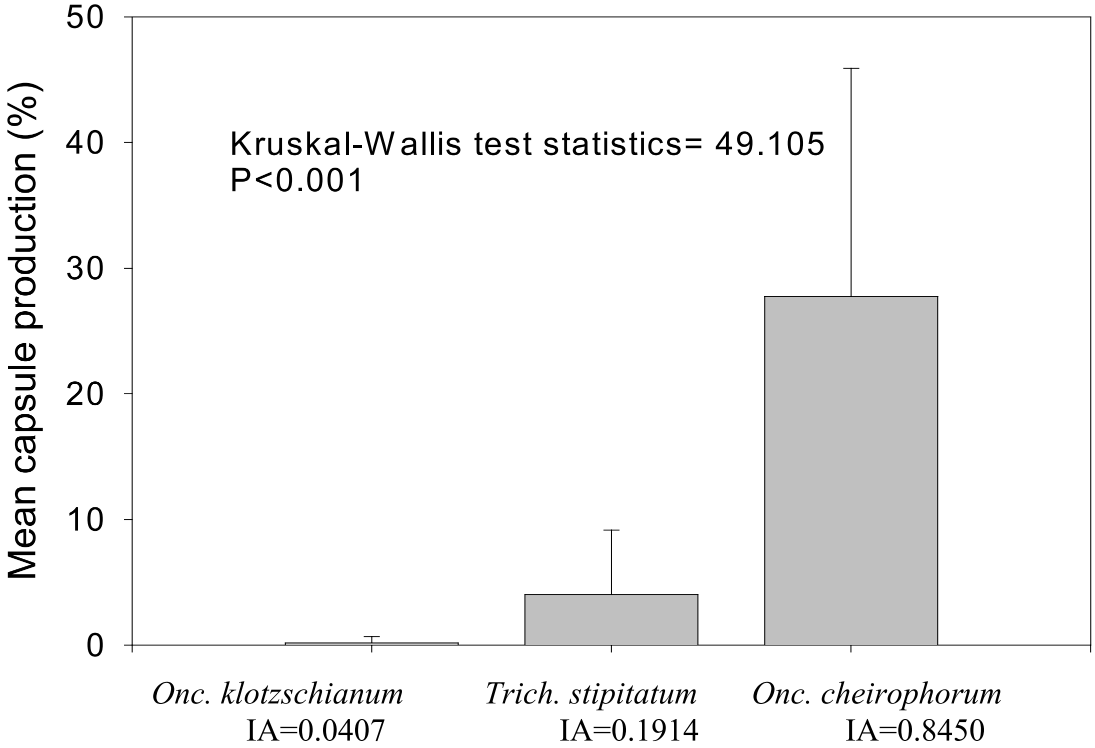

Figure 3-3. Female reproductive success (capsule formation) of three orchid species that mimic members of the Malpighiaceae; IA=Ion abundance.

CHAPTER 4
CONCLUSION

In conclusion, I present a brief summary of the most important results relating to the chemistry, pollination, and visitation of oil-secreting species. I conclude by giving insight into the evolution of oil-secretions in flowering plants.

The biochemical convergence found among various groups of orchids and malpighiaceous species suggests an evolutionary pressure towards utilizing a specific suite of pollinators. In other words, species containing similar oil-compounds share similar pollinators in a geographical region. Geographical regions and pollinator diversity are, therefore, the best predictors of biochemical convergence in this study. Phylogeny cannot be used to explain biochemical convergence of oil-reward compounds in these orchid species. Chemically, both malpighiaceous species and oil-secreting orchids may produce similar oil-related compounds that can attract similar pollinators in a given habitat. This type of mutualistic relationship can best be described as Müllerian mimicry. There is convergence, first in producing floral lipids in different clades, and second in producing flowers and oil-reward compounds similar to those of malpighiaceous species.

Biochemical, physiological and behavioral evidence suggests that the pollination of orchids with oil-secreting flowers is truly based on a floral-lipid reward system rather than an attraction-deception system, as is commonly found in orchids. Müllerian mimicry between orchids and malpighiaceous species seems to best describe the biochemical relationship between those groups. More field studies and further chemical analysis of flowers of Malpighiaceae and orchid species that grow in the same habitat may confirm the patterns found in this study.

The production of oils in orchid species is a polyphyletic trait, which has evolved at least five times in the subtribe Oncidiinae (Fig. 4-1). This independent production of floral lipids in different clades of orchids can be explained as an evolutionary strategy to enhance pollination by oil-collecting bees. This same pattern has been observed among angiosperms. Oil-secreting species have evolved independently in non-related angiosperm families, and in all cases seem to be an adaptation to attract oil-collecting bees (Buchmann 1987). It is possible that nectar, as a form of reward, evolved first and gave rise to floral lipids as a derived form of reward. It would be interesting to test this hypothesis using different phylogenies of families with oil-secreting species. Future studies should address a combination of chemical analysis with pollination observations in the field to understand the radiation of oil-secreting flowers in angiosperms and especially in orchids, a group with a high number of pollination syndromes.

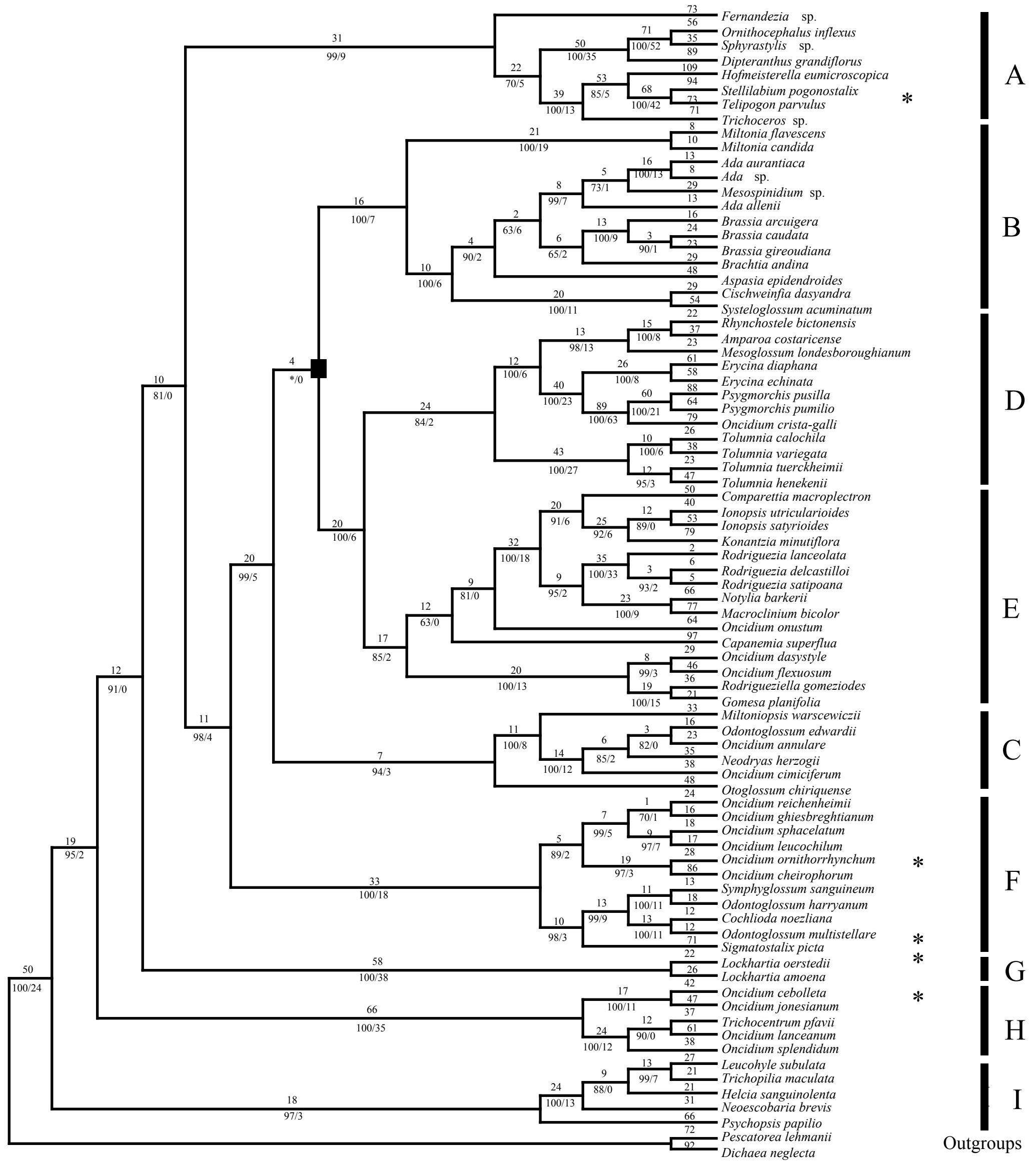

Figure 4-1. Single shortest tree for the Subtribe Oncidiinae from successively weighted analysis of combined data set (ITS/*matK*/*trnL-F*, Williams et al. 2001). * Representatives of oil-secreting orchid species. Major clades are indicated by letters. Fitch branch lengths (ACTRAN optimization) are indicated above the branches and bootstrap support percentages are indicated below the branches. More details are explained in Williams et al. (2001) (Reprinted with permission of the author).

APPENDIX A
LIST OF COMPOUNDS AND ION ABUNDANCE PER MASS OF FRESH FLOWER

Name of compound	**TS1**	**TS2**	**BC**	**OCH**	**BEE**	**SPO**	**SMA**	**SOX**	**OKL**	**TAS**	**LMI**	**OCO**	**ECR**	**OO1**	**OO2**	**SPI**	**OBI**	**LOE**	**EPU**
tetradecanoic acid, methyl ester	0	0	0	0	0.16	0	0	0	0	0	0	0.86	5.20	0	0	0	0.35	0.86	0
unknown 1	0	0	0	0.26	0	0	0	0	0	0	1.48	0	0	0	0	0.60	0	0	0
unknown 2	0	0	0	1.16	1.85	0	0	0	0	0	0	0	0	0	0	0	0	0	0
hexadecanal	0	0	0	2.20	1.42	0	0	0	0	0	0	0.46	0	11.81	3.82	0.45	0.34	0	0
2,4 Diphenyl-4-methyl-2(E)-pentene	0	0	0	0.69	0	0	0	0	0	0	1.68	0.69	0	0	0	0.29	0	0	0
phenol, 4-(1-methyl-1-phenylethyl)-	0	0	0	0	0	0	0	0	0	0	1.11	0	0	0	0	0	0	0	0
1,2-Benzenedicarboxylic acid, dibutyl ester	0	0	0	0	0	0	0	0	0	0	0	0	0	0.75	3.06	1.02	0.36	0	0
unknown 3	0	0	0	3.28	3.93	6.61	3.28	0	0	0	0	3.55	0	0	0	5.82	4.47	0	0
octadecanal	0	0	0	1.47	1.13	0	0	2.09	0	0	0	0.49	0	0.90	1.69	5.28	1.98	0	0
2-t-Butyl-4-(dimethylbenzyl)phenol	0	0	0	1.66	0	0	0	0	0	0	4.31	2.07	0	1.57	1.53	0.74	0.45	0	0
unknown 4	0	0	0	0.55	0	0	0	0	0	0	0	1.11	0	0.63	0	0	0	0	0
unknown 5	0	0	0	0	1.61	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 6	0	0	0	1.15	0	0	0	0	0	0	1.38	0	0	0	0	0	0	0	0
unknown 7	0	0	0	4.11	2.83	0	0	0	0	0.44	4.45	3.09	0	1.88	1.69	1.10	0.33	0	0
unknown 8	0	1.20	1.44	0	0.86	0	0	0	0	0.4	0	0	0	0.85	0	0	0.56	0	56.34
heneicosane	0	0	0	0	0	0	0	0	0	0	0	2.12	0	0	0	0	0	0	0
unknown 9	0	0	0	0	0	0	0	0	0	0	3.76	0	0	0	2.57	0.92	0	0	0
9-Octadecenoic acid (Z)- methyl ester	0	5.28	0	3.91	1.69	0	0	0	0	1.89	0	0	0	0	0	0	0	3.56	43.65
unknown 10	0	0	0	0	0	0	1.78	0	0	0	0	0	0	0	0	0	0	0	0
unknown 11	0	0	0	0	0	0	2.14	0	0	0	0	0	0	0	0	0	0	0	0
unknown 12	0	0	0	0	0	0	0	0	0	0	1.75	0	0	0	0	0.47	0	0	0
unknown 13	0	0	0	3.60	0	0	0	0	0	0	0	0	0	7.04	3.22	0	0	0	0
unknown 14	0	0	0	0	2.34	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 15 (isomeres)	0	0	7.49	0	0	0	0	0	0	0	0	1.98	0	0	0	1.47	4.34	0	0
docosane	0	0	0	1.56	0	0	0	0	0	0.52	2.83	2.15	0	0.69	1.85	1.11	0.90	0	0
unknown 16	0	0	0	0.39	0.56	0	0	0	0	0	0	0	0	0	0	1.16	0.81	0	0
unknown 17	0	0	0	0	0	9.47	3.93	0	0	0	0	0	0	0	0	2.29	0.45	0	0
unknown 18	0	0	0	0	0	6.86	3.29	5.99	0	0	0	0	0	0	0	0	0	0	0
unknown 19	0	0	0	0	2.53	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 20	0	0	0	0	0	0	0	0	0	0.42	0	0	0	0	0	0	0	0	0
octadecanoic acid, 3-hydroxy-methyl ester	0	0	0	5.32	3.01	0	0	0	0	0	0	0	0	15.73	0	1.04	0	0	0

Name of compound	TS1	TS2	BC	OCH	BEE	SPO	SMA	SOX	OKL	TAS	LMI	OCO	ECR	OO1	OO2	SPI	OBI	LOE	EPU
unknown 21	0	0	0	5.68	5.30	0	0	0	0	1.49	0	4.20	0	0	0	6.95	4.37	0	0
tricosane	1.49	2.74	0	4.73	2.01	0	0	0	0	3.08	4.95	6.54	0	4.92	4.05	3.9	4.06	2.04	0
eicosanoic acid, methyl ester	0	0	1.16	0	0	0	0	0	0	0.91	0.60	0	0	0	0	0	0	1.09	0
unknown 22	0	0	0	0.43	0.51	0	0	0	0	0	0	0	0	0	0	0.65	0.44	0	0
unknown 23	0	0	0	3.82	2.30	0	0	0	0	0	0	0	0	9.01	2.81	0	0.41	0	0
unknown 24	0	0	0	0	0	0	0	0	0	0	0	1.13	0	0	0	0.46	0	0	0
unknown 25	0	0	0	2.43	3.57	0	0	0	0	0	0	0	0	0	0	1.1	0.81	0	0
unknown 26	0	0	1.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0.45	0	0
unknown 27	0	1.89	0	0	0.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0
tetracosane	0	0	0.65	3.58	0	0	0	0	0	2.45	6.11	3.86	0	2.27	7.20	4.27	3.77	2.29	0
unknown 28	0	0	0	0	0	0	0	0	0	0	0.68	0	0	0	0	0	0	0	0
unknown 29	0	0	0	0	0	0	0	0	0	3.40	0	0	0	0	0	0	0	0	0
unknown 30	0	0	0	2.24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 31	0	0	0	0	3.42	0	0	0	0	0	0	0	0	0	0	0	0	0	0
hydroxy fame	0	0	0	0	0	6.67	6.97	8.93	0	0	0	0	0	0	0	0	0	0	0
unknown 32	2.06	1.3	0	0	0	0	0	0	0	0.35	0	0	0	0	0	0	0	0	0
unknown 33	0	0	0	2.54	6.18	0	0	0	0	0	0	0	0	0	0	4.32	0	0	0
unknown 34	0	0	8.44	0	0	0	0	0	0	5.07	0	1.03	0	0	0	0	3.94	0	0
docosanoic acid, methyl ester (1)	0	0	0	0	0	0	1.12	0	0	0	0	0	0	0	0	0	0	0	0
pentacosane	4.21	4.32	0	0	0	0	0	0	0	6.57	6.56	5.88	7.62	5.61	12.24	7.73	7.43	4.02	0
docosanoic acid, methyl ester (2)	0	0	0	0	0	0	0	0	0	0	0	0.9	0	0	0	1.30	2.12	0	0
unknown 35	0	0	0	1.71	4.71	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 36	0	0	0	0	0	0	0	3.06	0	0	0	0	0	0	0	0	0	0	0
unknown 37	0	0	0	0	0	4.76	2.65	0	0	0	0	0	0	0	0	0	0	0	0
unknown 38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.56	0	0.96	0	0
unknown 39	0	0	0	0	1.44	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 40	0	0	0	3.47	2.56	0	0	0	0	0	0	0.80	0	0	0	1.06	1.73	0	0
docosanoic acid, methyl ester (3)	1.25	1.52	2.97	0	0	0	0	0	9.59	0.98	0	0	0	0	0	0	0	0	0
unknown 41	0	0	0	3.10	6.25	0	0	0	0	0	0	0	0	0	0	4.02	3.08	0	0
unknown 42	0	0	0	0	0	0	1.14	1.45	0	0	0	0	0	0	0	0	0	0	0
hexacosane	1.71	2.43	1.02	4.38	0	0	0	0	0	5.22	6.267	6.83	0	3.46	11.48	9.75	6.21	2.63	0
unknown 43	0	0	0	3.99	4.89	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 44	0	0	0	0	0	0	0	0	0	1.03	0	0	0	0	0	0	0	0	0
unknown 45	0	0	0	4.71	5.42	0	0	0	0	0	0	3.29	0	0.86	0	5.45	3.97	0	0
unknown 46	0	0	0	0	0	0	0	0	0	2.94	0	0	0	0	0	0	0	0	0
unknown 47	0	0	0	0	0	28.92	33.8	26.37	0	0	0	0	0	0	0	0	0	0	0
1,13-Tetradecadiene	0	0	0	0	0	0	0	0	0	1.75	0	0	0	0	0	0	0	0	0
unknown 48	0	0	0	0	0	0	0	0	0	0	1.52	8.03	0	0.59	0	0	4.28	1.63	0
unknown 49	3.20	1.62	0	0	0.80	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1-Eicosanol	0	0	0	0	0	0	0	0	0	1.42	0	0	0	0	0	0	0	0	0
unknown 50	0	0	0	0.42	0	0	0	0	5.7	0	0	0	0	2.09	0	0	0	0	0
unknown 51	3.04	1.45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.31	0
unknown 52	0	0	0	0	0	0	0	0	0	0.55	0	0	0	0	0	0	0	0	0
unknown 53	0	1.69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Name of compound	TS1	TS2	BC	OCH	BEE	SPO	SMA	SOX	OKL	TAS	LMI	OCO	ECR	OO1	OO2	SPI	OBI	LOE	EPU
unknown 54	0	0	0.88	0	2.02	0	0	0	0	3.71	0	0	0	0	0	0	0	0	0
tetracosanoic acid, methyl ester	0	0	0	0	0	0	1.55	0	0	0	0	0	0	0	0	0	0	0	0
unknown 55	0	0	0	0	0	15.29	15.3	17.29	0	0	0	0	0	0	0	0	0	0	0
unknown 56	0	0	0	0	1.69	0	0	0	0	0	0	0	0	0	0	0	0	0	0
heptacosane	14.8	8.58	1.44	3.63	0	0	0	0	12.87	9.80	5.61	4.25	4.46	5.60	12.23	7.19	6.30	3.66	0
11,13-Eicosadienoic acid, methyl ester	0	0	1.51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 57	0	0	0	0.37	0	0	0	0	0	0	2.00	0	0	0	0	0.706	0	0	0
unknown 58	0	0	0	0	0	13.96	12.9	12.18	0	0	0	0	0	0	0	0	0	0	0
tetracosanoic acid, methyl ester	3.74	3.44	6.29	2.77	5.67	0	0	0	9.25	1.78	0	2.86	31.82	0	0	4.148	7.779	0	0
unknown 59	0	0	0.88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 60	0	0	0	0	0.86	7.43	7.56	17.67	0	0	0	0	0	0	0	0	0	0	0
unknown 61	0	0	0	0.97	1.57	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.152	1.48	0	0
unknown 63	0	0	1.08	0	0	0	0	0	0	0	0	0	0	0	0	0	3.27	0	0
unknown 64	0	0	6.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0.82	0	0
unknown 65	0	0	0	0	1.52	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 66	0	0	0	0	0	0	0	0	0	1.20	0	0	0	0	0	0	0	0	0
octacosane	3.34	3.77	6.15	0	0	0	0	0	0	5.18	3.37	0	0	3.31	6.31	7.25	0	2.84	0
unknown 67	0	0	0	3.74	4.90	0	0	0	0	0	0	5.17	0	0	0	0	0	0	0
unknown 68	0	0	0	0	0	0	0	0	0	2.01	0	0	0	0	0	0	0	0	0
unknown 69	0	0	7.42	0.54	1.09	0	0	0	0	0	0	1.03	0	0	0	0.53	3.24	0	0
unknown 70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.02	0	0	0	0
unknown 71	0	0	0	0	1.35	0	0	0	0	3.65	0	0	0	0	0	0	0	0	0
unknown 72	0	0	0	0	0	0	0	0	0	0	1.82	9.82	0	0.94	0	0	4.86	2.58	0
nonacosanol	3.97	0	0	0	0.58	0	0	0	0	1.4	0	0	0	0	0	0	0	0	0
unknown 73	5.11	2.42	0	0.55	0	0	0	0	12.86	0.92	0	0.70	2.05	2.64	0	0	0	1.90	0
nonacosane	21.8	19.3	0	1.66	0	0	2.42	0	49.72	7.6	18.89	4.28	0	4.92	8.52	2.46	3.79	64	0
unknown 74	0	0	2.18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 75	0	0	0	0	0	0	0	0	0	0	2.37	0	0	0	0	0	0	0	0
hexacosanoic acid, methyl ester	2.13	0	0	0	0.15	0	0	0	0	0.99	0	0	18.02	0	2.35	0	0.85	0	0
unknown 76	0	0	9.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 77	0	0	3.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 78	0	0	0	0	0	0	0	0	0	1.52	0	0	0	0	0	0	0	0	0
triacontane	2.54	3.70	0	1.47	0	0	0	0	0	0	1.78	2.74	0	2.77	2.85	1.33	1.15	2.22	0
nonadecane	0	0	0	0	0	0	0	0	0	2.92	0	0	0	0	0	0	0	0	0
unknown 79	0	0	4.69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 80	0	0	1.19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2,4,6-Tri (dimethylbenzyl)phenol	0	0	0	0.48	0	0	0	0	0	0	1.04	0.84	0	0	0	0	0	0	0
unknown 81	0	0	0	0	0.39	0	0	0	0	2.30	0	0	0	0	0	0	0	0	0
unknown 82	0	0	0	0	0	0	0	0	0	2.28	0	0	0	0	0	0	0	0	0
unknown 83	0	0	0	0.88	0	0	0	0	0	0	1.54	1.13	0	0	0	0	0	0	0
1-Dotriacontanol	2.74	0	0	0	0	0	0	0	0	1.73	0	1.76	0	0	0	0	1.45	0	0
unknown 84	0	0	0	0.98	0	0	0	0	0	0	1.04	0	0	0	0	0	0	0	0
unknown 85	0	0	0	0	0	0	0	0	0	0	0	0	0	3.79	0	0	0	0	0
unknown 86	0	0	0	0.93	0	0	0	0	0	0	1.64	0.97	0	0	0	0	0	0	0

Name of compound	**TS1**	**TS2**	**BC**	**OCH**	**BEE**	**SPO**	**SMA**	**SOX**	**OKL**	**TAS**	**LMI**	**OCO**	**ECR**	**OO1**	**OO2**	**SPI**	**OBI**	**LOE**	**EPU**
hexatriacontane	19.2	24.6	1.84	0.54	0	0	0	0	0	5.94	0.90	1.48	0	2.80	2.95	0	0.64	2.59	0
tetratriacontane	1.05	2.78	0	0.64	0	0	0	0	0	1.01	0	0.90	0	1.36	0	0	0	0	0
unknown 87	0	0	1.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 88	0	0	1.50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 89	0	0	1.94	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 90	0	0	0	0	0	0	0	4.93	0	0	0	0	0	0	0	0	0	0	0
tetratetracontane	2.48	5.76	0.53	0	0	0	0	0	0	1.31	0	0	0	1.07	0	0	0	0	0
unknown 91	0	0	0	0	0	0	0	0	0	0	1.05	0	0	0	0	0	0	0	0
unknown 92	0	0	3.86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
unknown 93	0	0	0	0	0	0	0	0	0	0	0	0	30.81	0	0	0	0	0	0

Each value represents the per cent composition of a given compound for that species.
Species codes: OS1=*Trichocentrum stipitatum* replicate 1; TS2=*Trichocentrum stipitatum* replicate 2; BC=*Byrsonima crassifolia*; OCH=*Oncidium cheirophorum*; BEE=Oils from a bee's leg (*Paratetrapedia* sp.); SPO=*Sigmatostalix portillae*; SMA=*Sigmatostalix marinii*; SOX=*Sigmatostalix oxyceras*; OKL=*Oncidium klotzschianum*; TAS=*Trichocentrum ascendens*; LMI=*Lockhartia micrantha*; OCO=*Ornithocephalus cochleariformis*; ECR=*Erycina cristagalli*; OO1=*Oncidium ornithorrhynchum* replicate 1; OO2=*Oncidium ornithorrhynchum* replicate 2; SPI=*Sigmatostalix picturatissima*; OBI=*Ornithocephalus bicornis*; LOE=*Lockhartia oerstedii*; EPU=*Erycina pusilla.*

APPENDIX B
LOCALITIES AND VOUCHER NUMBERS FOR PLANT TAXA

Taxon	**Localities**	**Voucher***
Byrsonima crassifolia Rich. ex Kunth.	Chilibre, Panama	*Silvera-30*
Erycina cristagalli (Rchb.f.) N.H.Williams & M.W.Chase	La mesa del Valle, Panama	*Silvera-87*
Erycina pusilla (L.) N.H.Williams & M.W.Chase	Gamboa, Panama	*Silvera-64*
Lockhartia micrantha Rchb.f.	Chilibre, Panama	*Silvera-72*
Lockhartia oerstedii Rchb.f.		*Whitten-99235*
Oncidium cheirophorum Rchb.f.	La Fortuna, Boquete, Panama	*Silvera-15*
Oncidium klotzschianum Rchb.f.	La Fortuna, Panama	*Silvera-20*
Oncidium ornithorrhynchum Kunth.		*Whitten s.n.*
Ornithocephalus bicornis Lindl. exBenth.	Campana	*Silvera-55*
Ornithocephalus cochleariformis C.Schweinf.	El Valle	*Silvera-43*
Sigmatostalix marinii Königer	Ecuador	*Whitten-99264*
Sigmatostalix oxyceras Königer & J.G.Weinm	Ecuador	*Whitten-99302*
Sigmatostalix picturatissima Kränzl	La Fortuna, El Valle	*Silvera-66*
Sigmatostalix portillae Königer	Ecuador	*Silvera-s.n.*
Trichocentum ascendens (Lindl.) M.W.Chase & N.H.Williams	Sona, Panama	*Silvera-89*
Trichocentrum stipitatum (Lindl.) M.W.Chase & N.H.Williams	Chilibre, Panama	*Silvera-88*

*All vouchers are deposited at the University of Florida Herbarium (FLAS).

APPENDIX C
LOCALITIES AND VOUCHER NUMBERS FOR BEE TAXA

Taxa	**Locality**	**Vouchers ***
Centris bicornuta Mocsary	Chilibre, Panama	*Silvera-9,12*
Centris inermis Friese	Chilibre, Panama	*Silvera-25,32,34,24,42*
Centris longimana Fabricius	Chilibre, Panama	*Silvera-28*
Centris trigonoides Lepeletier	Chilibre, Panama	*Silvera-15,63*
Cephalotrigona capitata Smith	Chilibre, Panama	*Silvera-13*
Epicharis maculata Smith	Chilibre, Panama	*Silvera-11,20*
Epicharis rustica Olivier	Chilibre, Panama	*Silvera-39,38*
Eulaema nigrita Lepeletier	Chilibre, Panama	*Silvera-44*
Frieseomelitta nigra Lepeletier	Chilibre, Panama	*Silvera-3,10,11*
Paratetrapedia moesta Cresson	Chilibre, Panama	*Silvera-36,23,3,1,2,14,6,* *Silvera-22,36,35,24,34,42*
Paratetrapedia sp.1	Chilibre, Panama	*Silvera-7,23,36,55,57,21,* *Silvera-19,8,60,40,31,29,* *Silvera-4,21,56,30,58*
Paratetrapedia sp. (undescribed)	La Fortuna, Panama	*Silvera-F1, F2*
Scaptotrigona luteipennis De la Torre	Chilibre, Panama	*Silvera-49*
Tetrapedia cf. maura Cresson	Chilibre, Panama	*Silvera-17,8*
Trigona angustula La Treille	Chilibre, Panama	*Silvera-53,47,41,52*
Trigona corvina Cockerell	Chilibre, Panama	*Silvera-54,2,28,962,15,61,* *Silvera-5,31,33,45,18,46*
Trigona fulviventris Guérin-Méneville	Chilibre, Panama	*Silvera-5,12,30*
Trigona fuscipennis Friese	Chilibre, Panama	*Silvera-14,16,25*
Trigona muzoensis Schwarz	Chilibre, Panama	*Silvera-12,21,41,51,22*

*Vouchers are kept in personal collection to be deposited at the Smithsonian Tropical Research Institute (STRI) Panama.

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BIOGRAPHICAL SKETCH

Katia Itzel Silvera was born in Panama City, Republic of Panama, on January 31, 1974. As a university student, she began working with the Smithsonian Tropical Research Institute (STRI), and shortly afterward, in 1998, she received a Bachelor of biology with specialization in plant biology from the University of Panama. Following graduation, she worked as a research assistant with Dr. Jim Dalling in Barro Colorado Island (BCI) who served as a co-advisor for her undergraduate thesis. Katia has been involved in several projects in STRI including plant physiology at the canopy crane, seedling ecology and water relations among others. She has also been involved in the visitors program working as a natural history interpreter for the public at BCI for several years. Her positive undergraduate experience, encouragement from her advisors, and interest in orchids brought her to the Florida Museum of Natural History to volunteer with Dr. Robert Dressler working in orchid systematics. She remained in the University of Florida Department of Botany to pursue a master's degree with Dr. Norris H. Williams, Dr. Robert Dressler and Dr. Mark Whitten, who are all world experts on neotropical orchid evolution and systematics. Katia plans to continue studying the evolution of orchids and their pollinators in the tropics, especially in Panama.