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DEL MAZO CANCINO, ALEJANDRO; DAMON, ANNE
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COMPARISON OF FLORAL FRAGRANCE COMPONENTS OF SPECIES OF *ENCYCLIA* AND *PROSTHECHEA* (ORCHIDACEAE) FROM SOCONUSCO, SOUTHEAST MEXICO

ALEJANDRO DEL MAZO CANCINO & *ANNE DAMON

*El Colegio de la Frontera Sur (ECOSUR), apdo. postal 36, Carretera Aeropuerto Antiguo, Tapachula, Chiapas, México. adamon@tap-ecosur.edu.mx

ABSTRACT. We analyzed the volatile fragrance components of flowers of four species of *Prosthechea* and three species of *Encyclia*, from the lower and middle altitudes of Soconusco region in the southeast of Mexico. The flowers of *P. cochleata*, reported to be an autogamic species, produced no volatiles. *P. baculus*, *P. chacaoensis* and *P. radiata*, all of which have highly fragrant flowers, shared various volatile components, most notably, *p*-methyl anisole, *e*-ocimene, oxophorone, α -farnesene and benzyl benzoate. *E. adenocarpa* has lightly fragrant flowers, with hexadecene and *e*-nerolidol the major components, whereas the physically similar *E. parviflora* produced mainly 3,4,5-trimethoxybenzoic acid, methyl ester and only one other compound. *E. cordigera* is a highly fragrant species and produced relatively large amounts of *cis*-geraniol. Various volatile fragrance components were shared by these two, recently separated genera, especially benzaldehyde, *cis*-ocimene and *e*-ocimene. Similarities were greater between the *Prosthecheas* (excluding *P. cochleata*) than the *Encyclias*.

RESUMEN. Se analizaron los compuestos volátiles de flores de cuatro especies de *Prosthechea* y tres especies de *Encyclia*, de altitudes bajas y medianas de la región del Soconusco en el sureste de México. Las flores de *Prosthechea cochleata*, una especie considerada autogámica, no produjeron ningún compuesto volátil. *P. baculus*, *P. chacaoensis* y *P. radiata*, todas con flores muy aromáticas, comparten varios compuestos volátiles, especialmente *e*-ocimeno, α -farneseno, *p*-metil anisol, oxofocono y benzoato de bencilo. *Encyclia adenocarpa* tiene flores ligeramente fragantes y los compuestos principales son hexadeceno y *e*-nerolidol, mientras *E. parviflora*, que es morfológicamente similar, produjo cantidades notables de metil-éster del ácido 3,4,5-trimetoxibenzoico y un solo compuesto más. *E. cordigera* es una especie muy fragante y produjo cantidades relativamente grandes de *cis*-geraniol. Varios compuestos volátiles son compartidos por estos dos géneros recientemente separados, especialmente benzaldehído, *cis*-ocimeno y *e*-ocimeno. Hubo mayor similitud entre los compuestos de *Prosthechea* (excluyendo *P. cochleata*) que entre los de *Encyclia*.

KEY WORDS / PALABRAS CLAVE: *Encyclia cordigera*, *Encyclia adenocarpa*, *Encyclia parviflora*, *Prosthechea baculus*, *Prosthechea radiata*, *Prosthechea chacaoensis*, *Prosthechea cochleata*, epiphytic orchids, floral fragrance components.

INTRODUCTION

Laeliinae is a neotropical subtribe of the Orchidaceae, forming a complex taxonomic group with continuing work to define genera (de Oliveira Pires *et al.* 2003, van der Cingel 2001). Within this subtribe, *Prosthechea* was previously included in *Encyclia*, but was separated in 2000 supported by a series of morphological and molecular studies (van den Berg *et al.* 2000, Higgins 1997). The genera *Anacheilium* and *Hormidium* described by Withner & Harding (2004) were recognized as synonymous with *Prosthechea* (Higgins 1997).

At the time that Kaiser (1993) presented the most important compilation of orchid fragrances to date, *Prosthechea* was not separated from *Encyclia* and flowers of the genus were described as producing a recognizable “*Encyclia*” scent. Only two species discussed by Kaiser coincide with our study, and *E. adenocarpa* was described as having a notable ionone-rich scent, attractive from a distance, with major components benzyl and butyl caproate, hydroquinone dimethyl ether y β -ionone. *Prosthechea baculus* was particularly noted for the large amount of an

oxoisophorone dihydro derivative and corresponding epoxy diketone 54, (E)-ocimene, benzaldehyde and 2,2,6-trimethylcyclohex-2-en-1,4-dione. Genders (1977) and Frowine (2005) suggested that scents may be similar within orchid genera, at least to the human nose, and to the horticulturist, *E. cordigera* is described as producing a strong honey and vanilla scent, and *P. radiata* a strong spicy floral, coconut cream pie, carnation and hyacinth scent (Frowine 2005).

In this study we chemically analyzed the fragrances of 7 species of orchids from the coast and lower altitudes of Soconusco region in the southeast of Mexico: *Encyclia cordigera* (Kunth) Dressler, *Encyclia adenocarpa* (La Llave & Lex.) Schltr., *Encyclia parviflora* (Regel) Withner, *Prosthechea baculus* (Rchb.f.) W.E. Higgins, *Prosthechea radiata* (Lindl.) W.E. Higgins, *Prosthechea chacaoensis* (Rchb.f.) W.E. Higgins and *Prosthechea cochleata* (L.) W.E. Higgins. Two of these species (*P. cochleata*, *E. cordigera*) are included in the cladogram determined by Higgins (2000). All species of *Prosthechea* in our study are described as *Anacheilium* by Withner and Harding (2004) (*A. baculus*, *A. chacaoense*, *A. cochleatum*, *A. radiatum*), and are described as having strong fragrances and glycoside flavonoid crystals to make flowers more visible in dark areas; however, in Soconusco, these orchids are usually found in well-illuminated sites.

P. chacaoensis is the most common and widely distributed orchid species in the agroecosystems, secondary vegetation and forest fragments of Soconusco and *E. cordigera* is still fairly common and found scattered among the remaining trees on the coastal plain. *P. cochleata*, often described as a common species in the literature, is now extremely scarce in the region, outside of private collections (Damon & Colin 2005), and like both *P. baculus* and *P. radiata* is restricted to tropical forest fragments and coffee plantations above 800 m altitude. *E. adenocarpa* and *E. parviflora* are coastal species, found in tropical humid forests at the edge of mangrove swamps.

The study of orchid pollination is notoriously difficult, and particularly so for out of reach epiphytic species, and reports of pollination in the wild are scarce (Widmer *et al.* 2000, Pridgeon *et al.* 2005, Johnson *et al.* 2003). *Encyclia* and *Prosthechea*, are presumed to be pollinated by bees and wasps, although *P. vitellina* has been seen receiving visits by humming birds (Hágsater *et al.* 2005), which

are attracted by color, not scent. *E. cordigera* is reported to be pollinated by female carpenter bees (*Xylocopa* sp., Hymenoptera: Anthophoridae) (van der Cingel 2001) but there are very few reports of the pollination of other species of these two genera. We know very little about the mechanisms of pollination of these plants, which includes the production of fragrance to attract their elusive pollinators. This study is a contribution towards an understanding of the mechanism of pollinator attraction of Soconusco species of *Prosthechea* and *Encyclia*, and towards the continuing discussion regarding the taxonomic placement of these genera and the species presently allocated to them.

MATERIAL AND METHODS

The present study was carried out in two sites:

1. Orchidarium "Santo Domingo", situated in Ejido Santo Domingo in the municipality of Unión Juárez, Chiapas, at 900 m (15°01'48"N and 92°06'21"W). Average annual temperature is 23°C with 4700 mm annual precipitation.

2. Botanical Garden "El Soconusco" (ECOSUR), situated in the community "La Guardianía", in the municipality of Tuzantán, Chiapas, at 180 m (15°08'44"N and 92°25'25"W). Average annual temperature is 28°C with 2300 mm annual precipitation.

Biological Material:

In this study, we collected fragrances from fully opened flowers of *E. cordigera*, *E. parviflora*, *E. adenocarpa*, *P. baculus*, *P. chacaoensis*, *P. radiata* and *P. cochleata*. The flowers were handled with great care to avoid bruising. Three fragrance samples were taken for each species of orchid.

The volatiles were collected using the technique "dynamic aeration" ("headspace") (Damon *et al.* 2002), in which the flowers were placed into specially designed glass chambers (40 x 13 cm for large inflorescences and 20 x 13 for small ones) and the fragrance was collected by an air flow passing through the chamber at 1 l/min. To avoid contamination, the air was purified using activated carbon before passing into the interior of the chamber. The fragrances were absorbed into 0.2 g of Porapak Q (100/120 mesh, Water Associates, Inc., Milford, USA) contained in clean Pasteur pipettes, and before each collection the Porapak Q was cleaned using the procedure described by Blight (1990).

The fragrance samples were collected during a period of at least 2 hours in the morning, between 9 and 12 am. After collection, the pipettes containing the samples were transported in ice packs to the laboratory in ECOSUR-Tapachula, where the volatiles contained in the Porapak Q were eluted with 200 µl diethyl ether (HPLC grade, Aldrich, Toluca, Mexico). The samples were stored at -20°C until the chemical analysis could be carried out.

Chemical analysis of the fragrances was carried out using a Gas Chromatograph CP-3800 coupled to Mass Spectrometer (SATURN 2000, VARIAN Palo Alto, CA, USA). The samples were analysed using a non-polar capillary column VF-5MS (30 m x 0.25 mm ID, VARIAN). The gas carrier was helium flowing at 1 ml/min. The injection port temperature was held at 200°C, with an analysis program that began at 50°C for 2 minutes, followed by a ramp of 10°C/min until 250°C which was maintained for 10 min. The compounds were identified by comparison with the Kovacs Index (KI), the mass spectra of the standards, and the NIST 05 library, incorporated into the equipment.

The synthetic chemical compounds that were used as standards were obtained from Sigma-Aldrich (Toluca, México) and Bedoukian Research (Danbury, CT, USA) with 98% chromatographic purity.

RESULTS

A total of 42 volatile compounds were found in the fragrances of the three species of *Encyclia* studied: 30 for *E. cordigera*, 20 for *E. adenocarpa* and 2 for *E. parviflora* (Table 1.). The chemical analysis of the fragrances showed that the major components for *E. cordigera* were *cis*-geraniol (49.76%), followed by benzyl benzoate, indole and *e*-ocimene; for *E. adenocarpa* major components were hexadecene (39.39%) and *e*-nerolidol (13.75%), followed by indole, methyl-methylaminobenzoate, *e*-ocimene and dodecanol, whereas *E. parviflora* had only two compounds, 3,4,5-trimethoxybenzoic acid, methyl ester (89.96%) and *p*-vinyl anisole (10.04%).

Similarly, for *Prosthechea* (Tabla 2), a total of 44 fragrance components were identified. The fragrance of *P. baculus* had 27 components, of which *e*-ocimene (28.12%) and phenethyl acetate (28.21%), followed by methyl salicylate, phenethyl alcohol, *p*-methyl anisole and *e*-nerolidol were the major ones. For *P. radiata*, with 26 components, *p*-methyl anisole (38.96%), *e*-

ocimene (27.15%) and benzyl benzoate (12.45%) were the most important and for *P. chacaoensis*, also with 26 components, *e*-ocimene (46.06%), *p*-methyl anisole (25.2%), cresol and benzyl benzoate were the most abundant. Headspace samples taken from flowers of *P. cochleata* contained no volatiles.

DISCUSSION

P. cochleata is irregular within *Prosthechea* and within *Anachaelium* (Withner & Harding 2004) as it produces a barely detectable scent, however, this species is reported to be autogamic (van der Cingel 2001) and indeed, no insects or birds have been seen visiting this species in Soconusco. As an autogamous species with no scent, it was not included in the inter- and intrageneric comparisons.

Various volatiles were shared by *P. baculus*, *P. chacaoensis* and *P. radiata*, all of which have highly fragrant flowers, most notably, *p*-methyl anisole, *e*-ocimene, oxophorone, α -farnesene and benzyl benzoate. Several more compounds were found in the fragrances of two of the species. Our fragrance samples of *P. baculus* contained (E)-ocimene, benzaldehyde as mentioned by Kaiser (1993), but other volatiles were absent.

The composition of the fragrances produced by the three species of *Encyclia* was very different. *E. adenocarpa* has lightly fragrant flowers, with hexadecene, *e*-nerolidol and phenylformide the major components, whereas the physically similar *E. parviflora* produced mainly 3,4,5-trimethoxybenzoic acid, methyl ester and only one other compound. *E. cordigera* is a highly fragrant species and produced relatively large amounts of *cis*-geraniol. None of the volatile components of the fragrance of *E. adenocarpa* reported by Kaiser (1993) (benzyl and butyl caproate, hydroquinone dimethyl ether and beta ionone) were found in our samples.

Excluding *P. cochleata*, the only volatile fragrance components shared by all the species studied of these two, recently separated genera were: *e*-ocimene which was a major component in the *Prosthecheas*, myrcene, produced in variable quantities, and benzaldehyde, 6-methyl-5-hepten-2-one, limonene, benzyl benzoate, *z*-ocimene, linalool, nonanal, *allo*-ocimene, *cis*-geraniol, geraniol, indole, α -farnesene and *e*-nerolidol, which were produced in small quantities. These substances are also reported in other orchids (Kaiser 1993) and

may be common in the Orchidaceae in general. Some of the volatile substances found in the fragrances of the *Encyclias* (e.g. eugenol, vanillin, benzyl benzoate and methyl salicylate) are important attractants for Euglossine bees, which may explain the occasional visit to these flowers by species of *Euglossa*, *Eulema*, *Eufriesia* and *Exaerate* bees. These substances that are attractive to non-habitual pollinators may be effectively "switched off" by the presence of other substances, but during times of pollinator scarcity may offer flexibility to orchids by facilitating a switch to a new pollinator, and result in a change of selection pressures leading to a change in fragrance composition.

Allo-ocimene was found in the fragrances of *P. radiata*, *P. chacaoensis* and *E. adenocarpa*. This substance occurs in nature in various isometric structures (Dunphy 2006) and this is the first time it has been reported in orchid fragrances.

Indole is one of the few nitrogenous compounds found in orchid scents (Kaiser 1993) and it was present in small quantities in *P. baculus* and *P. radiata* and larger quantities in *E. cordigera* and *E. adenocarpa*. The presence of nitrogen in fragrances of epiphytic orchids which are often resource limited is interesting and may act either as a repellent to prevent ants accessing flowers (Ghazoul 2001), or as a volatile present in extrafloral nectaries. This compound is also found in the fragrance of *Acacia* sp. (Leguminosae, Flath *et al.* 1983) which are notorious for their interactions with ants. Indole is present in glandular secretions of some species of ants. Other components of orchid fragrances, such as limonene and geraniol are alarm pheromones for different species of ants (Holldobler & Wilson 1990).

Encyclia parviflora only produces two components in its scent, and the major component, 3,4,5-trimethoxybenzoic acid, methyl ester has not been previously reported in orchids but has been found in ether extracts of *Leptospermum scoparium* Forst. & Forst., *Leptospermum ericoides* A. Rich. (Myrtaceae) and *Trifolium repens* L. (Leguminosae) (Seng *et al.* 1988) and has been shown to have antibacterial activity (Russell *et al.* 1990).

It remains to be seen whether other species of *Prosthechea* share similarities with *P. baculus*, *P. chacaoensis* and *P. radiata*, and whether other species of *Encyclia* also have such widely differing fragrance composition, which reflects the various

pollination syndromes found within this group and argues for continued research into the taxonomic affinities of these species. Furthermore, the study of orchid fragrances is in its infancy and requires detailed analysis of the chemical composition and function of each component, each of which must be justified within the plant's energy budget, especially in the case of resource-limited tropical epiphytic orchids.

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Table 1. Relative amounts of fragrance components of species of *Encyclia* from Soconusco Region, Chiapas, Mexico. 1. *E. cordigera*; 2. *E. adenocarpa*; 3. *E. parviflora*.

Compound	Relative amount (%)			
	KI	1	2	3
benzaldehyde **	977	0.79	0.33	-
6-methyl-5-hepten-2-one **	989	0.15	0.57	-
myrcene **	992	0.07	-	-
mesitylene **	1005	-	0.24	-
limonene **	1032	0.04	-	-
benzyl alcohol **	1046	2.38	-	-
<i>z</i> -ocimene**	1038	0.75	0.69	-
salicylaldehyde **	1048	0.41	-	-
<i>e</i> -ocimene *	1050	4.45	6.12	-
linalool **	1104	2.28	0.19	-
nonanal **	1108	0.1	0.76	-
<i>allo</i> -ocimene *	1133	-	0.21	-
<i>p</i> -vinylanisole *	1165	-	-	10.04
benzyl acetate **	1168	0.14	-	-
methyl salicylate **	1201	1.99	1.68	-
<i>cis</i> -geraniol **	1232	49.76	-	-
neral *	1246	2.78	-	-
geraniol **	1256	0.18	-	-
phenethylacetate **	1268	0.54	-	-
geranial **	1274	3.58	-	-
indole **	1319	5.57	7.1	-
propylaniline *	1308	-	0.72	-
benzyl butyrate **	1351	0.05	-	-
eugenol **	1364	1.54	1.44	-
neryl acetate **	1366	3.78	-	-
geranyl acetate **	1384	0.26	-	-
β -cubebene *	1403	-	0.14	-
1-tetradecene **	1407	-	0.89	-
vanillin **	1407	0.17	-	-
methyl-methylaminobenzoate *	1435	-	6.3	-
dodecanol **	1479	-	5.31	-
α -farnesene**	1511	-	0.37	-
<i>e</i> -nerolidol **	1571	0.67	13.75	-
hexadecene **	1607	-	39.39	-
2- <i>cis</i> ,6- <i>trans</i> -farnesol **	1706	0.77	-	-
β -sinensal *	1706	1.67	-	-
<i>trans</i> -farnesol *	1724	1.77	-	-
3,4,5-trimethoxybenzoic acid, methyl ester	1727	-	-	89.96
<i>cis</i> -farnesol *	1751	1.66	-	-
benzyl benzoate **	1781	6.73	-	-
1-octadecene **	1807	-	1.22	-
methyl hexadecanoate *	1928	3.81	-	-
KI = Kovacs Index - Absent Chemical identification ** Synthetic standards * Computer library NIST 05				

Table 2. Relative amounts of fragrance components of species of *Prosthechea* from Soconusco Region, Chiapas, Mexico. 1. *P. baculus*; 2. *P. radiata*; 3. *P. chacaoensis*; 4. *P. cochleata*.

Relative amount (%)					
Compound	KI	1	2	3	4
α -thujene *	931	2.54	0.45	-	-
α -pinene **	945	-	0.76	T	-
benzaldehyde **	977	T	0.38	0.64	-
sabinene *	984	-	0.29	-	-
6-methyl-5-hepten-2-one **	987	0.34	-	-	-
myrcene **	992	T	1.61	3.12	-
<i>p</i> -methyl anisole *	1032	4.77	38.96	25.2	-
limonene **	1032	T	0.63	0.34	-
<i>z</i> -ocimene **	1038	T	0.73	1.08	-
benzyl alcohol **	1046	T	-	-	-
1,8-cineole **	1047	T	-	-	-
<i>e</i> -ocimene *	1050	28.12	27.15	46.06	-
<i>m</i> -cresol **	1076	-	2.46	-	-
α -pinene oxide **	1077	T	-	-	-
cresol **	1077	-	-	9.88	-
isoterpinolene *	1083	-	0.43	-	-
<i>trans</i> -linalool oxide **	1097	-	0.56	-	-
linalool **	1104	2.08	-	-	-
nonanal **	1108	-	0.51	1.11	-
methyl benzoate **	1109	0.51	-	-	-
tetrahydro linalool *	1114	0.52	-	-	-
phenethylalcohol **	1128	4.87	-	-	-
<i>allo</i> -ocimene *	1133	-	0.71	0.77	-
<i>trans</i> -pinocarveol **	1139	-	0.15	-	-
<i>o</i> -dimethoxybenzene *	1151	3.22	-	-	-
oxophorone *	1154	1.14	4.46	3.03	-
<i>cis</i> -verbenol **	1164	-	1.3	-	-
<i>p</i> -dimethoxybenzene **	1176	-	0.34	-	-
decanal **	1211	-	0.32	1.33	-
methyl salicylate **	1214	7.09	-	-	-
<i>cis</i> -verbenone **	1232	-	1.26	-	-
phenethylacetate **	1268	28.21	-	-	-
geranial **	1277	-	0.59	-	-
indole **	1319	0.75	0.19	-	-
cinnamyl alcohol **	1330	-	2.38	-	-
1,2,4-trimethoxybenzene *	1378	1.13	-	-	-
(+)-longifolene **	1456	-	-	0.55	-
isoamyl benzoate **	1457	0.33	-	-	-
<i>e</i> -cinnamyl acetate **	1461	-	0.57	-	-
α -farnesene **	1511	2.99	0.37	0.27	-
phenylethyl tiglate **	1516	1.23	-	-	-
benzyl tiglate **	1516	3.11	-	-	-
<i>e</i> -nerolidol **	1571	4.04	-	-	-
benzyl benzoate **	1781	3.02	12.45	6.62	-
KI = Kovacs Index T – Trace - Absent Chemical identification ** Synthetic standards * Computer library NIST 05					