

**STRONG PHYLOGENETIC EFFECTS ON FLORAL SCENT VARIATION
 OF OIL-SECRETING ORCHIDS IN SOUTH AFRICA¹**

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- *Premise of the study:* Evolution involves the interplay between natural selection and phylogenetic constraint. This is particularly evident among the flowering plants where form and diversity of flowers attest to the importance of both pollinator-mediated selection and phylogenetic constraint. Although this has been studied mostly using visible floral characters, invisible volatile chemicals emitted by the flowers should be subject to these same evolutionary forces. Unfortunately, most analyses of floral volatiles have over-emphasized the importance of natural selection and underplayed phylogenetic constraint without quantifying their respective roles in the evolution and composition of floral scents.
- *Methods:* We used multivariate analyses to test the relative importance of pollinators vs. phylogeny in determining the composition of floral scents among oil-secreting orchids in southern Africa. Floral scents of 42 oil-secreting taxa/ecotypes distributed among 12 subclades in the tribe Deseae were sampled using headspace adsorption and gas chromatography-mass spectroscopy.
- *Key results:* We identified 257 scent compounds distributed over nine different compound classes, with the majority of scents dominated by aliphatic or benzenoid compounds. The only significant predictor of floral scent among these orchids above the species level was phylogeny. Nevertheless, in two of the clades there were differences in scent profiles at the species and ecotype level that corresponded to different pollinators and were thus suggestive of pollinator-mediated selection.
- *Conclusions:* Scent variation was greater than expected and phylogeny was more important than pollinator-mediated selection in predicting the composition of floral scents of oil-secreting orchids, despite the specialized nature of the pollinator reward system.

Key words: Coryciinae; floral scents; oil-collecting bees; oil-secreting flowers; orchids; phylogeny; pollination; *Rediviva*

Patterns of evolution often reflect a balance between natural selection and phylogenetic constraint where the latter is defined as "...any result or component of phylogenetic history of a lineage that prevents an anticipated course of evolution in that lineage." (McKittrick 1993). In the flowering plants, phylogenetic constraints have played an obvious role in defining the characteristics of flowers within specific genera, families, or even orders in the face of similar selection by pollinators (e.g., the cyathium in *Euphorbia*, the composite flower in Asteraceae, or the zygomorphic flowers of the Lamiales. Constraints on morphological characters of flowers are much better known than constraints on the composition of floral fragrances, because the latter are invisible and more difficult to characterize than the former.

Floral scent constituents often correlate strongly with specific groups, families, or orders of pollinators, such as beetles, flies, and butterflies (Dobson, 2006). This suggests that pollinator-mediated selection plays a prominent role in the evolution of floral scents and that convergence in scent composition among unrelated species may predominate among flowering plants (Knudsen and Tollsten, 1995). For example, most bat-pollinated species contain Dimethyl disulphide as a prominent scent constituent (Knudsen and Tollsten, 1995; Bestmann et al., 1997; Helversen et al., 2000) even in unrelated groups where bat-pollination has evolved independently. Moth-pollinated flowers exhibit similar scent convergence based on relatively few chemical constituents, including Benzaldehyde, Phenylacetaldehyde, Methyl benzoate, Lilac aldehyde, and Linalool (Dobson, 2006). Additional examples of pollinator-mediated selection involve scent ecotypes within species (Chess et al., 2008; Anderson et al., 2009), correlations between various scent components and specific pollinators among closely related plant species (Hills et al., 1972; Manning and Goldblatt, 2005; Peakall et al., 2010) and species whose scent composition varies independently from morphological characters (Lindberg et al., 2000).

Yet, despite the strongly implied role of pollinator selection for the evolution of floral scents, scent profiles are rarely identical among distantly related plant species and even the signature compounds that define a pollinator group can occasionally be missing. For example, floral scents of bat-pollinated species do not always contain sulfur compounds (Petterson et al., 2004; Piechowski et al., 2010). Pollinators are therefore not necessarily tied to only one or a few specific compounds in a scent bouquet.

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Furthermore, the interpretation of scent profiles may be complicated by conflicting selective pressures on the various constituents from multiple pollinators or because some of the constituents serve functions other than pollinator attraction, such as chemical defense (Kessler and Halitschke, 2009). Various constituents may also represent background chemical “noise” generated from poor enzyme specificity in one or more biochemical pathways (Raguso et al., 2003) or result from phenotypic plasticity caused by differences in abiotic factors (Majetic et al., 2008; 2009).

An important constraint on pollinator-mediated selection of scent compounds is phylogenetic inertia. Assessing the importance of plant phylogeny in affecting floral scent composition comes mainly from studies that have considered the utility of scent data for taxonomic or phylogenetic reconstruction (i.e., Barkman et al., 1997; Barkman, 2001; Levin, et al., 2003; Feulner et al., 2009) rather than in an ecological context as a constraint on pollinator-mediated selection per se (see Peakall et al., 2010). The consensus from these studies is that scent data can provide some phylogenetic signal, but most scent compounds are homoplasious. The several studies that have suggested that phylogeny plays a role in scent evolution (i.e., Dahl et al., 1990; Nogueira et al., 2001; Raguso et al., 2003; Jürgens, 2004) did not compare directly its importance relative to pollinators.

The few studies that have attempted to assess pollinator-mediated selection vs. phylogenetic constraint in the evolution of floral scents have typically examined groups that vary in major pollinator type or syndrome (but cf. Peakall et al., 2010). This complicates analyses of this issue because if phylogeny and pollinator-type are examined simultaneously, and pollinators are very different, pollinator effects might mask or swamp out the phylogenetic effects. However, if pollinators are too similar in their scent preferences, phylogenetic effects may appear more significant. Furthermore, plants with generalized pollination systems are expected to have different and often conflicting effects on floral scents, whereas specialized pollination systems should exhibit little or no conflict in selection on scent components (Knudsen et al., 2006). Ideally, therefore, one should compare the relative roles of pollinator-mediated selection and phylogenetic constraint among closely related species with a specialized pollination system and within a particular pollination type (Peakall et al., 2010).

The Coryciinae sensu Linder and Kurzweil (1994) represent one such specialized pollination system. With approximately 118 species in five genera, each of the species in this group is pollinated by one or at most two species of pollinator (Steiner, 1989; Steiner, 1998; Pauw, 2006; Johnson and Steiner, 2003; Waterman et al., 2011). The majority of these orchids provide nonvolatile oil as a pollinator reward (Steiner, 1989; Steiner, 1998; Pauw, 2006; Johnson and Steiner, 2003; Steiner and Liltved, 2011). This oil is collected solely by female *Rediviva* (Melittidae) bees (Figs. 1A, 1B) using modified legs with specialized spatulate and branched setae. The setae facilitate the harvest and temporary retention of oil until it is transferred to the hind legs for transport to the nest, where it becomes part of the cell lining and/or larval food provisions (Steiner and Whitehead, 1988; 1990; 1991; unpublished data).

The flowers of these orchids exhibit less overall morphological variation superficially than species in the other subtribes, yet exhibit unique and complex structures (Kurzweil et al., 1991). There are two main types of flowers in this group that place pollinaria on different parts of the pollinators. The globose or galeate type has the reproductive parts hidden within the flower

(*Corycium* and *Disperis*) and deposits pollinaria on the forelegs of the bees as they collect the oil that is also hidden in the flower. The open type usually has an erect oil-secreting structure that the bees rub with their forelegs while their midlegs, hindlegs, or ventral side of their abdomen contacts the pollinaria as the bee steadies itself on the flower (i.e., *Pterygodium*, *Evothella*, *Ceratandra*; Steiner, 1989; Pauw, 2006; Waterman et al., 2009).

Anecdotal accounts characterize the scents of some of these orchids as unpleasant, soapy, cloying, or even pungent (Steiner and Liltved, 2011), leading us to hypothesize that some of their chemical constituents help effect pollinator attraction and contribute to reproductive isolation and speciation. *Rediviva* is closely related to *Macropis* oil-collecting bees of the Holarctic (Michez et al., 2009), one species of which has been shown to be effectively attracted by the floral scent of its host plants (Dötterl and Schäffler, 2007). There is still little information on the role of scent in the pollination of Coryciinae (Kurzweil et al., 1991; Linder and Kurzweil, 1999), however, and scent constituents have neither been systematically sampled nor quantitatively or semiquantitatively analyzed. The purpose of this study was to: (1) identify the floral scent constituents of these orchids; (2) determine the extent to which scent profiles vary among species; and (3) assess whether pollinators or phylogenetic patterns are more important in explaining scent variation.

Because the oil-secreting orchids are highly specialized for pollination and because the same species of oil-collecting bee often visits one or more species or genera of oil-secreting orchids (Steiner, 1989; Pauw, 2006), we predicted that scent profiles would be convergent for a particular guild of orchids pollinated by a given oil-collecting bee, irrespective of phylogenetic relationships. Also, because there are two biogeographic regions in southern Africa that are separated by over 700 km (i.e., Winter and Summer Rainfall regions), with different oil-collecting bee species (Steiner, 1989; Whitehead and Steiner, 2001; Whitehead et al., 2008), we predicted that the scents of the orchids within a region would be more similar to each other than to related species that occur in different regions.

MATERIALS AND METHODS

We sampled the scents of oil-secreting orchids (39 of the 54 known species) using at least one representative from each of the eleven clades identified by Waterman et al. (2009) for Coryciinae (sensu Linder and Kurzweil, 1994), and the one oil-secreting species in the *Satyrium* clade (i.e., *S. rhynchanthum* Bolus) (Fig. 2, see Appendix 1 for voucher information). Five of the twelve clades with oil-secreting orchids occur in both Winter and Summer Rainfall geographic regions, five occur only in the Winter Rainfall region and two occur only in the Summer Rainfall region (Table 1). We modified the cladogram of Waterman et al. (2009) by adding an additional ecotype for each of three species (*Pterygodium catholicum* (L.) Sw. “late”, *P. caffrum* (L.) Sw. “late”, *P. hallii* (Schelpe) Kurzweil & H. P. Linder “coastal”) as well as one additional species (*P. cf. cooperi* Rolfe) that has yet to be recognized based on morphological and nuclear and chloroplast DNA sequence data. These species occurred in three different clades: *Anochilus*, *Ommatodium*, and *Eupterygodium* (Steiner and Cruz, 2009; Steiner and Cruz, unpublished data). All orchids sampled (with the exception of *Corycium flanaganii* (Bolus) Kurzweil & H. P. Linder) are pollinated exclusively by *Rediviva* bees (Steiner, 1989; Whitehead and Steiner, 2001; Whitehead et al., 2008; Steiner, unpublished data, Pauw, 2006; see also Table 1).

Scent sampling and identification of compounds—The small number of flowers per plant and relatively weak (to the human nose) scents in some species required the use of long sampling times (8–12 hours after the plants were cut and placed in water) and pooling of samples from individual plants (1–33) to obtain an adequate sample for analysis. The number of plants and flowers used per sample varied in accordance with scent intensity as perceived by the

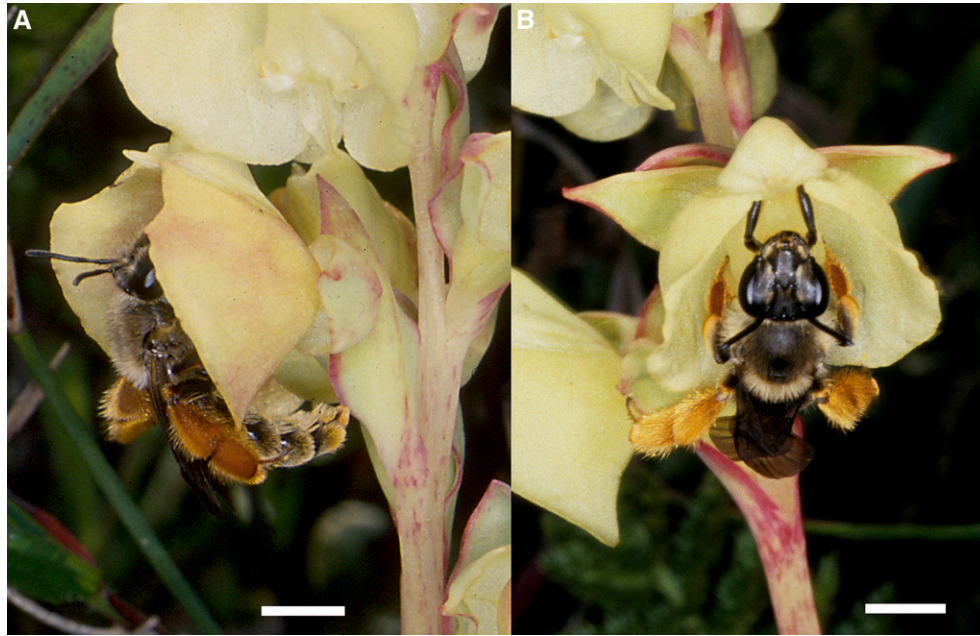


Fig. 1. *Rediviva peringueyi* visiting the flowers of *Pterygodium catholicum*. (A) Side view; (B) Front view. Scale bars = 4 mm.

human nose. Single-individuals were used to sample the flowers of *Pterygodium hallii*, *P. magnum* Reichenb.f., and *Corycium orobanchoides* (L.f.) Sw., because these species have large inflorescences bearing numerous flowers. We minimized the contributions that leaves and other vegetative tissues contributed to the scent profiles by excluding all vegetative material that was not intimately associated with inflorescences (e.g., inflorescence axis, bracts, pedicels, etc) and by sampling only the upper parts of inflorescences. However, we did not sample nonflowering vegetative stems and leaves as controls, so cannot entirely rule out their contributions to the overall scent profiles. To the human nose, these accessory structures contributed little to the overall scent and pollinators were never observed to be attracted to nonflowering plants. Because both within- and between-population variability in floral scent is well-known both among orchids (Moya and Ackerman, 1993; Tollsten and Bergström, 1993; Mant et al., 2005; Salzman et al., 2007) and nonorchids (Dötterl et al., 2005b; Azuma et al., 2001), it is possible that pooling of samples from multiple individuals within a population may have resulted in a scent profile with more compounds than are typically detected in any one individual.

Both Kaiser (1994) and Johnson et al. (2005) found little difference in scent profiles of intact flowers sampled in situ from plants cut, placed in water, and sampled within one or two days. Schiestl et al. (1997) found a significantly lower emission rate and a different composition (relative amount) of compounds in *Ophrys sphegodes* Mill. for picked vs. unpicked flowers, however they reported that picked flowers were still attractive to pollinators over several days. To determine how a scent profile might be affected by our sampling method, we compared scent from an intact flowering plant of *Pterygodium schelpei* H. P. Linder sampled for 3 hours in situ with the scent from a flowering plant placed in water immediately after cutting, and sampled the next day. These two methods gave very similar scent profiles (Bray-Curtis similarity: 77%, Clarke and Gorley 2006).

All samples were collected using headspace adsorption. Flowering stems were enclosed in a glass vessel and the scented air in the chamber was pumped through a glass capillary tube containing 3 mg of Poropak Super Q absorbent, Sigma-Aldrich, www.sigmaaldrich.com with an SKC 222-4 (SKC Inc., www.skinc.com) personal air sampler. The pump flow rate was maintained at 1.9–2.2 l/hr. After sampling, the traps were eluted with 20–30 μ l of a 5:1 high-grade mixture of Hexane puriss. (p. a. Sigma-Aldrich, www.sigmaaldrich.com): Acetone puriss. (p. a. Sigma-Aldrich, www.sigmaaldrich.com). The eluate was analyzed directly by injecting 1–2 μ l into the gas-chromatograph (Carlo Erba Fractovap 4160, Thermo Scientific, www.thermoscientific.com) or GC-MS (Thermo Finnigan Voyager Mass Spectrometer combined with a Trace GC and Xcalibur software, Thermo Scientific, www.thermoscientific.com). The analyses were made on a DB-Wax column (J & W Scientific, Folsom, California, USA) 30 m \times 0.32 mm i.d. with a film thickness of 0.25 μ m. Compounds were identified

by comparisons with mass spectra and retention times of authentic reference samples available from a collection of reference compounds (cf. Kaiser and Tollsten, 1995).

Statistical Analyses—For each compound, the percentage of the total peak area in each sample was calculated (= relative amount) and used for further analyses. Semiquantitative similarities in floral scent patterns among taxa/ecotypes were calculated with the Bray-Curtis similarity index in the statistical software PRIMER 6.1.11 (Clarke and Gorley, 2006). Nonmetric multidimensional scaling (NMDS) was used to depict variation in floral scent among the samples (Clarke and Gorley, 2006).

To test for differences in scent profiles within and among taxa and between regions, we used PERMANOVA analyses implemented in PRIMER 6.1.11 based on Bray-Curtis similarities. PERMANOVA is a technique for testing the simultaneous response of one or more variables to one or more factors in an ANOVA experimental design on the basis of a (dis)similarity (distance) matrix with permutation methods (Anderson et al., 2008). We used 10 000 permutations in each of the analyses. To test for differences in scent of taxa within the Winter Rainfall region, we calculated a PERMANOVA analysis based on the Bray-Curtis similarity matrix with the fixed factors *clade* and *pollinator* in a two-way crossed design. This layout allows testing for effects of one of the factors and simultaneously controlling for the other factor. To test for differences in scent of taxa in the Summer Rainfall region, we calculated a one-way PERMANOVA analysis with the factor *clade* only because variation in the pollinators was too low for meaningful comparison. Only the *Ommatidium* and *Disperis* clades have more than one pollinator in this region. Most of the orchids in these clades are pollinated by *Rediviva neliana* (Cockerell) and *R. brunnea* Whitehead & Steiner (Table 1). To test for regional effects on scent patterns, we performed a PERMANOVA analysis using region and clade as factors in a two-way crossed design with taxa of clades occurring only in both regions. For all these three PERMANOVA analyses we determined the mean relative amount of compounds in cases where more than one sample per taxa/ecotype was available. We did not test whether the scent is species specific because multiple population samples for most of the taxa/ecotypes were not available (none were available for the Summer Rainfall region). However, we used a one-way PERMANOVA to test for differences in scent between the two ecotypes occurring in *Pterygodium catholicum*. For these two ecotypes we used samples from five different populations of the “early” ecotype and samples from two different populations of the “late” ecotype (Table 1).

Evolution of the most important scent compounds was inferred with Fitch parsimony optimization (Fitch, 1971) onto the modified tree of Waterman et al. (2009). Character states were scored for each species and the characters were optimized with the program MacClade 4.0 (Maddison and Maddison, 2000).

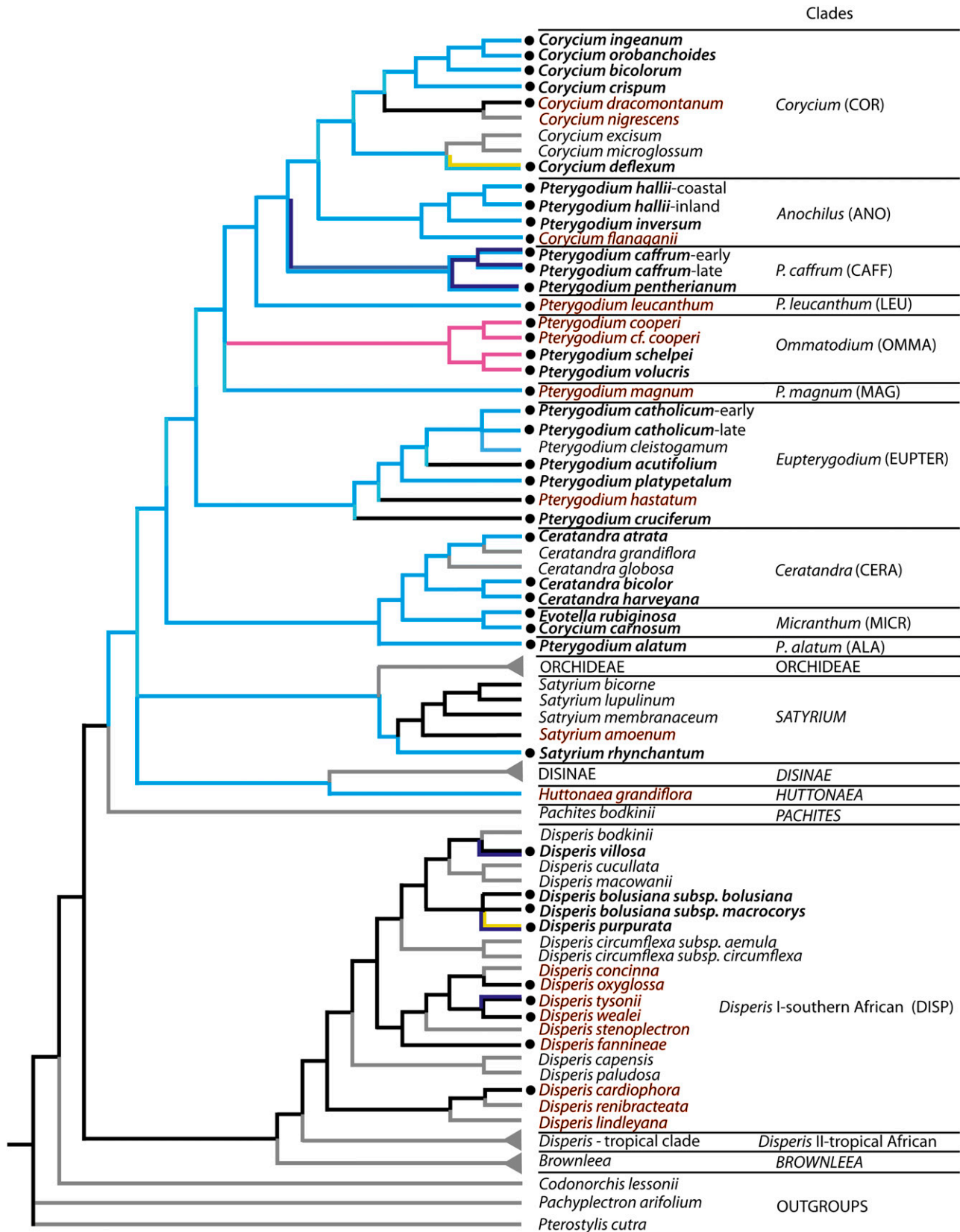


Fig. 2. Cladogram of Discae modified from Waterman et al. (2009) showing taxa sampled for floral scent (solid dots). Examples of important compounds with various distributions across the cladogram are indicated by colored branches: 2-Methoxy-6-methyl-acetophenone (pink), Z-4-Octenoic acid (yellow), 2-Tridecanone (blue), and (Z)-9-Hexadecenal (purple). Branches in gray lack scent data. Presence of 2-Tridecanone in *Huttonaea* is based on floral extracts (Dötterl and Steiner, unpublished data). Summer Rainfall region taxa are brown.

TABLE 1. Oil-secreting orchids from Linder and Kurzweil (1999), clade names (Waterman et al., 2009), regions (WR = Winter Rainfall, SR = Summer Rainfall), pollinators (Whitehead and Steiner 2001, Whitehead et al., 2008, Waterman et al., 2011, Steiner, unpublished data) and number of populations sampled. Bee names followed by “?” are the likely pollinator based on the known distribution of oil-collecting bees active during the flowering period of the orchid and on visits observed to other nearby oil-orchids.

Orchid Species	Clade name	Region	Pollinator(s)	Populations
<i>Pterygodium cruciferum</i>	<i>Eupterygodium</i>	WR	<i>Rediviva peringueyi</i>	1
<i>P. hastatum</i>	<i>Eupterygodium</i>	SR	<i>R. neliana</i> , <i>R. brunnea</i>	1
<i>P. platypetalum</i>	<i>Eupterygodium</i>	WR	<i>R. peringueyi</i>	1
<i>P. catholicum</i> “early”	<i>Eupterygodium</i>	WR	<i>R. peringueyi</i>	5
<i>P. catholicum</i> “late”	<i>Eupterygodium</i>	WR	<i>R. gigas</i>	2
<i>P. acutifolium</i>	<i>Eupterygodium</i>	WR	<i>R. gigas</i>	1
<i>P. caffrum</i> “early”	<i>Caffrum</i>	WR	<i>R. peringueyi</i>	1
<i>P. caffrum</i> “late”	<i>Caffrum</i>	WR	<i>R. gigas</i>	1
<i>P. pentherianum</i>	<i>Caffrum</i>	WR	<i>R. longimanus</i>	1*
<i>P. volucris</i>	<i>Ommatodium</i>	WR	<i>R. peringueyi</i>	1
<i>P. schelpei</i>	<i>Ommatodium</i>	WR	<i>R. longimanus</i>	1*
<i>P. cf. cooperi</i>	<i>Ommatodium</i>	SR	<i>R. colorata</i>	1
<i>P. cooperi</i>	<i>Ommatodium</i>	SR	<i>R. neliana</i> , <i>R. brunnea</i>	1
<i>P. magnum</i>	<i>Magnum</i>	SR	<i>R. neliana</i> , <i>R. brunnea</i>	1
<i>P. leucanthum</i>	<i>Leucanthum</i>	SR	<i>R. neliana</i>	1
<i>P. hallii</i> “inland”	<i>Anochilus</i>	WR	<i>R. macgregori</i>	1
<i>P. hallii</i> “coastal”	<i>Anochilus</i>	WR	<i>R. peringueyi</i>	1
<i>P. inversum</i>	<i>Anochilus</i>	WR	<i>R. peringueyi</i>	1*
<i>Corycium flanaganii</i>	<i>Anochilus</i>	SR	<i>Immanthidium</i> sp.	1
<i>C. crispum</i>	<i>Corycium</i>	WR	<i>R. parva</i> , <i>R. rufipes</i>	1
<i>C. orobanchoides</i>	<i>Corycium</i>	WR	<i>R. peringueyi</i>	1*
<i>C. ingeanum</i>	<i>Corycium</i>	WR	<i>R. macgregori</i>	1
<i>C. deflexum</i>	<i>Corycium</i>	WR	<i>R. macgregori</i>	1
<i>C. dracomontanum</i>	<i>Corycium</i>	SR	<i>R. neliana</i> , <i>R. brunnea</i>	1
<i>C. nigrescens</i> × <i>C. dracomontanum</i>	<i>Corycium</i>	SR	<i>R. neliana</i> , <i>R. brunnea</i>	1
<i>C. bicolorum</i>	<i>Corycium</i>	WR	<i>R. gigas?</i>	1
<i>C. carnosum</i>	<i>Micranthum</i>	WR	<i>R. gigas</i>	1
<i>Evotella rubiginosa</i>	<i>Micranthum</i>	WR	<i>R. gigas?</i>	1
<i>P. alatum</i>	<i>Alatum</i>	WR	<i>R. peringueyi</i>	1
<i>Ceratandra atrata</i>	<i>Ceratandra</i>	WR	<i>R. gigas</i>	1
<i>C. harveyana</i>	<i>Ceratandra</i>	WR	<i>R. gigas</i>	1
<i>C. bicolor</i>	<i>Ceratandra</i>	WR	<i>R. gigas</i>	1
<i>Disperis bolusiana</i> subsp. <i>bolusiana</i>	<i>Disperis</i>	WR	<i>R. peringueyi</i>	1
<i>D. bolusiana</i> subsp. <i>macrocoris</i>	<i>Disperis</i>	WR	<i>R. longimanus</i>	1
<i>D. purpurata</i>	<i>Disperis</i>	WR	<i>R. macgregori</i>	1
<i>D. villosa</i>	<i>Disperis</i>	WR	<i>R. peringueyi</i>	1
<i>D. tysonii</i>	<i>Disperis</i>	SR	<i>R. neliana</i> , <i>R. brunnea</i>	1
<i>D. cardiophora</i>	<i>Disperis</i>	SR	<i>R. neliana</i> , <i>R. brunnea</i>	1
<i>D. fannineae</i>	<i>Disperis</i>	SR	<i>R. colorata</i>	1
<i>D. oxyglossa</i>	<i>Disperis</i>	SR	<i>R. neliana</i> , <i>R. brunnea</i>	1
<i>D. wealei</i>	<i>Disperis</i>	SR	<i>R. neliana</i> , <i>R. brunnea</i>	1
<i>Satyrium rhynchanthum</i>	<i>Satyrium</i>	WR	<i>R. gigas</i>	1

* Two samples from the same population were collected and the mean relative amount of the compounds was used for the scent analyses.

RESULTS

The total number of compounds identified from the scents of the 42 oil-secreting orchids [39 taxa/ecotypes, two subspecies, one presumed hybrid (*Corycium nigrescens* Sond. × *C. dracomontanum* Parkman & Schelpe)] was 257 and these comprised nine compound classes (Table 2, Appendix 2). Nearly half (48%, N = 124) were aliphatics (i.e., derived from fatty acid metabolism) and these represented more than double the number of compounds present in any other compound class. The other main classes were benzenoids (19%; N = 49), monoterpeneoids (16%; N = 41), sesquiterpenoids (7%; N = 19) and phenylpropanoids (5%; N = 13), with the remaining four minor classes contributing together only 5% (N = 11) of the total (Table 2). The number of compounds detected per taxon ranged from nine in *C. crispum* (Thunb.) Sw. to 58 in *C. orobanchoides* with a mean and standard deviation of 26.5 ± 10.0 . Aliphatics were the dominant compounds

(relative amounts = 48%–99%) in two-thirds of the clades (N = 8), benzenoids were dominant (48%–94%) in one-quarter of the clades (N = 3), and sesquiterpenes were dominant (46%) in only one of the 12 clades (Table 2). More than 60% of the compounds occurred in only one or two taxa/ecotypes, whereas only 3% of the compounds occurred in more than half of the taxa/ecotypes (see Fig. 3). Among the latter were several aliphatics (viz., Octanal, Decanal, 2-Tridecanone, et al.), a benzenoid (Benzaldehyde), and a monoterpene (Limonene).

Scent variation in the Winter Rainfall region—In the Winter Rainfall region, scent patterns were significantly correlated with clade (PERMANOVA: Pseudo- $F_{9,28} = 2.58$, $P < 0.001$) but not with pollinators (PERMANOVA: Pseudo- $F_{4,28} = 1.11$, $P = 0.38$), and there was no significant interaction effect between the two (PERMANOVA: Pseudo- $F_{6,28} = 0.83$, $P = 0.70$). Therefore, the significant clade effect is not influenced by the

TABLE 2. Summary of scent composition (mean relative amount, %) by compound group and by clade. EUPTER = *Eupterygodium*, CAFF = *Caffrum*, LEU = *Leucanthum*, ANO = *Anochilus*, MAG = *Magnum*, OMMA = *Ommatodium*, COR = *Corycium*, ALA = *Alatum*, MICR = *Micranthum*, CERA = *Ceratandra*, DISP = *Disperis*, SAT = *Satyrium*; mean relative amounts $\geq 10\%$ are in bold; tr: trace (= relative amount $< 0.5\%$).

Clade	EUPTER	CAFF	LEU	ANO	MAG	OMMA	COR	ALA	MICR	CERA	DISP	SAT
Region SR/WR	1/5	0/3	1/0	1/3	1/0	2/2	2/5	0/1	0/2	0/3	5/4	0/1
Aliphatics												
Alkanes	tr	—	—	—	—	2	tr	—	tr	tr	tr	—
Alkenes	—	—	—	—	—	—	—	—	—	—	1	—
Alcohols	16	1	tr	6	4	3	2	3	2	9	7	tr
Aldehydes	35	52	1	3	15	28	1	tr	tr	tr	60	tr
Ketones	tr	45	2	39	tr	tr	37	78	49	78	1	3
Acids	23	tr	—	tr	40	11	1	3	—	tr	12	—
Esters	tr	—	27	—	tr	tr	3	—	tr	tr	8	—
Benzenoids												
Alcohols	8	—	tr	19	14	1	23	tr	tr	2	1	20
Aldehydes	tr	—	tr	1	2	1	3	tr	tr	tr	1	4
Ketones	—	—	—	—	—	46	tr	—	—	—	—	—
Ethers	5	tr	—	—	—	tr	20	—	tr	tr	tr	69
Esters	tr	—	—	10	—	tr	4	—	—	tr	—	1
Phenylpropanoids												
Alcohols	—	—	—	—	—	—	tr	—	—	—	—	—
Aldehydes	—	—	—	—	—	—	1	—	—	—	—	—
Ethers	8	—	—	tr	—	—	—	—	2	3	—	—
Esters	tr	—	—	—	—	—	—	—	—	—	—	—
Carotenoid-derived												
Alcohols	—	—	—	—	—	—	—	—	—	—	tr	—
Aldehydes	—	—	—	—	—	—	—	—	—	—	tr	—
Monoterpenes												
Hydrocarbons	tr	1	12	12	1	5	tr	tr	1	tr	2	tr
Alcohols	tr	—	1	tr	tr	tr	tr	tr	33	5	tr	1
Aldehydes	—	—	—	—	—	—	—	—	1	tr	—	—
Ketones	—	—	—	—	tr	tr	—	—	—	—	—	—
Acids	—	—	—	—	—	—	tr	—	—	—	—	—
Ethers	tr	tr	10	—	tr	tr	tr	—	tr	tr	tr	tr
Esters	—	—	—	—	—	—	—	—	1	tr	tr	—
Monoterpene-derived												
Alcohols	tr	—	1	—	—	—	—	—	—	—	—	—
Ketones	tr	—	1	tr	tr	2	tr	1	tr	tr	tr	—
Sesquiterpenes												
Hydrocarbons	1	—	tr	7	23	tr	tr	tr	4	—	tr	tr
Alcohols	—	—	44	—	—	—	1	7	6	1	2	—
Aldehydes	—	—	—	—	—	—	—	tr	tr	tr	—	—
Ethers	1	—	2	2	tr	—	—	—	tr	—	—	tr
Esters	—	—	—	—	—	—	tr	8	tr	—	1	—
Sesquiterpene-derived												
Ketones	—	tr	—	—	—	tr	4	tr	—	—	4	—
N-bearing compounds												
Totals	—	—	—	—	—	—	tr	—	—	tr	—	—
Aliphatics	75	99	29	48	59	44	45	84	51	88	88	4
Benzenoids	14	tr	tr	29	16	48	49	tr	tr	2	2	94
Phenylpropanoids	8	—	—	tr	—	—	1	—	2	3	—	—
Carotenoid-derived	—	—	—	—	—	—	—	—	—	—	tr	—
Monoterpenes	1	1	23	13	2	6	tr	tr	36	6	3	1
Monoterpene-derived	tr	—	2	tr	tr	2	tr	1	tr	tr	tr	—
Sesquiterpenes	2	—	46	9	23	tr	1	15	10	1	3	1
Sesquiterpene-derived	—	tr	—	—	—	tr	4	tr	—	—	4	—
N-bearing compounds	—	—	—	—	—	—	tr	—	—	tr	—	—

pollinator effect. Taxa belonging to the same clade had a similar scent pattern and therefore occur in a similar region in the NMDS ordination space, whereas the bees (e.g., *Rediviva peringueyi* (Friese), *R. longimanus* Michener, *R. macgregori* Whitehead & Steiner, and *R. gigas* Whitehead & Steiner) that pollinate taxa in more than one clade are scattered throughout the space (Fig. 4A). Specific bees pollinate taxa that emit quite different scents. Also, taxa with similar scent profiles can be pollinated by different species of bees. As an example, the two taxa analyzed in the clade *Ommatodium* (*Pterygodium schelpei* and *P. volucris* (L.f.) Sw.) are

very close in the scent ordination space (Fig. 4A) but are pollinated by different *Rediviva* species (i.e., *R. longimanus* and *R. peringueyi*, respectively (Table 1)). Similarly, the three taxa/ecotypes analyzed in the clade *Caffrum* (*Pterygodium pentherianum* Schltr., *P. caffrum* “early”, *P. caffrum* “late”) have very similar scents (Fig. 4A), and are pollinated by three different *Rediviva* species (i.e., *R. longimanus*, *R. peringueyi*, and *R. gigas*, respectively).

With the exception of aliphatic 2-ketones, 18 major scent compounds occurred in large amounts in taxa from only one or a few clades, though some also occurred as minor constituents

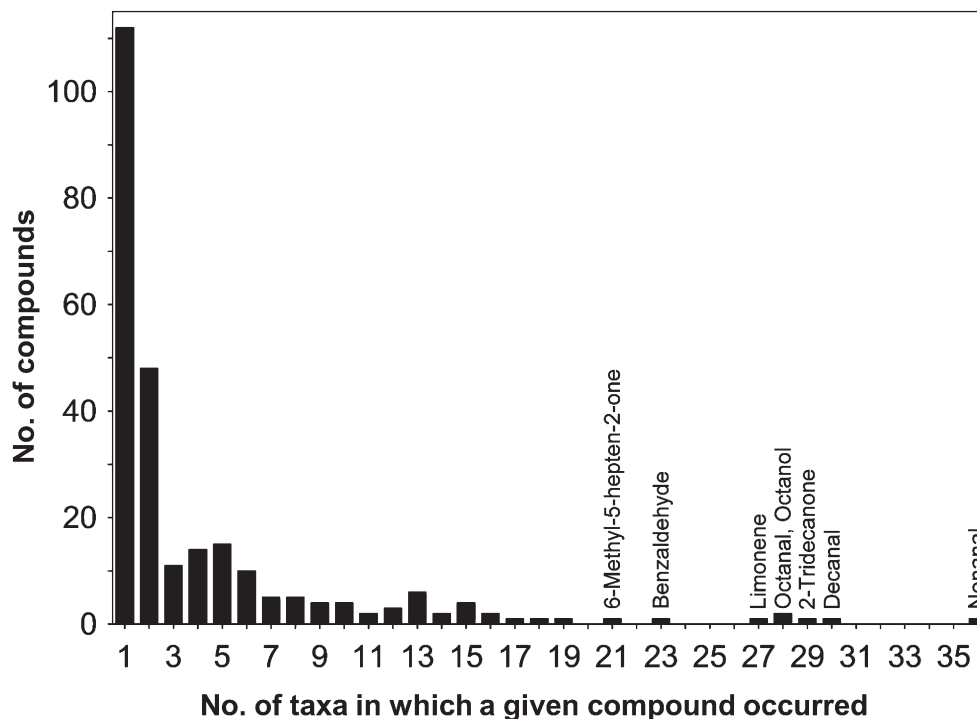


Fig. 3. Plot of number of compounds vs. a given number of taxa/ecotypes [e.g., 112 (43.6%) of the total scent compounds occurred in only one taxon/ecotype whereas single compounds occurred in from one to 36 taxa/ecotypes]. For example, the compounds Benzaldehyde, Limonene and Nonanal occurred in 23, 27 and 36 taxa/ecotypes respectively.

in taxa from other clades (Fig. 4B). Overall, seven of the compounds were quite widespread, occurring in at least ten of the 29 taxa distributed among five to eight clades (aliphatics circled and labeled as 1, 2 and 3 in Fig. 4B). The most abundant of these was 2-Tridecanone, which occurred in 21 taxa distributed across eight of the 12 clades (Fig. 2). The other six compounds occurred in as few as two taxa (i.e., Z-4-Octenoic acid; Fig. 2) or as many as nine (e.g., Anisyl alcohol) distributed in one to five clades.

Though we could not identify a pollinator effect at the clade level in the Winter Rainfall region, we could identify both temporal and spatial ecotypes in scent profiles that correlated with pollinator species. In the *Eupterygodium* clade, *Pterygodium catholicum* temporal ecotypes have different pollinators (*Rediviva peringueyi* and *R. gigas* for early and late flowering ecotypes, respectively) and emit different relative amounts of compounds (Table 2, Fig. 5A) (PERMANOVA: Pseudo- $F_{1,6} = 32.67$, $P = 0.046$) even though their scents are both dominated by aliphatic compounds and most of the compounds are shared (Table 3). Both ecotypes emit high relative amounts of Octanoic acid and additionally either high amounts of the aldehydes Dodecanal and Decanal (*P. catholicum* “early”) or the alcohols Dodecanol and Decanol (*P. catholicum* “late”) (Table 3). In the *Anochilus* clade, there are scent ecotypes within *P. hallii* that occur in different habitats (coastal vs. inland) and these are pollinated by different pollinators (Table 4, Fig. 5B).

Scent variation in the Summer Rainfall region—In the Summer Rainfall region there was also a significant clade effect on scent composition (PERMANOVA: Pseudo- $F_{6,12} = 1.72$, $P = 0.002$), but we could not analyze for a pollinator effect (see Materials and Methods for more details). Most of these orchids share the same

pollinator guild (i.e., *Rediviva neliana/R. brunnea*) and this is scattered throughout the ordination space of floral scent compounds.

Thirteen compounds occurred with a relative amount of $\geq 20\%$ in at least one taxon, but only one compound (Dodecanal) was found abundant ($\geq 20\%$) in more than one clade (Fig. 6). Seven of the 13 compounds were generally very specific, occurring in only one or two taxa and in only one or two clades. Only Benzyl alcohol (10 taxa, 6 clades), Dodecanal (7 taxa, 4 clades), Limonene (5 taxa, 3 clades), Anisyl alcohol and (E)-Nerolidol (both 4 taxa, 3 clades), and (E, Z)-2,6-Dodecadienal (3 taxa, 2 clades) were more common.

Scent variation between Winter and Summer Rainfall region—With one exception, oil-secreting orchids in the Summer Rainfall region emit more or less the same scent pattern as plants in the Winter Rainfall region even though they are pollinated by different *Rediviva* species in the two regions. There was a significant clade effect in the combined analysis of scents from clades occurring in both Winter and Summer Rainfall regions (PERMANOVA: Pseudo- $F_{4,29} = 3.48$, $P < 0.001$), a significant region effect (PERMANOVA: Pseudo- $F_{1,29} = 1.91$, $P = 0.032$), and a significant interaction effect (Clade*Region, PERMANOVA: Pseudo- $F = 1.41$, $P = 0.045$). Although the clade effect is not influenced by region, the region effect is influenced by clade. This significant region effect was caused only by the *Corycium* clade (PERMANOVA: Pseudo- $F_{1,6} = 3.84$, $P = 0.047$), as other clades did not have a significant effect (PERMANOVA: $P > 0.25$ each) (Fig. 7). For the *Corycium* clade, taxa of the Summer Rainfall region emit scent dominated by Benzyl alcohol and Anisyl alcohol, while taxa in the Winter Rainfall region emit aliphatic 2-ketones, Hydroquinone monomethyl ether or 3,5-Dimethoxytoluene (compare with Figs. 4B, 6).

TABLE 3. Relative scent composition (mean relative amount, %) of “early” and “late” flowering populations of *Pterygodium catholicum*. Localities: 1. Kirstenbosch; 2. Roman’s River; 3. Bot River; 4. Gydo Pass; 5. Chapman’s Peak-1 6. Chapman’s Peak-2; and 7. Franschoek Pass.

	<i>P. catholicum</i> “early”					Ave.	<i>P. catholicum</i> “late”		
	1	2	3	4	5		6	7	Ave.
Aliphatics									
Tetradecane	—	—	—	—	—	—	0.1	—	0.1
Pentadecane	—	—	—	—	—	—	0.2	—	0.2
Hexadecane	—	—	—	—	—	—	0.1	—	0.1
Octadecane	—	—	—	0.8	—	0.8	—	—	-
Hexanoic acid	2.6	1.1	4.8	6.4	4.3	3.8	6.4	2.7	4.5
Octanoic acid	6.6	2.8	17.2	13.5	13.7	10.7	22.3	30.1	26.2
Nonanoic acid	—	—	—	—	—	-	—	—	-
Decenoic acid	0.4	0.1	—	2.3	0.31	0.8	0.3	—	0.3
Dodecanoic acid	—	—	—	1.1	—	1.1	—	—	-
Hexanal	—	—	—	0.1	—	0.1	—	—	-
Octanal	0.5	0.3	0.2	0.3	1.1	0.5	0.1	—	0.1
Nonanal	0.7	0.3	—	0.8	0.5	0.6	—	0.1	0.1
(E)-2-Nonenal	—	—	—	—	—	-	0.2	—	0.2
Decanal	11.4	3.8	4.4	3.5	7.2	6.1	0.1	0.2	0.2
Dodecanal	69.6	80.0	61.0	47.8	62.9	64.2	0.7	1.6	1.1
Undecanal	0.3	0.1	—	—	0.1	0.1	—	—	—
Tetradecanal	—	0.2	0.1	0.4	0.3	0.3	—	—	—
Hexadecanal	—	—	—	0.1	0.1	0.1	—	—	—
a Octadecenal	—	—	—	3.40	—	3.4	—	—	—
2-Tridecanone	0.3-	—	—	—	—	0.3	—	0.1	0.1
Hexanol	—	0.10	0.1	—	0.4	-	1.4	—	1.4
Octanol	0.4	0.3	—	0.2	0.8	0.4	9.7	—	9.7
Nonanol	0.1	—	—	—	0.0	0.1	0.2	0.1	0.2
Decanol	0.8	0.6	0.1	0.8	0.9	3.3	9.2	9.0	9.1
Undecanol	1.0	—	—	0.3	—	0.5	—	—	—
Dodecanol	1.0	0.9	1.2	1.7	0.6	1.1	39.7	45.2	42.5
Methyl caproate	—	—	—	—	—	-	—	—	—
Dodecyl formate	—	—	—	—	—	-	—	0.1	—
Benzenoids									
Phenol	—	—	—	—	—	-	—	—	—
Benzaldehyde	—	—	—	—	—	-	—	—	—
para-Cresol	3.2	2.4	6.6	5.7	1.6	3.9	6.7	2.1	-
Creosol	0.5	3.7	3.7	7.6	2.8	3.7	2.1	4.9	-
Anisyl alcohol	0.1	—	—	—	—	0.1	—	—	—
Methyl benzoate	—	0.2	—	—	—	0.2	—	—	—
3,4-Dimethoxytoluene	0.1	0.9	0.1	2.3	0.3	0.7	—	0.1	0.1
Hydroquinone dimethyl ether	—	0.4	0.2	—	—	0.3	—	—	—
para-Methyl anisole	—	1.0	—	—	0.4	0.4	—	—	—
Phenylpropanoids									
Eugenol	—	—	—	—	1.03	1.0	—	—	—
Coumarine	—	—	—	—	—	-	0.1	—	0.1
Monoterpene-derived									
6-Methyl-5-hepten-2-one	—	—	—	0.1	0.21	0.1	—	—	0.0
Monoterpenoids									
Limonene	—	0.1	0.1	0.1	—	0.1	0.1	—	0.1
Linalool	—	0.3	—	0.3	—	0.1	—	3.5	1.8
Sesquiterpenoid									
Caryophyllene	—	—	—	—	—	—	—	—	—
(E,E)- α -Farnesene	—	—	—	—	0.1	0.2	—	—	—

(Waterman et al., 2009) were very similar. Both pairs had scents dominated by 2-Tridecanone and the other compounds were very similar as well (Fig. 4B).

DISCUSSION

The importance of phylogeny—This study demonstrates the importance of quantifying the relative contribution of natural selection and phylogenetic constraint in understanding patterns of evolution. It is especially true in situations that might be

counterintuitive with respect to the relative importance of natural selection in determining the final pattern of evolution (i.e., floral scent composition). Among the oil-secreting orchids of South Africa, where many species are pollinated by a single bee species, one would expect pollinator selection (in this case convergence on a particular scent type) to be more important than for a plant with a more generalized pollination syndrome, where conflicting scent preferences from unrelated pollinators would make it far less likely for convergence on a limited number of scent constituents. However, despite the specialized nature of the pollination system of the oil-secreting orchids of

TABLE 4. Relative scent composition (%) of *Anochilus* clade for compounds present in $\geq 1.0\%$ for at least one collection. Locations for multiple collections: 1. Bot River; 2. Darling; 3. Oorlogskloof road; and 4. Langebaan.

	<i>Corycium flanaganii</i>	<i>Pterygodium inversum</i> -1	<i>P. inversum</i> -2	<i>P. hallii</i> "inland"3	<i>P. hallii</i> "coastal"4
Aliphatics					
Nonanal	1.1	4.6	3.7	1.8	0.4
Decanal	-	0.5	1.1	-	-
Dodecanal	-	-	2.9	-	-
2-Tridecanone	4.3	3.0	5.6	1.6	40.4
2-Tridecanol	-	-	-	-	1.6
Tetradecane	2.4	-	-	-	-
2-Petadecanol	-	0.9	1.8	-	0.6
2-Petadecanone	2.5	0.6	1.0	5.3	34.1
a Heptadecen-2-ol	-	8.0	20.4	-	5.5
2-Heptadecanone	-	-	-	1.7	1.2
a Heptadecen-2-one	-	-	-	49.1	8.4
para-Cresol	1.8	-	0.1	-	-
Anisyl alcohol	-	51.8	28.4	32.7	0.9
Anisyldehyde	-	2.2	1.1	1.3	-
Anisyl angelate	-	1.5	-	-	-
Anisyl tiglate	-	24.7	31.8	5.2	4.8
Monoterpenes					
Limonene	33.3	-	-	-	-
α -Pinene	6.2	-	-	-	-
β -Pinene	1.2	-	-	-	-
Sabinene	1.6	-	-	-	-
Myrcene	3.4	-	-	-	-
para-Cymene	2.7	-	-	-	-
Terpinen-4-ol	1.6	-	-	-	-
Sesquiterpenes					
Caryophyllene	27.3	-	-	-	-
Caryophyllene epoxide	8.6	-	-	-	-

southern Africa and our prediction that scent profiles would be convergent, at least within clearly recognizable guilds pollinated by single bee species (i.e., the *Rediviva peringueyi* guild—Pauw, 2006), we found no significant effect of pollinators on floral scent at the clade level. Instead, we found that phylogeny was more important in controlling scent variation even within this group of closely related plant species pollinated by a single class of pollinators.

Variation in overall scent constituents within this group of orchids is surprisingly large, although confined primarily to aliphatic and benzenoid compounds (rarely to terpenoid compounds). With a few possible exceptions (see pollinator-mediated selection), *Rediviva* oil-collecting bees do not appear to have specific compound preferences within a class of compounds. This indicates that pollinator-mediated stabilizing selection may not be very strong. If convergence on a relatively small number of compounds were important, one might expect less overall variation in the scents of these orchids and a consistent presence of signature compounds.

We found that different *Rediviva* species can pollinate different orchids with similar scents (e.g., the allopatric sister pair *Pterygodium caffrum* and *P. pentherianum* in the Caffrum clade) or the same orchid species (e.g., *P. catholicum*) with different scents, and a single *Rediviva* species may pollinate different orchid species with similar or different scents. This is most obvious from the scattered scent profiles of orchids pollinated by *R. peringueyi* and *R. gigas* in the NMDS ordination space (Fig. 4A).

Among the 257 floral scent compounds identified from the samples, many were widespread (Knudsen et al., 2006), and some were very rare or unique to orchids such as 2-Methoxy-6-methylacetophenone, Anisyl tiglate and Anisyl angelate. Besides

its presence in the *Ommatodium* clade, 2-Methoxy-6-methylacetophenone has only been described from the floral scents of the unrelated *Cyphomandra divaricata* C. Martius ex Sendtner (Solanaceae) and *Colocasia gigantea* Schott (Araceae) (Kaiser, 2010). Anisyl tiglate and its accompanying (Z) isomer (Anisyl angelate) have not yet been found in other floral scents.

Compounds that occurred in at least half of the taxa/ecotypes analyzed (e.g., Benzaldehyde, Limonene), as well as compounds present in fewer taxa, but in relatively large amounts (e.g., 2-Tridecanone, Decanal, Benzyl alcohol, Anisyl alcohol), have been found previously not only in oil-orchids, but also in non-oil plants (Knudsen et al., 2006). Among the rare or novel floral scent compounds discovered were the uncommon but abundant (Z)-4-Octenoic acid, 2-Methoxy-6-methylacetophenone, (Z)-9-Hexadecenal, and (E, Z)-2,6-Dodecadial, compounds that may provide specific cues that allow the bees to detect appropriate oil-hosts and discriminate oil- from nonoil-plants.

Overall, our samples contained several widespread and well-known compounds, some that occur in the scents of other oil-secreting plants pollinated by oil-collecting bees, and others that have rarely or never been found in the floral scents of other flowering plants. Floral scents have been described from only a few other oil-secreting plants, including Oncidiine orchids from South America (Kaiser, 1993) pollinated by anthophorid bees (Vogel, 1974), *Tritoniopsis parviflora* (Jacq.) G.J.Lewis (Iridaceae), pollinated by a *Rediviva* bee (Manning and Goldblatt 2005), and *Lysimachia punctata* L. (Primulaceae) pollinated by *Macropis* oil-collecting bees (Dötterl and Schäffler, 2007). *Macropis* bees are closely related to *Rediviva* (Michez et al., 2009), but occur in the Holarctic and not in southern Africa (Vogel, 1986). Several of the compounds emitted by *Tritoniopsis parviflora* and *Lysimachia punctata* were also found in

our scent samples (e.g., Benzaldehyde, 2-Tridecanone, 3,5-Dimethoxytoluene), yet there are notable differences as well. *Tritoniopsis* emits Benzyl benzoate and *Lysimachia* emits 1-Phenyl-1,2-propanedione (together with 1-Hydroxy-1-phenyl-2-propanone) as abundant compounds, both of which were absent from our samples.

Pollinator-mediated selection—Although our analyses above the species level suggest that phylogeny is more important than pollinators for explaining scent composition among the oil-secreting orchids, we have identified four potential cases of pollinator-mediated scent selection at the species level and below where pollinator shifts correspond to differences in scent profiles. First, in the *Anochilus* clade, there has been a shift from pollination by oil-collecting bees to leaf-cutter bees in the Summer Rainfall region in at least one population even though the flowers of the Summer Rainfall species, *Corycium flanaganii*, retain the ability to secrete floral oil (Steiner, unpublished data). This corresponds to a shift from a scent strongly dominated by aliphatic and benzenoid compounds to one strongly dominated by terpenoids (Table 4, Fig. 5B). This change may have occurred as a result of strong pollinator limitation caused by its flowering prior to the emergence of resident oil-collecting bees (Steiner, unpublished data). A second pollinator shift but with a partial loss of the ability to secrete oil has occurred in *Ceratandra grandiflora* Lindl. in the *Ceratandra* clade. In this species, there has been a shift from pollination by *Rediviva* bees to pollination by beetles that do not forage for the oil (Steiner, 1998; Lahti et al., 2009). The scent of *C. grandiflora* has not yet been sampled, but qualitatively it is much weaker and less dominated by the types of aliphatic compounds that give its sister taxon, *Ceratandra atrata* (L.) Dur. & Schinz (Fig. 2), its distinctive fragrance (Steiner, unpublished data). For *C. grandiflora*, the pollinator shift can be attributed to colonization of a habitat

where oil-collecting bees do not occur (Whitehead and Steiner, 2001) and the weaker scent is due, presumably, to relaxed selection by pollinators that respond primarily to visual rather than olfactory cues (Picker and Midgley, 1996; Steiner, 1998).

A third likely example of pollinator-mediated selection of floral scent below the species level occurs in *Pterygodium catholicum*, in the *Eupterygodium* clade. It exhibits intraspecific variation in both morphological (Steiner and Cruz, 2009) and scent characters that correlate with flowering time, habitat, and pollinator (Steiner and Liltved, 2011). The differences are subtle and appear to have evolved in response to selection by two different oil-collecting bees, *Rediviva peringueyi* and *R. gigas* (Whitehead and Steiner, 2001; Steiner, unpublished data). These bees are the major pollinators of two different orchid guilds in the Winter Rainfall region that are separated primarily by flowering time (early vs. late) and floral color (Steiner and Cruz, 2009; Steiner and Liltved, 2011). The scents of orchids pollinated by these bees can be similar or different (Fig. 4). The “early” guild flowers at the beginning of spring and has greenish white to greenish yellow flowers (Pauw, 2006), whereas the “late” guild flowers at the end of spring and mostly has bright yellow flowers or occasionally purplish, pink, or white flowers. *Pterygodium catholicum* bridges these two pollination guilds by having “early” and “late” flowering ecotypes. The differences in the scent profiles of these two ecotypes (Fig. 5A) appear to be the result of pollinator-mediated selection based on the preferences of the two *Rediviva* species for the ecotype with which they normally co-occur (Waterman et al., 2011), yet the chemicals within the scents that are responsible for these preferences remain to be determined (e.g., Does *R. peringueyi* prefer the aliphatic alcohols Dodecanol and Decanol over the aliphatic aldehydes Dodecanal and Decanal and *R. gigas* the reverse?). If this is the case, it suggests that a relatively minor change in the reaction pathway (the reduction of the aldehyde to the alcohol) may reflect differences in pollinator preference. In the *Caffrum* clade, paradoxically, the “early” and “late” forms of *P. caffrum* belong to the same two guilds as the two forms of *P. catholicum*, yet they lack any obvious scent differences and group closely together in the scent ordination space based on the presence of (Z)-9-Hexadecenal in relatively large (30–62%) amounts.

In the *Anochilus* clade, scent ecotypes of *Pterygodium hallii* are associated with different habitats and pollinators and a large spatial separation (Table 4, Fig. 5B). The scent of the coastal form of *P. hallii* is strongly dominated (74.5%) by 2-Tridecanone and 2-Pentadecanone, whereas the inland form is strongly dominated (81.8%) by Anisyl alcohol and a-Heptadecen-2-one. However, spatial separation alone does not guarantee the development of scent ecotypes, since populations of the related *P. inversum* can occur over 115 km apart with little variation in scent profile (Table 4, Fig. 5B).

A fourth potential example of pollinator-mediated scent evolution can be observed from convergence in scents of two sympatric, but only distantly related, Coryciinae orchids (*Corycium deflexum* and *Disperis purpurata*) that belong to a guild pollinated by *Rediviva macgregori* (Steiner 1989, Steiner, unpublished data). These two orchids were the only species tested in the Coryciinae that have relatively large amounts (48.2 and 9.0%, respectively) of (Z)-4-Octenoic acid in their scents (Fig. 2).

Data on floral scents in the Iridaceae also suggests pollinator-mediated selection of scent constituents by oil-collecting bees (Manning and Goldblatt, 2002; 2005). Manning and Goldblatt (2002) found that the major scent component in *Tritoniopsis*

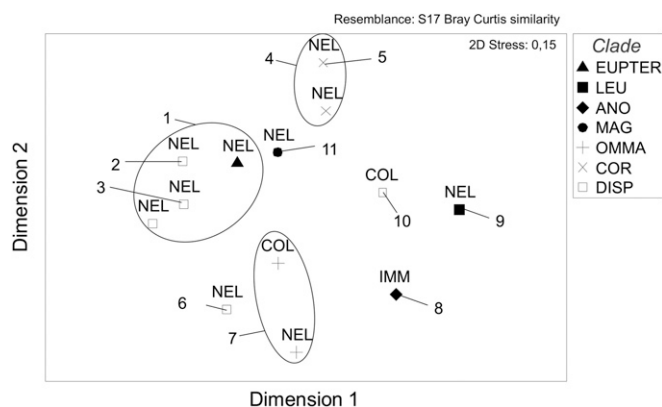


Fig. 6. Overlay of important scent compounds on plot of relative scent composition of oil-secreting orchid species in the Summer Rainfall region using NMDS, with orchid clades (symbols), major scent components (numerals), and pollinators (abbreviations) indicated. Only scent compounds constituting $\geq 20\%$ of total sample are plotted. Pollinator abbreviations: NEL = *Rediviva neliana* and *R. brunnea* guild, COL = *R. colorata*, IMM = *Immanthidium* sp. (Megachilidae). 1: Dodecanal (25–83%); 2: (Z,Z)-9,11-Hexadecadienal (tentative) (30%); 3: (Z)-5-Tetradecenyl acetate (40%); 4: Benzyl alcohol (34–61%); 5: Anisyl alcohol (37%); 6: (E,Z)-2,6-Dodecadienal (27%); 7: 2-Methoxy-6-methyl acetophenone (28–65%); 8: Limonene (33%) + Caryophyllene (27%); 9: (E)-Nerolidol (44%); 10: (E)-Geranylacetone (22%); 11: (Z)-4-Decenoic acid (32%) + Longifolene (20%)

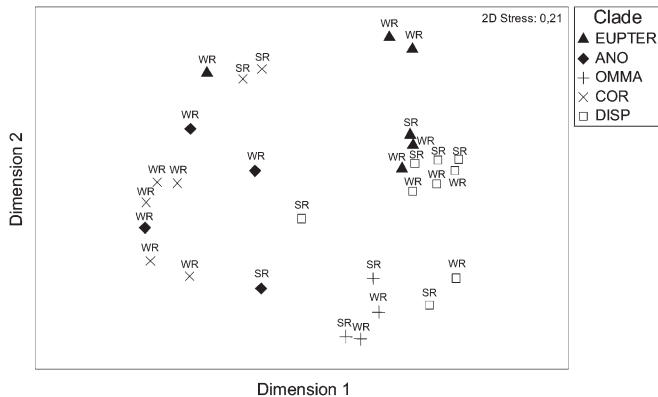


Fig. 7. Plot of relative scent composition of oil-secreting orchid species from both Winter and Summer Rainfall regions using NMSD, with orchid clades (symbols) and regions (abbreviations) indicated. Abbreviations: WR = Winter Rainfall region and SR = Summer Rainfall region.

parviflora, the only oil-secreting species of Iridaceae in southern Africa, is 3,5-Dimethoxytoluene, a benzenoid compound that is also present in substantial amounts in the scent of the oil-secreting orchid *Corycium orobanchoides* (also in present study), but not in the scents of the two other groups of insect-pollinated *Tritoniopsis* species (bee-flowers and moth-flowers, respectively). This led them to suggest that this compound or compound class (i.e., benzenoids) might be characteristic of oil-secreting orchids in general and, by implication, the result of pollinator-mediated selection. Our results indicate that benzenoid compounds, including 3,5-Dimethoxytoluene, are abundant scent components in some oil-secreting orchids but are not as frequent as aliphatic compounds (e.g., 2-Tridecanone, 2-Pentadecanone, (Z)-9 Hexadecenal, Decanal and Dodecanal), nor signature compounds for the group as a whole.

Morphology vs. scent—We expected greater evidence for pollinator-mediated selection among these highly specialized (in terms of pollination) orchids, particularly within the large guilds pollinated by *Rediviva peringueyi*, *R. gigas*, and *R. neliana/brunnea*. Except for the examples already discussed, however, surprisingly little evidence exists for the convergent evolution of scents that one would expect if pollinator-mediated selection were pervasive within these guilds. This is counter to the situation in the sexually deceptive orchids of Peakall et al. (2010), where there is little morphological variation among species and scent plays the primary role in reproductive isolation. In the oil-secreting orchids of southern Africa morphology appears to play an important role in reproductive isolation and speciation. This takes the form of variation in the overall shape of the flowers (i.e., globose vs. open) and the relative position of the oil reward and pollinaria within the flower. Species within a clade usually deposit pollinaria on the same part of the bee pollinator whereas species in different clades tend to deposit pollinaria on a different part of the bee (Waterman et al., 2011). Thus, within a clade one would expect differences in scent to be more important than morphology and between clades, one would expect the opposite. However, even within most clades, sister species are ecologically isolated by having different pollinators due either to differences in habitats or flowering times (Steiner and Cruz, 2009). Thus, scent variation may be more important for

species-specific recognition and the development of flower constancy by the bee pollinators (Wright & Schiestl, 2009) than in reproductive isolation and speciation.

Other studies have shown that some scent compounds in headspace samples are not detected by pollinators (e.g., Dötterl et al., 2006; Dötterl, 2008; Riffell et al., 2009; Schiestl and Marion-Poll, 2002; Salzmann et al., 2007), and that these non-biologically active compounds show less intraspecific variation than the biologically active scent compounds (Ibanez et al., 2010). If this is the case in the Coryciinae, then divergence among scents might only involve small changes in one or a few of the active scent compounds that constitute the scent profile; these changes might then be pollinator-mediated.

The scents of most Coryciinae, *sensu lato*, are strong to the human nose compared to nonorchid oil-secreting plants visited by some of the same oil-collecting bees. For example, most oil-secreting Scrophulariaceae in southern Africa have weak or barely detectable scents to the human nose, especially *Diascia* (sect. *Racemosae*) and *Hemimeris* spp. (Steiner, unpublished data). Even some oil-secreting orchids (e.g., *Huttonaea* spp.) have weak or barely detectable scents, yet the flowers are still pollinated by oil-collecting bees (Steiner, 2010). Similarly, some oil-secreting *Lysimachia* species (Primulaceae) (e.g., *L. vulgaris* L. and *L. congestiflora* Hemsley) lack perceptible scent even though they are pollinated by oil-collecting bees, whereas others such as *L. punctata*, *L. nummularia* L., and *L. ciliata* L. have relatively strong scents (Dötterl and Schäffler, unpublished). This suggests that some of the compounds attractive to oil-collecting bees may not be detectable to the human nose. Further, flowers may also emit compounds of low volatility that may not be detectable by headspace methods but which nevertheless influence bee attraction, especially when the bees are near the flowers (e.g., Schiestl et al., 1999). Solvent extracts of flowers from several of the orchids studied here and from *Lysimachia* species have revealed such low-volatility compounds, the importance of which are currently under investigation (Dötterl et al., unpublished data).

Electroantennographic responses from other bees (Dötterl et al., 2005a; Dötterl and Vereecken, 2010; Salzmann et al., 2007; Schiestl and Roubik, 2003), including oil-collecting bees related to *Rediviva* (i.e., *Macropis*, Dötterl, 2008; Dötterl and Vereecken, 2010), strongly suggest that *Rediviva* bees cannot detect all of the compounds in the scents of their oil-secreting host plants. However, we would expect a response to many of the more abundant compounds listed in Figs. 5 and 7. For example, the widespread occurrence and abundance of 2-Tridecanone suggests that it is a biologically active compound for *Rediviva*, especially because it is active for *Macropis* bees (Dötterl, 2008; Dötterl and Vereecken, 2010). Similarly, Benzyl alcohol, 1,4-Dimethoxybenzene, Eugenol, Geraniol, and (E)-Nerolidol, each dominate the scent of at least one orchid species and are electrophysiologically and/or behaviorally active for other bees (Dötterl et al., 2005a, Dötterl and Vereecken, 2010; Salzmann et al., 2007; Schiestl and Roubik, 2003), and may therefore also be biologically active in *Rediviva*. These may be used by *Rediviva* to locate host plants and discriminate rewarding from nonrewarding species.

The scents of many of the oil-secreting orchids are heavily dominated (in number as well as amount) by aliphatic compounds (i.e., products of fatty acid metabolism; see also Table 2), unlike the scents of plants visited by bees that do not collect oil (Dobson, 2006). This may be due to shared genetic and metabolic pathways needed by the flowers for the production of both volatile aliphatic compounds in the scent and nonvolatile floral

oils that constitute the food reward. Some of the oil-secreting orchids, however, produce only small amounts of aliphatics in their scents (e.g., *Satyrium rhynchanthum*), as do oil-secreting flowers in other plant families (e.g., *Tritoniopsis parviflora* (Iridaceae) and *Lysimachia punctata* (Primulaceae) (Dötterl and Schäffler, 2007; Manning and Goldblatt, 2005).

Conclusions—Scents of oil-secreting orchids in southern Africa are unexpectedly diverse but mostly dominated by aliphatic, sometimes by benzenoid, and very rarely by terpenoid compounds. This implies that oil-collecting bees have selected for scents within broad classes of compounds (i.e., aliphatics or benzenoids) rather than specific compounds within these classes. Nonetheless, with a few exceptions the diversity within these compound classes is not correlated with particular *Rediviva* species nor biogeographic regions (e.g., Summer vs. Winter Rainfall). Instead, it is best explained as a direct consequence of orchid phylogeny, because significant effects were found only for orchid clades and not for pollinators. Pollinator-mediated selection, as suggested by both convergence in response to the same pollinator and divergence in response to different pollinators, is thus not as pervasive in the Coryciinae as expected. Both suggest pollinator-mediated selection, yet these effects are minor relative to the much more extensive effect of phylogeny. We could not distinguish between biologically active and inactive compounds, nor were we able to test the attractiveness of these compounds in bioassays with different *Rediviva* species. Developing tests for these aspects of this pollination system represent the next steps toward a better understanding of the functional significance of these unusual orchid scents and those of other oil-secreting plants.

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APPENDIX 1. Taxa used in this study with author(s), collector, collector's number, and acronym of herbarium where voucher specimens are housed (Thiers, 2011).

Ceratandra atrata (L.) Dur. & Schinz, <i>Steiner 3318</i> (NBG); <i>C. bicolor</i> Sond. ex Bolus, <i>Steiner 3430</i> (NBG); <i>C. harveyana</i> Lindl., <i>Steiner 3314</i> (NBG). Corycium bicolorum (Thunb.) Sw., <i>Steiner 3424</i> (NBG); <i>C. carnosum</i> Lindl. (Rolfe), <i>Steiner 3317</i> (NBG); <i>C. crispum</i> (Thunb.) Sw., <i>Steiner 3399</i> (NBG); <i>C. deflexum</i> (Bolus) Rolfe, no voucher; <i>C. dracomontanum</i> Parkman & Schelpe, <i>Steiner 3478A</i> (NBG); <i>C. flanaganii</i> (Bolus) Kurzweil & H. P. Linder, <i>Steiner 3336</i> (NBG); <i>C. ingeanum</i> Oliver, <i>Steiner 3398</i> (NBG); <i>C. nigrescens</i> Sond. × <i>C. dracomontanum</i> Parkman & Schelpe, <i>Steiner 3478</i> (NBG); <i>C. orobanchoides</i> (L.f.) Sw., <i>Steiner 4326</i> (CAS). Disperis bolusiana Schltr. ex Bolus subsp. bolusiana, <i>Steiner 3298</i> (NBG); <i>D. bolusiana</i> subsp. macrocorys (Rolfe) J. C. Manning, <i>Steiner 3898</i> (CAS); <i>D. cardiophora</i> Harv., <i>Steiner 3535</i> (NBG); <i>D. fannineae</i> Harv., <i>Steiner 3504</i> (NBG); <i>D. oxyglossa</i> Bolus, <i>Steiner 3536</i> (NBG); <i>D. purpurata</i> Reichenb.f. subsp. purpurata, <i>Steiner 3960</i> (CAS); <i>D. tysonii</i> Bolus <i>Steiner 3502</i> (NBG); <i>D. villosa</i> Sw., <i>Steiner 3367A</i> (NBG); <i>D. wealei</i> Reichenb.f., <i>Steiner 3493</i> (NBG);	Evotella rubiginosa (Sond. ex Bolus) Kurzweil & H. P. Linder, <i>Steiner 3441</i> (NBG). Pterygodium acutifolium Lindl., <i>Steiner 3313A</i> (NBG); <i>P. alatum</i> (Thunb.) Sw., <i>Steiner 3712</i> (CAS); <i>P. caffrum</i> (L.) Sw. "early", <i>Steiner 3411</i> (NBG); <i>P. caffrum</i> "late", <i>Steiner 3425</i> (NBG); <i>P. catholicum</i> (L.) Sw. "early", <i>Steiner 3918</i> (CAS), <i>3829</i> (CAS), <i>3892</i> (CAS), <i>3939</i> (CAS); <i>P. catholicum</i> "late", <i>Steiner 3308</i> (NBG); <i>P. cooperi</i> Rolfe, <i>Steiner 3577</i> (CAS); <i>P. cf. cooperi</i> , <i>Steiner 3569</i> (CAS); <i>P. cruciferum</i> Sond., <i>Steiner 3301</i> (NBG); <i>P. hallii</i> (Schelpe) Kurzweil & H. P. Linder "inland", <i>Steiner 3397</i> (NBG); <i>P. hallii</i> "coastal", <i>Steiner 4367</i> (CAS); <i>P. hastatum</i> H. Bol., <i>Steiner 3492</i> (NBG); <i>P. inversum</i> (Thunb.) Sw., <i>Steiner 3416</i> (NBG), <i>3894</i> (CAS); <i>P. leucanthum</i> Bolus, <i>Steiner 3455</i> (NBG); <i>P. magnum</i> Reichenb.f., <i>Steiner 3494</i> (NBG); <i>P. penterianum</i> Schltr., <i>Steiner 3362</i> (NBG), <i>3365</i> (NBG); <i>P. platypetalum</i> Lindl., <i>Steiner 3365A</i> (NBG); <i>P. schelpei</i> H. P. Linder, <i>Steiner 3291A</i> (NBG); <i>P. volucris</i> (L.f.) Sw., <i>Steiner 3297</i> (NBG). Satyrium rhynchanthum Bolus, <i>Steiner 3429</i> (NBG).
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APPENDIX 2. Occurrence of the compounds in the clades and taxa of the orchids analyzed from the Winter (WR) and Summer (SR) Rainfall regions.

	Occurrence WR		Occurrence SR			Occurrence WR		Occurrence SR	
	Clade (10)	Taxa (29)	Clade (7)	Taxa (13)		Clade (10)	Taxa (29)	Clade (7)	Taxa (13)
Aliphatics (124)									
<i>Alkanes (8)</i>									
Undecane	1	1	0	0					
Dodecane	1	1	0	0					
Tridecane	2	2	1	1					
Tetradecane	6	7	1	1					
Pentadecane	4	4	1	1					
Hexadecane	2	2	1	1					
Octadecane	1	1	0	0					
Heneicosane	0	0	1	1					
<i>Alkenes (2)</i>									
1-Pentadecene	0	0	1	2					
(Z)-1,8-Pentadecadiene	0	0	1	1					
<i>Alcohols (31)</i>									
1-Penten-3-ol	0	0	1	1					
Hexanol	4	7	1	2					
(Z)-3-Hexenol	4	4	1	1					
Heptanol	4	5	0	0					
2-Heptanol	1	2	0	0					
2-Methylheptanol	1	1	0	0					
Octanol	9	19	6	9					
(E)-2-Octenol	2	2	0	0					
(Z)-4-Octenol	1	1	0	0					
1-Octen-3-ol	2	4	3	3					
Nonanol	6	14	2	3					
2-Nonanol	3	3	0	0					
1-Decen-3-ol	0	0	1	1					
Decanol	4	7	2	3					
(Z)-3-Decenol	0	0	1	2					
(Z)-6-Decen-1-ol	0	0	1	1					
(E,Z)-2,4-Decadienol	1	1	0	0					
(E,E)-2,4-Decadienol	1	1	0	0					
(E)-3-Methyl-4-decen-1-ol	1	2	1	2					
Undecanol	2	4	1	1					
2-Undecanol	6	13	0	0					
Dodecanol	5	10	2	4					
(Z)-3-Dodecenol	3	3	0	0					
(Z,Z)-3,6-Dodecadienol	3	4	2	3					
(E,E)-2,6-Dodecadienol	1	1	0	0					
2-Tridecanol	7	15	0	0					
					Tetradecanol	1	1	0	0
					2-Pentadecanol	3	4	0	0
					(Z)-8-Pentadecen-2-ol	1	1	0	0
					(Z)-9-Hexadecenol	1	2	0	0
					a Heptadecen-2-ol	1	2	0	0
					<i>Aldehydes (31)</i>				
					Hexanal	6	9	4	7
					Heptanal	4	5	4	8
					(E)-2-Heptenal	1	1	1	1
					(E,E)-2,4-Heptadienal	0	0	1	1
					Octanal	8	17	7	11
					(E)-2-Octenal	3	3	2	3
					(E,Z)-2,4-Octadienal	2	2	0	0
					Nonanal	9	24	7	12
					(E)-2-Nonenal	1	2	3	4
					(Z)-2-Nonenal	0	0	1	1
					Decanal	8	19	5	11
					(E)-2-Decenal	2	2	1	1
					(E)-4-Decenal	1	1	0	0
					(Z)-4-Decenal	2	3	0	0
					(E,Z)-2,4-Decadienal	2	2	0	0
					(E,E)-2,4-Decadienal	2	4	0	0
					(E)-3-methyl-4-decenal	1	2	1	2
					Undecanal	2	6	3	4
					Dodecanal	6	12	4	7
					(E)-2-Dodecenal	1	1	1	2
					(Z,Z)-3,6-Dodecadienal	2	2	0	0
					(E,Z)-2,6-Dodecadienal	3	5	2	3
					(Z,Z,Z)-3,6,9-Dodecatrienal	0	0	1	1
					Tridecanal	0	0	1	2
					Tetradecanal	2	4	2	4
					Hexadecanal	4	8	1	1
					(Z,Z)-7,10-Hexadecadienal	2	4	0	0
					(Z,Z)-9,11-Hexadecadienal (tentative)	0	0	1	1
					(Z)-7-Hexadecenal	2	4	0	0
					(Z)-9-Hexadecenal	2	5	1	1
					a Octadecenal	1	1	0	0
					<i>Ketones (16)</i>				
					2-Heptanone	4	6	0	0
					1-Octen-3-one	0	0	2	2
					2,3-Octadione	1	1	0	0

APPENDIX 2. Continued

	Occurrence WR		Occurrence SR			Occurrence WR		Occurrence SR	
	Clade (10)	Taxa (29)	Clade (7)	Taxa (13)		Clade (10)	Taxa (29)	Clade (7)	Taxa (13)
2-Nonanone	7	13	1	2	2-Oxy-6-methylacetophene	0	0	1	1
1-Nonen-3-one	0	0	1	1	2-Methoxy-6-methylacetophenone	2	3	1	2
2-Decanone	1	1	0	0	<i>Ethers (13)</i>				
2-Undecanone	7	18	0	0	Anisole	1	1	0	0
2-Dodecanone	5	9	0	0	para-Methylanisole	5	10	3	4
2-Tridecanone	8	21	5	8	1,4-Dimethoxybenzene	3	7	3	4
2-Tetradecanone	2	2	0	0	Hydroquinone monomethyl ether	2	3	1	1
2-Pentadecanone	6	12	3	3	3,4-Dimethoxytoluene	1	4	0	0
(Z)-8-Pentadecen-2-one	1	2	0	0	3,5-Dimethoxytoluene	4	5	1	2
2-Hexadecanone	1	1	0	0	para-Methoxybenzyl methyl ether	3	4	0	0
2-Heptadecanone	3	5	0	0	para-Methoxyphenylethyl methyl ether	1	2	0	0
a Heptadecen-2-one	1	2	0	0	3,4-Dimethoxybenzyl methyl ether	1	1	0	0
2-Oxy-5-methylhexan-3-one	1	1	0	0	1,2,4-Trimethoxybenzene	2	2	0	0
<i>Acids (12)</i>					1,3,5-Trimethoxybenzene	1	2	0	0
Acetic acid	2	2	0	0	3,4,5-Trimethoxytoluene	1	1	0	0
Pentanoic acid	1	1	0	0	Benzyl methyl ether	1	1	0	0
Hexanoic acid	5	11	1	1	<i>Esters (17)</i>				
Heptanoic acid	3	3	0	0	Benzyl formate	0	0	1	2
Octanoic acid	7	12	1	1	Benzyl acetate	2	2	2	3
(E)-4-Octenoic acid	1	1	0	0	Methyl benzoate	1	1	0	0
(Z)-4-Octenoic acid	2	2	0	0	Methyl salicylate	0	0	1	1
Nonanoic acid	5	8	0	0	Methyl anisate	1	1	0	0
Decanoic acid	4	9	0	0	Anisyl formate	4	4	1	1
(Z)-4-Decenoic acid	0	0	1	1	Anisyl acetate	4	6	1	1
(E)-3-Methyl-4-decenoic acid	1	2	1	1	Anisyl propionate	1	1	0	0
Dodecanoic acid	2	6	0	0	Anisyl isobutyrate	1	1	0	0
<i>Esters (20)</i>					Anisyl butyrate	1	1	0	0
Methyl caproate	1	1	1	1	Anisyl isovalerate	1	1	0	0
Methyl 2-ethylcaproate	0	0	1	1	Anisyl angelate	1	1	0	0
Methyl octanoate	1	1	0	0	Anisyl tiglate	2	6	0	0
Methyl (Z)-4-octenoate	1	1	0	0	Anisyl caproate	1	2	0	0
Octyl acetate	0	0	1	1	Anisyl caprylate	1	1	0	0
Nonyl acetate	0	0	1	1	para-Methoxyphenylethyl acetate	1	1	0	0
Decyl acetate	0	0	1	1	Ethyl paramethoxyphenylacetate	1	1	0	0
Methyl (E)-3-methyl-4-decenoate	1	1	1	1	Phenylpropanoids (13)				
2-Undecyl acetate	3	5	0	0	<i>Alcohols(3)</i>				
Dodecyl formate	1	1	0	0	para-Methoxyphenylpropyl alcohol	1	1	0	0
Dodecyl acetate	0	0	2	3	(Z)-para-Methoxycinnamic alcohol	1	1	0	0
(Z)-3-Dodecenyl acetate	0	0	1	2	(E)-para-Methoxycinnamic alcohol	1	1	0	0
Tridecyl acetate	1	1	1	1	<i>Aldehydes (3)</i>				
2-Tridecyl acetate	1	2	0	0	(E)-Cinnamic aldehyde	1	1	0	0
Tetradecyl acetate	0	0	1	1	(Z)-para-Methoxycinnamic aldehyde	1	1	0	0
(Z)-5-Tetradecenyl acetate	0	0	1	1	(E)-para-Methoxycinnamic aldehyde	1	1	0	0
2-Pentadecyl acetate	0	0	1	1	<i>Ethers (6)</i>				
(Z)-9-Hexadecenyl acetate	1	2	0	0	Estragole	1	1	0	0
γ -Decalactone	0	0	1	1	Methyl eugenol	3	3	1	1
γ -Dodecalactone	0	0	1	1	Eugenol	4	6	1	1
Benzenoids (48)					(E)-Isoeugenol	1	1	1	1
<i>Alcohols (8)</i>					Elemicine	2	2	0	0
Phenol	2	2	0	0	6-Methoxyeugenol	1	1	0	0
Benzyl alcohol	4	5	6	10	<i>Esters (1)</i>				
para-Cresol	6	10	2	3	Coumarine	1	2	0	0
Creosol	1	4	2	2	Carotenoid-derived (5)				
2-Phenylethyl alcohol	4	5	4	5	<i>Alcohols (2)</i>				
Anisyl alcohol	4	9	3	4	β -Ionol	0	0	1	1
para-Methoxyphenylethyl alcohol	2	3	1	2	(E)-6,10-Dimethyl-5,9-undecadien-2-ol	0	0	1	1
4-Ethylguaiaicol	0	0	1	1	<i>Ketones (3)</i>				
<i>Aldehydes (6)</i>					β -Ionone	0	0	1	1
Benzaldehyde	7	13	6	10	(E)-9(10)-Epoxy-6,10-dimethyl-5-undecen-2-one	1	1	1	1
Anisylaldehyde	4	7	2	3	(E)-Geranylacetone	3	4	2	2
3,5-Dimethoxybenzaldehyde	1	1	0	0	Monoterpenes (42)				
3,4,5-Trimethoxybenzaldehyde	1	1	1	1	<i>Hydrocarbons (9)</i>				
Cumin aldehyde	1	1	0	0	α -Pinene	6	11	4	5
Vanilline	1	1	0	0	β -Pinene	7	8	3	4
<i>Ketones (4)</i>					Sabinene	2	2	1	1
Acetophenone	1	1	1	1	Myrcene	5	7	5	5
para-Methoxyacetophenone	1	2	0	0					

APPENDIX 2. Continued.

	Occurrence WR		Occurrence SR			Occurrence WR		Occurrence SR	
	Clade (10)	Taxa (29)	Clade (7)	Taxa (13)		Clade (10)	Taxa (29)	Clade (7)	Taxa (13)
Limonene	10	22	3	5	Geranyl formate	2	2	0	0
(Z)-Ocimene	0	0	1	1	Geranyl acetate	2	3	1	1
(E)-Ocimene	1	1	5	5	Geranyl butyrate	1	1	0	0
γ -Terpinene	0	0	1	1	Geranyl caproate	1	1	0	0
para-Cymene	2	2	2	2	Monoterpene-derived (5)				
<i>Alcohols (13)</i>					<i>Alcohols (2)</i>				
(trans)-Sabinene hydrate	0	0	1	1	6-Methyl-5-hepten-2-ol	1	1	1	1
(cis)-Sabinene hydrate	0	0	1	1	2-Methyl-2-vinyl-tetrahydrofuran-5-ol	0	0	1	1
α -Fenchol	0	0	1	1	<i>Ketones (3)</i>				
Linalool	5	8	4	5	6-Methyl-5-hepten-2-one	7	12	6	9
Ipsdienol	0	0	1	1	Cryptone	1	1	1	1
Terpinen-4-ol	0	0	1	1	2-Methyl-2-vinyl- tetrahydrofuran-5-one	0	0	1	1
2-Methyl-6-methylene-3,7-octadien- 2-ol	0	0	2	2	Sesquiterpenes (19)				
Citronellol	2	2	0	0	<i>Hydrocarbons (8)</i>				
Nerol	2	2	0	0	Longicyclene	0	0	1	1
Geraniol 10	5	6	0	0	Longifolene				
2,3-Epoxy geraniol	2	2	0	0	Caryophyllene	3	3	2	2
6,7-Epoxy geraniol	1	1	0	0	(E)- β -Farnesene	2	2	2	2
para-Cymen-8-ol	0	0	1	1	Humulene				
<i>Aldehydes (3)</i>					Germacrene D	0	0	1	1
Citronellal	1	1	0	0	Germacrene A	0	0	1	1
Neral	1	1	0	0	(E,E)- α -Farnesene	3	3	2	2
Geranial	2	2	0	0	<i>Alcohols (3)</i>				
<i>Ketones (3)</i>					(E)-Nerolidol	4	4	3	4
Camphor	0	0	1	1	(E)-2-(3)-Dihydrofarnesol	1	1	0	0
Ipsdienone	0	0	1	1	(E,E)-Farnesol	4	4	1	1
Carvone	1	1	0	0	<i>Aldehydes (3)</i>				
<i>Acids (1)</i>					(E)-2-(3)-Dihydrofarnesal	1	1	0	0
Geranic acid	1	1	0	0	(Z,E)-Farnesal	1	1	0	0
<i>Ethers (7)</i>					(E,E)-Farnesal	3	3	0	0
1,8-Cineole	5	8	3	3	<i>Ethers (3)</i>				
Perillene	1	1	0	0	Caryophyllene β -epoxide	0	0	1	1
Myrcene epoxide	1	1	3	4	Caryophyllene epoxide	2	2	3	3
(trans)-Linalool oxide (furanoid)	1	1	1	1	(E)- α -Farnesene epoxide	1	1	1	1
(cis)-Linalool oxide (furanoid)	1	1	1	1	<i>Esters (2)</i>				
(E)-Ocimene epoxide					(E)-2(3)-Dihydrofarnesyl acetate	1	1	0	0
2-Hydroxy eucalyptol	0	0	1	1	(E,E)-Farnesyl acetate	3	3	1	1
<i>Esters (7)</i>					N-bearing compounds (2)				
Linalyl acetate	0	0	1	1	Phenylacetone nitrile	1	1	0	0
Methyl geranate	1	1	0	0	Indole	0	0	1	1
Citronellyl acetate	1	1	0	0					