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SPECIALIZATION FOR POLLINATION BY BEETLES AND WASPS: THE ROLE OF LOLLIPOP HAIRS AND FRAGRANCE IN *SATYRIUM MICRORRHYNCHUM* (ORCHIDACEAE)[1]

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Exposed nectar presentation is a key trait in flowers specialized for pollination by short-tongued insects. We investigated the pollination of *Satyrium microrrhynchum,* a rare South African orchid in which nectar is secreted as droplets on long floral hairs ("lollipop hairs") at the mouth of a shallow labellum. Our observations indicate that this orchid is pollinated specifically by two insect species: a cetoniid beetle (*Atrichelaphinus tigrina*) and a pompilid wasp (*Hemipepsis hilaris*). Both insects have short mouthparts and remove nectar from the hairs with sweeping motions of their mouthparts. Pollinaria become attached to the upper surface of their heads while they feed on the nectar. Beetles damage the hairs while feeding, which may explain the positive relationship between hair damage and pollination success in plants of *S. microrrhynchum* from populations where beetles were common. The orchid has cryptic green-yellow flowers with spectral reflectance similar to that of its leaves. The fragrance from plants in three populations, analyzed using gas chromatography coupled to mass spectrometry, was dominated by various terpenoids; linalool was the most abundant. Plants in different populations emitted similar compounds, but eugenol and derivatives of this compound were found in only one of the three populations. In an electrophysiological study (gas chromatography coupled to electroantennography), using antennae of *A. tigrina*, clear signals were elicited by some of the floral scent compounds.

Key words: bimodal pollination; floral scent; GC-EAD; GC-MS; nectar; nectary; Orchidaceae; pollination syndrome.

Flowers with exposed nectar are usually exploited by a wide range of short- and long-tongued insects, resulting in generalized pollination systems (Waser et al., 1996; Johnson and Steiner, 2000). However, specialized pollination systems can occur in plants with exposed floral nectar if they possess traits that filter flower visitors. Traits that have been suggested or shown to act as filters include cryptic flower coloration (Johnson, 2005), nectar that is unpalatable to certain visitors (Adler, 2000; Johnson et al., 2006; Shuttleworth and Johnson, 2006), and a floral scent with unusual compounds or blends of compounds (Raguso, 2004).

With a few exceptions, beetles and wasps have short tongues and are thus unable to exploit nectar in deep tubular flowers. They are recorded most frequently as components of the visitor fauna of generalist flowers with a shallow perianth and exposed nectar. Nevertheless, there are many examples of nectar-producing flowers that are specialized for pollination by these insects (Nilsson, 1978, 1979; Singer and Cocucci, 1997; Goldblatt et al., 1998; Steiner, 1998a, b; Sakai and Inoue, 1999; Bernhardt, 2000; Ollerton et al., 2003; Johnson, 2005; Shuttleworth and Johnson, 2006). Fragrance is a key floral attractant for most beetles and wasps (Bergstrom et al., 1991; Gottsberger and Silberbauer-Gottsberger, 1991; Schiestl et al., 1999), although visual cues are undoubtedly also important for some beetles (Dafni, 1997; Goldblatt et al., 1998).

Satyrium Sw., a largely African orchid genus of about 90 species, shows remarkable diversification in pollination systems, with pollination by moths, butterflies, bees, flies, and birds having been recorded in previous studies (Garside, 1922; Johnson, 1996, 1997a, b; Harder and Johnson, 2005). The flowers are unusual in having twin spurs; these are usually elongated with deeply concealed nectar accessible only to animals with long mouthparts. Phylogenetic analyses show that spurs have become reduced or even lost altogether in several lineages of *Satyrium* (T. van der Niet, University of Zurich, unpublished data). This is evident in the grassland species *Satyrium microrrhynchum* Schltr., which has vestigial sac-like spurs. Preliminary field observations indicated a number of other unusual features of *S. microrrhynchum*, including the cryptic green-yellow coloration of the perianth, long hairs at the mouth of the labellum that often has signs of damage, and a strong fruity fragrance emitted from the flowers.

The aim of this study was to determine whether *S. microrrhynchum* possesses a suite of modifications for pollination by short-tongued insects. We specifically asked (1) Which insects pollinate this species? (2) What are the properties of the nectar? (3) Is there a correlation between damage to the hairs and pollination success? (4) What is the chemical composition of the floral fragrance? (5) Do the main pollinators respond electrophysiologically to compounds in the floral fragrance?

MATERIALS AND METHODS

The study species—*Satyrium microrrhynchum* Schltr. (Fig. 1A) has been recorded from just eight localities along the eastern escarpment of South Africa. It is consequently listed in the red data book of threatened plants in southern Africa (Victor, 2002). Populations of *S. microrrhynchum* occur in short, moist grassland that is usually burnt during the winter months. Flowers of *S. microrrhynchum* are green-yellow with the labellum spurs absent or vestigial. The floor of the entrance to the labellum is lined with long hairs (Fig. 1A).

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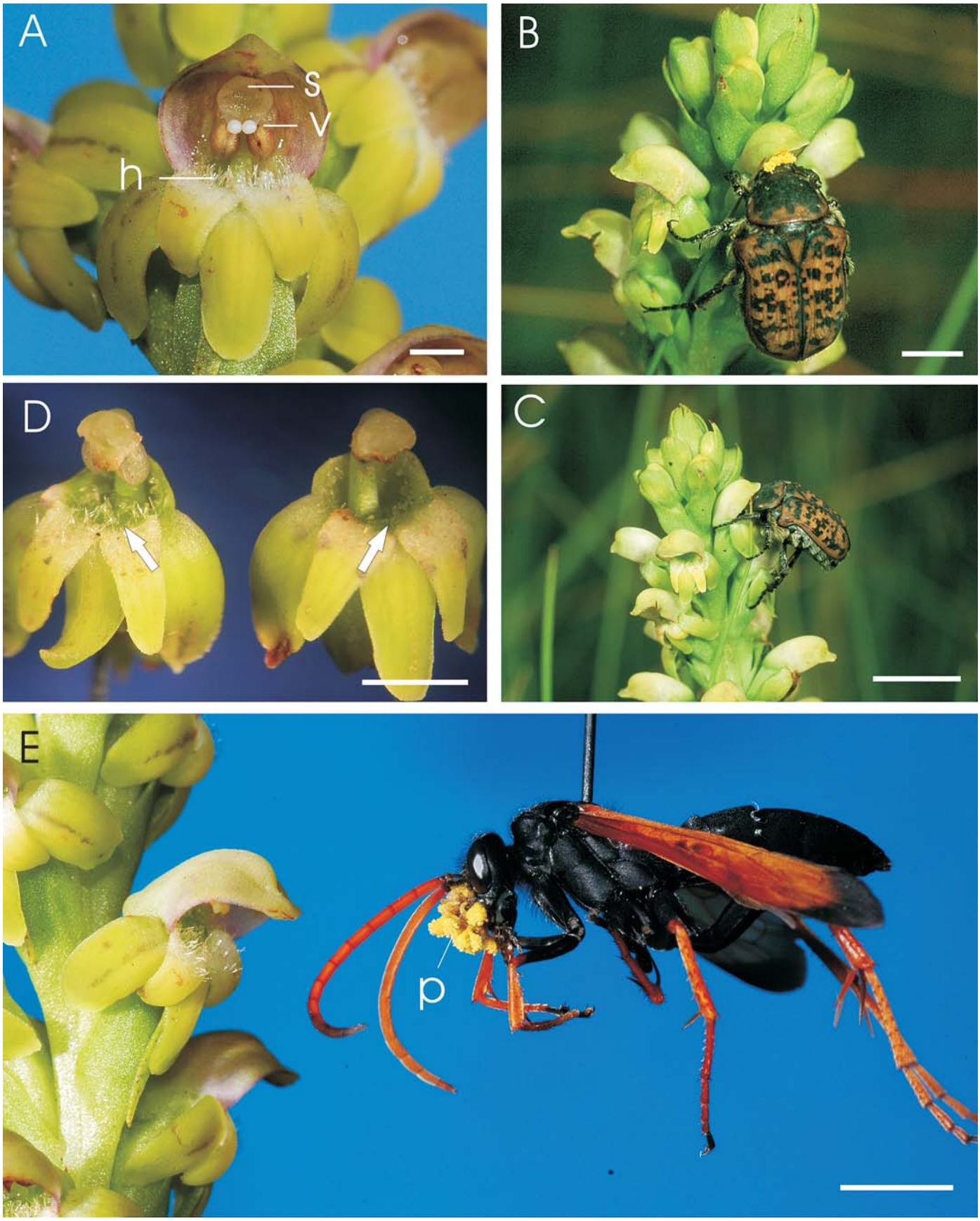
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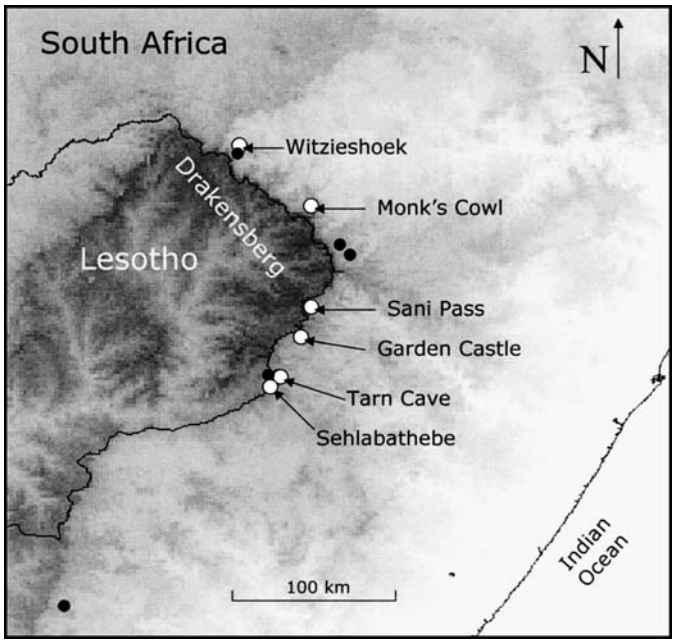

Fig. 2. Known localities of *Satyrium microrrhynchum* (filled circles) based on existing collections in herbaria and study sites (open circles).

Pollinia can only be withdrawn from the anther sacs if the caudicle is firmly pulled, thus preventing any autonomous self-pollination from taking place. Flowering takes place in January and February.

Study sites—We conducted studies at six sites encompassing almost the entire range of *S. microrrhynchum* in the Drakensberg Mountains (Fig. 2). The altitudes of these populations range from c. 2200–2800 m a.s.l. Most of the study populations are represented by existing herbarium specimens, but we deposited additional voucher specimens from the Garden Castle and Tarn Cave populations in the Bews Herbarium, University of KwaZulu-Natal, Pietermaritzburg.

Pollinator observations—We conducted 70 h of pollinator observations at the study sites, spread across 10 days between 2001 and 2006. Observations typically took place from 0800 to 1500 hours. The sites where observations were conducted most intensively were Tarn Cave (3 days in 2004 and 2006), Garden Castle (3 days in 2001 and 2002), and Sani Pass (2 days in 2006). Observations at the remaining sites were confined to a single day each. Insects visiting the flowers were captured and the number of *S. microrrhynchum* pollinaria on their bodies counted. In the case of well-worn pollinaria we counted the viscidia adhering to the exoskeleton. To establish the functional fit between the insects and the labellum of the orchid, measurements were taken of the lengths and widths of the heads of the insects captured at the Tarn Cave site using digital calipers. Voucher specimens of insect pollinators are deposited in the Natal Museum, Pietermaritzburg.

Floral morphology and nectar—The morphology of the labellum hairs of *S. microrrhynchum* was investigated using scanning electron microscopy (SEM). Flowers fixed in FAA (70% ethanol : 40% formalin : glacial acetic acid = 85 : 10 : 5) were dehydrated through a graded ethanol series and then critical-point dried in liquid carbon dioxide in a Hitachi HCP2 criticalpoint drier. Dried samples were then mounted onto a specimen stub, sputter-coated with gold palladium and viewed at 15 kV in a Philips XL30 Environmental Scanning Electron Microscope (ESEM). Width and depth of the labellum of flowers of living plants in the Tarn Cave population were measured using TA digital calipers. Spectral reflectance of *S. microrrhynchum* flowers and leaves was measured using an Ocean Optics (Dunedin, Florida, USA) S2000 spectrophotometer as described by Johnson and Andersson (2002). The volume of the standing crop of nectar in *S. microrrhynchum* flowers at the Garden Castle and Tarn Cave populations was determined using calibrated 5-μL pipettes. A 0–50% refractometer was used to establish the sugar concentration of the nectar. Because of the very small volumes of nectar, samples from several flowers had to be combined for concentration measurements.

Hair damage and pollination success—In each population except for Witsieshoek, we recorded the extent of damage to labellum hairs, deposition of pollen massulae on the stigma, and removal of pollinaria. Data were expressed in terms of the proportion of flowers per plant. After arcsine-square root transformation, these variables were compared among populations using one-way ANOVA. We also used linear regression to establish the relations between hair damage and various measures of pollination success.

Volatile collection—To characterize the floral scent composition of *S. microrrhynchum*, scent was collected using dynamic headspace methods as described by Dötterl et al. (2005b) at three different populations (Sani Pass, Monk's Cowl, Tarn Cave). At each population, scent was collected from two different inflorescences. Each flowering inflorescence was enclosed for 10 min within a polyethylene oven bag (size: 10 × 10 cm, Toppits, Toronto, Ontario, Canada), and the emitted volatiles were trapped for 2 min in an adsorbent tube using a membrane pump (G12/01 EB ASF, Rietschle-Thomas Inc., Puchheim, Germany). The flow rate was adjusted to about 200 mL/min using a 9 V battery. The closed end of ChromatoProbe quartz microvials (length: 15 mm; inner diameter: 2 mm; Varian Inc, Palo Alto, California, USA) were cut for use as adsorbent tubes, which were then filled with a mixture (1 : 1) of 3 mg of Tenax-TA (mesh 60–80, Supelco, Bellefonte, Pennsylvania, USA) and Carbotrap (mesh 20–40, Supelco). The adsorbents were fixed in the tubes using glass wool prior to scent collection.

Floral scent samples for the GC-EAD (gas chromatography coupled to electroantennography) analyses (described next) were collected using a different dynamic headspace method. For each sample, three inflorescences were cut, immediately placed in water, and enclosed in an oven bag, and volatiles were collected for 4 h in an adsorbent tube filled with 30 mg of the adsorbent mixture described previously. Volatiles were eluted with 70 μL acetone (SupraSolv, Merck KgaA, Germany). One sample was collected at Sani Pass, and two samples were collected at Tarn Cave.

Chemical analysis—The samples were analyzed with a Varian Saturn 2000 System using a 1079 injector that had been fitted with the ChromatoProbe kit (see Dötterl et al., 2005b; Dötterl and Jürgens, 2005). A quartz microvial was loaded into the probe, which was then inserted into the modified GC injector.The injector split vent was opened (1/20) and the injector heated to 40°C to flush any air from the system. The split vent was closed after 2 min, and the injector was heated at 200°C/min, then held at 200°C for 4.2 min, after which the split vent was opened (1/10) and the injector cooled down.

A ZB-5 column (5% phenyl polysiloxane) was used for the analyses (length 60 m, inner diameter 0.25 mm, film thickness 0.25 μm; Phenomenex, Torrance, California, USA). Electronic flow control (EFC) was used to maintain a constant helium carrier gas flow of 1.8 mL min^{-1}. The GC oven temperature was held for 7 min at 40°C, then increased by 6°C per min to 250°C and held for 1 min. The MS interface was 260°C, and the ion trap worked at 175°C. The mass spectra were taken at 70 eV (in EI mode) with a scanning speed of 1 $scan^{-1}$ from m/z 30 to 350. The GC-MS data were processed using the Saturn Software package 5.2.1 (Varian Inc.). Components were identified using the NIST 02 mass spectral database (National Institutes of Standards and Technology [NIST] algorithm, Gaithersburg, Maryland, USA) or MassFinder 3.0 (http://www.massfinder.com) and confirmed by comparing retention times

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Fig. 1. *Satyrium microrrhynchum* and its pollinators from South Africa. (A) Flower showing globose viscidia (v), stigma (s), and labellum hairs (h). Bar = 2 mm. (B) Cetoniid beetle *Atrichelaphinus tigrina* emerging from a flower with pollinaria adhering to its head. Bar = 5 mm. (C) *A. tigrina* in nectar feeding position. Bar = 10 mm. (D) Dissected flowers (labellum hood removed) showing the difference between undamaged hairs with nectar droplets (left arrow) and damaged hairs (right arrow). Bar = 5 mm. (E) Spider-hunting wasp *Hemipepsis hilaris* with large load of pollinaria (p) posed in front of a *S. microrrhynchum* inflorescence. Bar = 5 mm.

TABLE 1. Damage to floral hairs, pollination success, and captured pollinators for six populations of *Satyrium microrrhynchum* (see Fig. 1). "Plants examined" refers to the sample size for measurements of hair damage and pollination success. Means for hair damage, pollen removal, and pollen receipt that share the same superscript letter are not significantly different (Tukey's test).

Site	Estimated population size (plants examined)	Hairs damaged (% of flowers)	Pollen removal (% of flowers)	Pollen receipt (% of flowers)	Insects carrying *S. microrrhynchum* pollinaria			
					Atrichelaphinus tigrina		*Hemipepsis hilaris*	
		$\bar{x} \pm 1$ SE	$\bar{x} \pm 1$ SE	$\bar{x} \pm 1$ SE	*N*	Pollinaria $\bar{x}$ (range)	*N*	Pollinaria $\bar{x}$ (range)
Sehlabathebe	150 (12)	12.1 ± 7.0^{a}	57.6 ± 7.1^{a}	26.1 ± 7.7^{a}	—	—	—	—
Tarn Cave	250 (10)	70.2 ± 10.8^{b}	73.4 ± 6.7^{ab}	69.5 ± 9.6^{ab}	9	15.7 (6–30)	1	3.0
Garden Castle	45 (19)	72.1 ± 6.3^{b}	83.4 ± 3.9^{ab}	38.2 ± 7.4^{a}	5	7.0 (1–16)	1	10.0
Sani Pass	4 (4)	0^{a}	90.1 ± 4.0^{b}	90.1 ± 4.0^{b}	—	—	1	2.0
Monk's Cowl	40 (12)	14.1 ± 5.9^{a}	85.8 ± 4.7^{ab}	73.3 ± 8.6^{ab}	2	3.5 (3–4)	2	18.5 (1–36)
Witsieshoek	8 (0)	—	—	—	—	—	1	4.0

with published data (Adams, 1995). Identification of individual components was confirmed by comparison of both mass spectrum and GC retention data with those of authentic standards.

Known amounts of different terpenoids, fatty acid derivatives, and benzenoids were injected into the column, and the mean response of these compounds was used for quantifying the unknowns. To identify the compounds eliciting signals in the GC-EAD study (described later), 1 μL of the acetone samples in a closed quartz vial was placed in the injector port by means of the ChromatoProbe and then analyzed as described.

Electrophysiology—Electrophysiological analyses of the floral scent extracts were performed with the GC-EAD system described by Dötterl et al. (2005a). Antennae from wild-caught females and males of *Atrichelaphinus tigrina* were tested. The GC-EAD system consisted of a gas chromatograph (Vega 6000 Series 2, Carlo Erba, Rodano, Italy) equipped with a flame ionization detector (FID) and an EAD setup (heated transfer line, 2-channel USB acquisition controller) provided by Syntech (Hilversum, Netherlands). An odor sample (1 μL) was injected splitless at 60°C, followed by opening the split vent after 1 min and heating the oven at a rate of 10°C/min to 200°C. The end temperature was held for 5 min. A ZB-5 column was used for the analyses (length 30 m, inner diameter 0.32 mm, film thickness 0.25 μm; Phenomenex). The column was split at the end by the four-arm flow splitter GRAPHPACK 3D/2 (Gerstel, Mülheim, Germany) into two pieces of deactivated capillary (length 50 cm, inner diameter 0.32 mm) leading to the FID and to the EAD setup. Makeup gas (He, 16 mL per min) was introduced through the fourth arm of the splitter. For measurements, the three lamella of an antenna were separated by small balls of dental wax. Subsequently, the pedicel of the excised antenna was mounted in one electrode, and the tip of the third lamella was mounted in the other glass micropipette electrode. Alternatively, the third lamella was cut from the antenna and mounted between the electrodes. The electrodes were filled with insect ringer's solution (8.0 g/L NaCl, 0.4 g/L KCl, 04 g/L $CaCl_2$) and connected to silver wires.

RESULTS

Pollinator observations—We captured 22 individual insects of just two species carrying *S. microrrhynchum* pollinaria at the six study sites (Table 1, Fig. 1C–E). Of the 16 captured cetoniid beetles *A. tigrina*, seven were female, seven were male, and in two the sex could not be determined. All six captured individuals of the pompilid wasp *Hemipepsis hilaris* were male. The only other visitors observed on *S. microrrhynchum* flowers were a single individual of a small unidentified pompilid wasp and c. 10 individuals of an unidentified muscid fly species (none of which carried pollinaria). Other flower-visiting insects, including various honeybees and solitary bees, were common at the study sites, but were never observed on *S. microrrhynchum* flowers. Pollinaria were attached to the frons of the beetles and wasps (Fig. 1C–E). Attachment occurs when the insect inserts its head into the labellum and the frons is pushed against the globular viscidia. There was no difference overall in the mean (±1 SE) number of pollinaria carried by beetles and wasps (11.5 ± 2.1 vs. 9.3 ± 5.5; $t = 0.46$; $P = 0.3$). Many of the pollinaria on these insects were heavily worn such that only the viscidium and caudicles remained. Beetles brought back to the laboratory and observed under a dissecting microscope were seen to use their maxillary palps to sweep nectar droplets from the labellum hairs of *S. microrrhynchum* flowers. As the insect feeds on nectar, pollinaria on its frons are pushed against the stigma depositing small numbers of individual massulae.

Floral morphology and nectar—The mean (±1 SE) dimensions of the labellum of flowers in the Tarn Cave population were as follows: width 3.92 ± 0.09 mm; depth 3.27 ± 0.16 mm ($N = 12$). This corresponds closely with the head dimensions of insects captured on *S. microrrhynchum* flowers at this site (*A. tigrina* width: 2.76 ± 0.05 mm, depth: 2.93 ± 0.03 mm, $N = 11$; *H. hilaris* width: 2.80 mm, depth: 2.53 mm, $N = 1$). Nectar in *S. microrrhynchum* is clearly secreted from the labellum hairs (evident from the formation of nectar droplets on the hairs of flowers brought to the laboratory). In SEM images these hairs, hereafter referred to as lollipop hairs because of their unusual mode of presenting nectar to insects, to be unicellular with a smooth cuticle, whereas underlying cells had a striated surface (Fig. 3). The mean (±1 SE) volume of the standing crop of nectar in *S. microrrhynchum* flowers was 0.27 ± 0.04 μL ($N = 14$) in the case of the Tarn Cave population and 0.37 ± 0.05 μL ($N = 13$) in the Garden Castle population. The mean sugar concentration of the nectar of the flowers from these populations was $8.6\% \pm 2.5\%$ ($N = 10$) and $7.3\% \pm 1.6\%$ ($N = 4$), respectively. The spectral reflectance of flowers did not differ markedly from the leaves (possibly indicating the presence of chlorophyll pigments in the flowers), although flowers showed more overall reflectance (brightness) than leaves (Fig. 4).

Hair damage and pollination success—Some degree of damage to floral hairs was evident in flowers at all populations, except at Sani Pass (Table 1). In some flowers, the hairs were completely removed, and the surface of the labellum was scoured to a smooth surface (Fig. 1D). Observations of live beetles feeding on cut flowers under a dissecting microscope indicated that damage to the hairs occurs when beetles sweep over them with their mouthparts. We found significant positive relationships between pollination success and hair damage in most of the populations (Fig. 5). The highest levels of hair

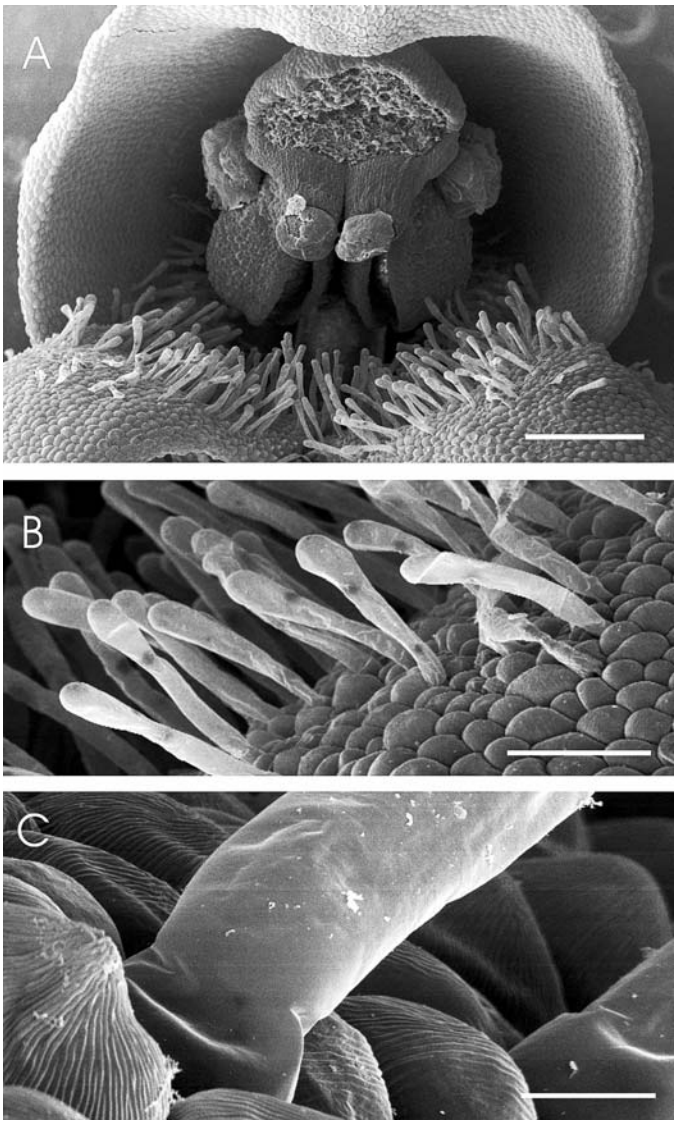

Fig. 3. Scanning electron micrographs of *Satyrium microrrhynchum* flowers. (A) Front view of a flower. Bar = 1 mm. (B) Labellum hairs. Bar = 200 μm. (C) Base of a single labellum hair. Note the difference in epidermal sculpturing between the hair and surrounding cells. Bar = 20 μm.

damage (c. 70% of flowers) as well as the highest numbers of beetles were recorded in the Garden Castle and Tarn Cave populations. No hair damage or beetle activity was recorded at Sani Pass. However, high levels of pollination success were recorded at this site, indicating that wasps are also effective as pollinators. In general, pollination levels in *S. microrrhynchum* flowers were remarkably high at most of the sites (Table 1). We recorded an average (±1 SE, range) of 8.43 ± 1.4, 0–58 massulae on the stigmas of 151 *S. microrrhynchum* flowers sampled at the Garden Castle site. Individual pollinia contained an average (±1 SE) of 100.3 ± 3.2 massulae ($N = 10$).

Volatile composition—Almost 70 compounds were detected in the scent of *S. microrrhynchum*, and more than 50 of these could be identified (Table 2). Many of the compounds were found to be monoterpenoids, but many sesquiterpenoids,

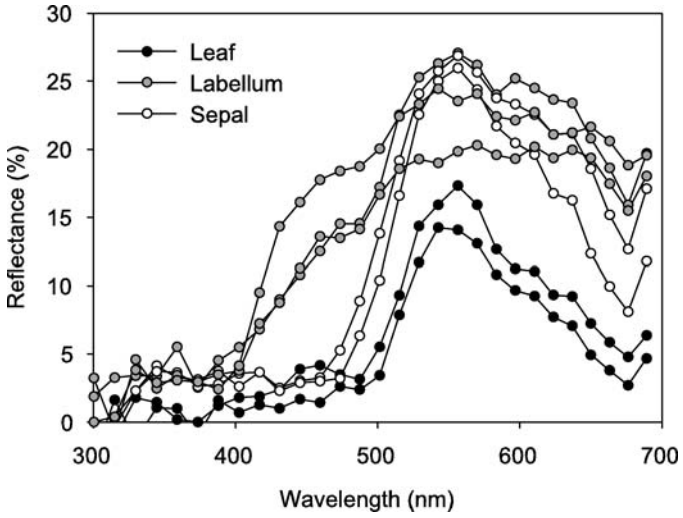

Fig. 4. Spectral reflectance of *Satyrium microrrhynchum* flowers and leaves.

benzenoids, some fatty-acid derivatives, and one irregular terpene were also found. Most of the compounds occurred in the scent from all three populations studied; however, eugenol and derivatives of eugenol were only detected in Tarn Cave samples. On the other hand, benzyl benzoate and myrtenol were only detected in samples from Sani Pass and Monk's Cowl.

Most of the compounds were emitted only in small amounts, and only six compounds reached a relative amount of at least 5% in any of the samples. The dominant compound in all samples was linalool with relative amounts of 37% in a Sani Pass sample and more than 70% in a Monk's Cowl sample. Eucalyptol was also abundant, especially in one Sani Pass sample, comprising 22% of the total. Elemicin, α-pinene, myrcene, and 2,6-dimethyl-1,5(*Z*),7-octatrien-3-ol reached relative amounts of 5–11%.

GC-EAD—In the electrophysiological study, we demonstrated that *A. tigrina* can detect (smell) at least some of the compounds emitted by *S. microrrhynchum* (Fig. 6). The biggest signal in the antennae is consistently elicited by linalool coeluting in the GC-EAD runs with 2,6-dimethyl-1,5(*E*),7-octatrien-3-ol. The antennae also responded to some of the eugenol derivatives from the Tarn Cave population. The male beetles seemed to respond more strongly to methyl salicylate than did female beetles.

DISCUSSION

The results of this study are consistent with floral specialization for pollination by beetles and wasps in *S. microrrhynchum*. Traits that appear to play a functional role in this pollination system include the shallow labellum (corresponding in dimensions with the heads of the pollinators), the nectar-secreting "lollipop" hairs (Fig. 3B); the globular viscidia (Figs. 1A, 3A), which are attached to the smooth surface of the frons of the beetles and wasps (by contrast, most other *Satyrium* species have plate-like viscidia, which attach to the proboscis [Johnson, 1997a]) and the emission of fragrance

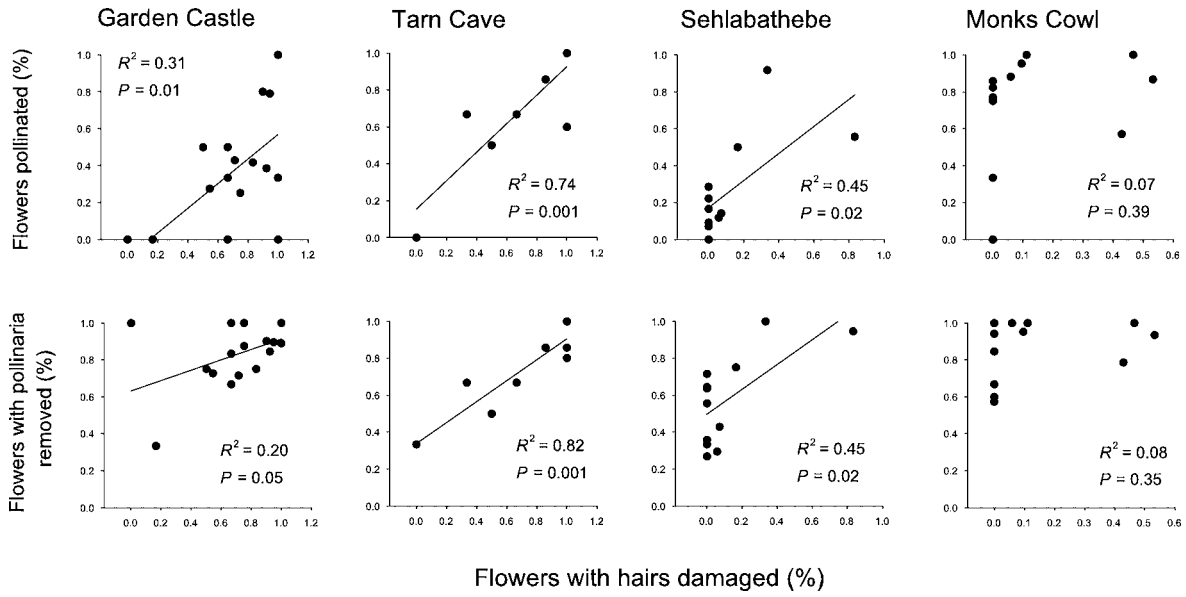

Fig. 5. Relationships between damage to labellum hairs and male and female components of pollination success in plants from four South African populations of *Satyrium microrrhynchum*.

compounds that elicit electrophysiological responses in the antennae of the beetles (Table 2, Fig 6). Although not investigated experimentally in this study, the cryptic coloration of *S. microrrhynchum* flowers may play a role in limiting visual attraction to insects that are morphologically unsuitable as vectors of the pollinaria. From a phylogenetic perspective, the traits in *S. microrrhynchum* are specialized in the sense that they were modified from ancestral traits that were adapted for pollination by long-tongued pollinators (T. van der Niet, University of Zurich, unpublished data).

Nectar-secreting hairs have been reported in several orchids (cf. Stipiczyńska, 1997), including *Satyrium coriifolium*, a long-spurred congener of *S. microrrhynchum* (Duthie, 1917). These hairs usually line the inner surface of a floral spur and are immersed in nectar. The presentation of nectar as individual droplets on the hairs in *S. microrrhynchum* flowers is highly unusual and appears to be a specialized trait that facilitates nectar feeding though the sweeping action of the mouthparts of beetles and wasps. Because of the damage to hairs in many populations (Table 1, Fig 1D), we initially thought that hairs were consumed as a reward. However, on closer inspection with a microscope of beetles feeding, the damage to the hairs occurs as an incidental consequence of the sweeping action of the mouthparts as the beetles feed on nectar. The nectar appears to be mopped up by the maxillary brushes, a mode of feeding on liquids that has also been reported to occur in another South African cetoniid beetle, *Trichostetha fascicularis* (Johnson and Nicolson, 2001). The nectar of *S. microrrhynchum* is surprisingly dilute (8%; see Results), but other plants specialized for pollination by *A. tigrina* also have nectar that is very dilute (S. Steenhuisen, unpublished data; Ollerton et al., 2003). Plants pollinated solely by *Hemipepsis* wasps, on the other hand, tend to produce more concentrated nectar (Ollerton et al., 2003; Johnson, 2005; Shuttleworth and Johnson, 2006). It is curious that the exposed nectar droplets in *S. microrrhynchum* flowers are not exploited by ants, which were common at all the study sites. Palatability tests with nectar and control sugar solutions (cf. Johnson et al., 2006; Shuttleworth and Johnson, 2006) should be conducted to establish whether there are compounds in the nectar of *S. microrrhynchum* that render it unpalatable to certain insects.

Although floral specialization for pollination by the beetle *A. tigrina* is evident in *S. microrrhynchum*, the beetle itself is a generalist, visiting flowers of many different plant species (cf. Ollerton et al., 2003). It is unlikely that the beetle responds only to a very specific scent compound. Indeed, our electrophysiological studies indicate that the antennae of this insect are responsive to several different compounds emitted by *S. microrrhynchum* flowers. Tests of the behavioral effectiveness of compounds that elicit an electrophysiological response in the antennae of *A. tigrina* have not yet been conducted. However, other published studies indicate that a wide range of compounds are attractive to cetoniid beetles (Donaldson et al., 1990; Larsson et al., 2003). Many of these compounds, such as linalool, methyl salicylate, geraniol and eugenol, are present in the fragrance of *S. microrrhynchum*. The chemical basis for the attraction of pompilid wasps in *S. microrrhynchum* is yet to be established. Given that *Hemipepsis* wasps are more specific than *A. tigrina* in their flower foraging, it is likely that they respond to a more restricted set of compounds.

A larger sample size would be required to test whether the unique presence of eugenol and its derivatives in fragrance samples from the Tarn Cave population (Table 2) represents a localized further specialization for beetle pollination. Eugenol is known to attract cetoniid beetles (Donaldson et al., 1990; Larsson et al., 2003). Interestingly, *Hemipepsis* wasps were common at the Tarn Cave site (c. 50 individuals observed in 2 days in 2006), yet only one individual was found to carry *S. microrrhynchum* pollinaria. By contrast, almost all the *A. tigrina* beetles captured at this site carried pollinaria.

Manning (2005) recently introduced the term bimodal pollination systems to describe pollination of plants by two completely unrelated pollen vectors, as is apparently the case in *S. microrrhynchum*. The possibility that other insect species,

TABLE 2. Floral scent composition for six *Satyrium microrrhynchum* plants representing three populations (Sani Pass, SP; Monk's Cowl, MC; Tarn Cave, TC).

Compound	SP 1	SP 2	MC 1	MC 2	TC 1	TC 2
Fatty acid derivatives						
cis-3-Hexen-1-ol*[a]	0.01	0.22	0.07	0.02	tr[b]	—
cis-3-Hexenyl acetate*	1.26	0.10	0.40	0.08	tr	tr
4-Oxoisophorone*	0.12	0.01	0.03	0.02	tr	0.14
Aromatics						
Benzaldehyde[c]*	0.69	0.11	0.08	0.06	0.31	0.49
1,4-Dimethoxybenzene*	—	0.09	0.31	0.07	0.04	0.48
Methyl salicylate*	0.40	0.49	0.47	0.39	0.04	0.53
Eugenol*	—	—	—	—	0.55	0.14
Methyl eugenol	—	—	—	—	4.51	1.83
trans-Methylisoeugenol	—	—	—	—	0.22	0.03
Elemicin	—	—	—	—	8.53	2.01
cis-Isoelemicin	—	—	—	—	0.22	0.03
Methoxyeugenol	—	—	—	—	0.42	0.06
trans-Isoelemicin	—	—	—	—	0.43	0.02
Benzyl benzoate*	0.03	0.01	0.06	tr	—	—
Monoterpenoids						
α-Thujene	2.09	0.97	0.01	0.27	0.05	1.16
α-Pinene*	7.73	4.92	1.13	3.53	0.13	0.45
Sabinene*	1.20	4.80	0.70	0.59	0.67	0.80
β-Pinene*	2.31	2.17	0.60	1.39	tr	0.03
Myrcene*	4.05	1.66	0.47	1.75	1.37	5.06
δ-3-Carene*	0.22	0.04	0.03	tr	0.01	—
α-Terpinene*	1.81	0.78	0.07	0.10	0.03	1.83
Karahanaenone	tr	0.08	0.05	tr	0.01	—
Limonene*	0.51	1.60	1.19	0.97	1.72	1.86
Eucalyptol*	22.31	14.22	5.03	9.82	6.86	13.31
trans-β-Ocimene*	1.54	0.57	1.84	1.88	0.69	3.11
γ-Terpinene*	1.17	0.60	0.10	0.36	0.12	1.49
cis-Linalool oxide (furanoid)*	0.51	0.32	0.24	0.12	0.12	0.81
2,6-Dimethyl-1,5(*Z*),7-octatrien-3-ol	0.11	0.02	0.15	0.05	tr	0.40
trans-Linalool oxide (furanoid)*	0.30	0.12	0.23	0.05	0.06	0.53
Terpinolene*	0.40	0.17	0.23	0.05	0.06	1.06
Linalool*	37.17	54.00	60.80	72.10	58.51	44.57
2,6-Dimethyl-1,5(*E*),7-octatrien-3-ol	7.74	6.02	6.09	1.95	2.74	11.01
Dehydro linalool oxide	0.01	0.01	tr	0.01	0.35	0.09
allo-Ocimene*	0.06	0.02	0.04	0.04	—	0.12
4-Terpineol	0.61	0.19	0.14	0.08	tr	0.73
cis-Linalool oxide (pyranoide)	0.20	0.10	0.08	0.08	0.10	0.08
2,6-Dimethyl-1,7-octadien-3,5-diol	0.18	0.50	0.33	0.06	0.54	0.37
α-Terpineol	1.04	0.69	1.15	0.38	2.48	1.71
Myrtenol	0.02	0.05	0.05	0.04	—	—
cis-Geraniol	0.05	0.02	0.02	0.12	0.11	0.32
Unknown monoterpenoids	0.49[4d]	2.77[4]	1.01[4]	0.36[4]	2.34[4]	0.91[4]
Sesquiterpenoids						
α-Cubebene	0.03	0.04	0.15	0.02	0.42	0.04
Aciphyllene	0.02	0.02	0.07	0.02	—	0.05
α-Ylangene	0.13	0.02	0.18	0.10	—	0.10
α-Copaene	0.18	0.05	0.42	0.17	0.20	0.18
α-Bourbonene	0.08	tr	0.07	0.05	—	0.07
β-Bourbonene	0.54	0.41	7.48	1.13	—	0.02
β-Ylangene	0.05	0.08	0.67	0.12	0.49	0.05
trans-β-Caryophyllene*	0.08	0.03	0.20	0.07	tr	0.08
γ-Amorphene	0.08	0.10	1.03	0.18	0.45	0.07
γ-Muurolene	0.07	0.12	0.38	0.12	0.21	0.03
Germacrene D*	0.08	—	0.17	tr	0.12	0.05
α-Panasinsene	0.94	0.20	0.84	0.21	0.43	0.51
cis-Calamene	0.02	0.01	0.02	0.02	0.06	0.03
β-Calacorene	0.08	0.02	0.07	0.02	0.04	0.06
Unknown sesquiterpenoids	1.18[8]	0.42[8]	4.97[8]	0.94[8]	2.88[6]	0.98[8]
Irregular Terpenes						
Geranyl acetone	0.10	0.07	0.05	0.02	0.36	0.08
Total volatiles (ng) emitted per inflorescence per hour	618	1510	668	1606	798	1175

[a] Compounds with asterisks were identified by the comparison of mass spectra and retention data with the data of authentic standards.
[b] tr = trace, relative amount is less than 0.005.
[c] Compound could be a degradation product of Tenax TA, one of the used adsorbents (see Peters et al., 1994).
[d] Unknowns within compound classes were pooled; superscript digit indicates number of pooled compounds.

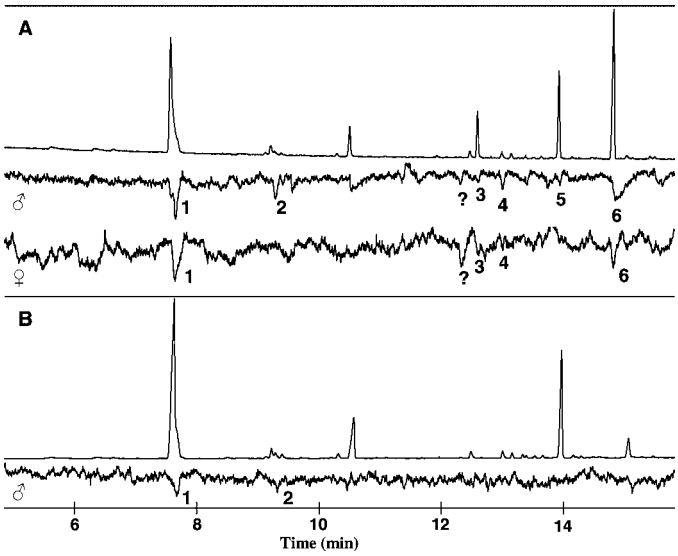

Fig. 6. Spectra from coupled gas chromatographic and electroantennographic detection (GC-EAD) of *Satyrium microrrhynchum* scent collected at (A) Tarn Cave and (B) Sani Pass in South Africa using antennae of *Atrichelaphinus tigrina* males and/or females. Peak 1, linalool/2,6-dimethyl-1,5(*E*),7-octatrien-3-ol; 2, methyl salicylate; ?, unknown compound; 3, methyl eugenol; 4, *trans*-β-caryophyllene; 5, γ-amorphene; 6, elemicin. The responses to linalool and methyl salicylate were confirmed using authentic standards.

besides the two observed, play a role in the pollination of *S. microrrhynchum* cannot be ruled out. However, the bimodality of this pollination system was consistent across many sites and years. Many other insect species, including other beetle and wasp species, were seen at the study sites, yet none of these were observed to visit *S. microrrhynchum* or to carry its pollinaria. The bimodality in the pollination system of *S. microrrhynchum* is likely due to fragrance components that are quite specifically attractive to both *Atrichelaphinus* beetles and *Hemipepsis* wasps. Nevertheless, most compounds found in the study populations are often found in floral scents (Knudsen et al., 1993). For example, linalool, the most abundant compound in the samples and eliciting the largest signal in the antennae of the beetles, is very widespread (Raguso and Pichersky, 1999). Therefore, a specific pattern of common compounds rather than a single compound may be responsible for specific attraction of the two pollinating species. Another possibility is that specific attraction is due to the occurrence of a specific pattern of different stereoisomers of a common compound. In linalool, for example, two stereoisomers are available, and both isomers are found in floral scents (Raguso and Pichersky, 1999; Dötterl et al., 2006). In a field biotest to analyze the female sex pheromone of a bee species, male bees responded differently to the different isomers of linalool (Borg-Karlson et al., 2003). Yet another possibility is that flowers of *S. microrrhynchum* emit compounds that repel most potential flower visitors other than the two primary pollinators, *A. tigrina* and *H. hilaris*. A deterrent effect of certain compounds on flower visitors has been established for other plants (e.g., Henning et al., 1992; Ômura et al., 2000).

Overlap in the floral syndromes associated with pollination by beetles and wasps appears to be a general pattern in nature (cf. Proctor et al., 1996). In a multivariate analysis of floral syndromes, Ollerton and Watts (2000) found that the classical wasp and beetle floral syndromes tend to cluster together in phenotypic space. Traits in common that caused this pattern include dull flower coloration, exposed nectar, and open perianth shape. The existence of common attractants for beetles and wasps is backed up by several empirical studies. For example, Nilsson (1981) found that the orchid *Listera ovata*, although seemingly a generalist with hundreds of insect species recorded as pollinators, is pollinated mainly by wasps and beetles. Another European orchid, *Coeloglossum viride,* which has a striking resemblance to *S. microrrhynchum,* is also pollinated mainly by wasps and beetles (C. I. Peter, Rhodes University, and S. D. Johnson). The wasp and beetle species that pollinate *S. microrrhynchum* also visit a number of other plant species in South Africa, including several asclepiads (Ollerton et al., 2003; Johnson, 2005). It would be particularly interesting to determine which traits, including scent chemistry, are shared among these largely unrelated species.

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