Diverse pollination systems of the twin-spurred orchid genus *Satyrium* in African grasslands

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Abstract The large terrestrial orchid genus *Satyrium* underwent evolutionary radiations in the Cape floral region and the grasslands of southern and eastern Africa. These radiations were accompanied by tremendous diversification of the unusual twin-spurred flowers that characterize the genus, but pollination data required to interpret these patterns of floral evolution have been lacking for grassland species in the genus. Here we document pollinators, nectar properties, and levels of pollination success for 11 grassland *Satyrium* species in southern and south-central Africa. Pollinators of these species include bees, beetles, butterflies, hawkmoths, noctuid moths, long-proboscid flies, and sunbirds. Most species appear to be specialized for pollination by one functional pollinator group. Long-proboscid fly pollination systems are reported for the first time in *Satyrium* (in *S. macrophyllum* and a high-altitude form of *S. neglectum*). Floral morphology, especially spur length and rostellum structure, differs markedly among plants with different pollinators, while nectar volume, concentration, and sugar composition are fairly uniform across species. Most taxa exhibited high levels of pollination success (>50% of flowers pollinated), a trend that can be attributed to the presence of nectar in the twin spurs.

Keywords Bird pollination, Floral syndromes, Flora of southern Africa, Functional morphology, Nectar sugar composition, Nyika Plateau, Pollinaria, Pollination success, Rostellum

Introduction

The terrestrial orchid genus *Satyrium* (Orchidaceae; Satyriinae) contains approximately 92 species and is confined almost entirely to mainland Africa, with just 5 species found in Madagascar and 4 species in Asia. Although the geographical origin of the genus is unknown, a recent molecular phylogenetic analysis revealed that there were separate large radiations of the genus in the grasslands of southern and south-central Africa (accounting for ca. 55 extant species) and in the fynbos shrublands of the south-western Cape (together accounting for ca. 32 extant species) (cf. Van der Niet et al. 2005; van der Niet and Linder 2008).

Pollinators and nectar traits of *Satyrium* species in the Cape region have been partly elucidated (Garside 1922;

Johnson 1996, 1997a), but information on the pollination biology of the more numerous grassland species has been almost entirely lacking. The only grassland *Satyrium* taxa for which pollination data have been available are *S. longicauda*, which is moth pollinated (Harder and Johnson 2005; Jersakova and Johnson 2007), *S. microrrhynchum*, which is beetle-pollinated (Johnson et al. 2007), and *S. hallackii* subsp. *ocellatum,* which is hawk-moth-pollinated (Johnson 1997b). Interestingly, *S. hallackii* subsp. *hallackii* in the Cape region is pollinated by carpenter bees (Johnson 1997b). This difference in pollinators of the Cape and grassland subspecies of *S. hallackii* is consistent with other studies that have shown strong differences in the pollinator faunas of these two regions (Goldblatt and Manning 2000; Manning and Snijman 2002).

Flowers of *Satyrium* are highly unusual among orchids on account of their twin-spurred labellum. The basic functional morphology of *Satyrium* flowers was worked out by Garside (1922), Vogel (1954; 1959), and Johnson (1997a). The flowers are nonresupinate, and the column is thus situated in front of the labellum spurs, which usually contain nectar. The column, with its unique tall basal part (Kurzweil 1996), fills the labellum galea, presenting the stigma and a shelf-like rostellum to the upper surface of animal visitors (nototrobic pollen placement). The pollinator passes to either side of the column while probing one of the floral spurs, typically removing a single pollinarium in the process. The flowers are thus subdivided into two functional pollination units, each consisting of a spur, a pollinarium, and a shared stigma, much like the flowers of *Iris* and *Moraea*, which are divided into three functional units (meranthia) that share a common stigma (cf. Steiner 2010). Rostellum structure in *Satyrium* tends to differ according to pollination system. Johnson (1996, 1997a) found that long-spurred species tend to have a (weakly) three-lobed rostellum with small laterally placed plate-like viscidia that fold partially around the proboscis of insects or large plate-like viscidia that attach to the bills of birds, while short-spurred species usually have pointed rostellum arms (either parallel or spreading) and globose terminal viscidia that attach to the eyes, head, or upper thorax of visiting insects. In addition, pollinators explain much of the overall variation in floral shape among *Satyrium* species (van der Niet et al. 2010).

The general aim of this study was to increase understanding of pollination systems in *Satyrium* species that inhabit the diverse grasslands of southern and south-central Africa. In particular, we sought to document flower visitors and their pollen loads, characterize nectar traits, and record levels of pollination success in flowers.

Materials and methods

Study species

We obtained pollination data for 11 grassland *Satyrium* species (Table 1). These species were selected because they are among the most widespread in the region. They all have similar inflorescence structure but differ widely in floral morphology, color, and scent (Fig. 1). The only one of these species for which some pollination data were previously available is *S. longicauda* (Harder and Johnson 2005; Jersakova and Johnson 2007), however, this species was included here as previous studies had focused on other aspects of its biology without thorough documentation of flower visitors and floral traits. Voucher specimens from plant populations studied were deposited in the Bews Herbarium (NU), Pietermaritzburg, and the Zurich Herbarium (Z), Zurich.

Study sites

Observations of the 11 study species were conducted at 16 localities in South Africa and Malawi between 1996 and 2009. Coordinates for these sites, together with dates and times of pollinator observations for each species, are given in the Electronic Suppementary Material.

Pollinator observations

Insect floral visitors were captured with a handnet and examined for the presence of pollinaria. Identification of pollinaria was usually straightforward as it was unusual for more than one *Satyrium* species to be in flower at a particular site, and it was often possible to make direct observations of removal of pollinaria from flowers. Voucher insects were set on pins, and their proboscides were measured to the nearest 0.5 mm with a steel ruler or digital calipers. Insect specimens were sent to taxonomic authorities for identification and voucher specimens were deposited in the National Collection of Insects in Pretoria, Albany Museum, Grahamstown, and the Natal Musem, Pietermaritzburg. Birds visiting *S. sceptrum* on the Nyika Plateau were observed using binoculars.

Nectar measurements

The length of floral spurs of each species was measured with a steel ruler to the nearest 0.5 mm from the tip of the spur to the viscidia on the rostellum. Volume and concentration of the standing crop of nectar in individual flowers of the study species were measured. Nectar volumes were measured with calibrated 0–5 µl micropipettes between 0800 and 1000 h

Table 1 Identity, abundance, pollen loads, and mouthpart dimensions of visitors to the flowers of grassland *Satyrium* species

Satyrium species	Study site	Flower visitor species (family)	Number observed (captured)	Pollinaria per captured individual, $\bar{x}$ (range)	Mouthpart length (mm) $\bar{x}$ ± SD
S. crassicaule	Nyika	*Macroglossum trochilus* (Sphingidae)	24(3)	ca. 3	20.0 ± 0.0
		Unidentified moth (Noctuidae)	1(1)	2	21.0 ± 0.0
S. cristatum var. *cristatum*	Ramas Gate	*Amegilla natalensis* (Anthophoridae)	4(3♂, 1♀)	0.5 (0–1)	10.0 ± 1.07
		Amegilla spilostoma (Anthophoridae)	2(2♂)	0.5 (0–1)	8.2 ± 0.0
	Tarn Cave	*Amegilla* sp. (Anthophoridae)	12(1)	3	Damaged
	Naudes Nek	*Amegilla natalensis* (Anthophoridae)	>20(2♂)	>2	–
S. cristatum var. *longilabiatum*	Entabeni	*Amegilla spilostoma* (Anthophoridae)	15(2♂, 2♀)	4 (0–10)	8.4 ± 0.34
	Kamberg	*Amegilla spilostoma* (Anthophoridae)	>20(6♂, 2♀)	Not recorded	–
	Highmoor	*Apis mellifera* (Anthophoridae)	(1) 1	5	3.4 ± 0.0
S. longicauda	Entabeni	*Basiothia schenkii* (Sphingidae)	3(3)	4.3 (0–13)	36.58 ± 0.0
		Empusada hutchisoni (Noctuidae)	20(20)	1.1 (0–3)	31.9 ± 4.2
		Hippotion celerio (Sphingidae)	1(1)	7	39.33 ± 0.0
	Wahroonga	*Basiothia schenkii* (Sphingidae)	>30(4)	3.3 (0–5)	39.5 ± 1.0
	Granny Mouse	*Basiothia schenkii* (Sphingidae)	5(3)	6.5 (0–11)	40.3 ± 2.9
		Empusada hutchisoni (Noctuidae)	5(5)	0	33.6 ± 2.3
		Hippotion celerio (Sphingidae)	5(5)	3.2 (2–10)	36.8 ± 1.8
		Unidentified moth (Noctuidae)	2(2)	0	11.0 ± 0.0
	Mt Gilboa	*Basiothia schenkii* (Sphingidae)	10(3)	6.3 (2–10)	42 ± 1.7
		Empusada hutchisoni (Noctuidae)	1(1)	0	32
	Garden Castle	*Basiothia schenkii* (Sphingidae)	1(1)	0	40
		Hippotion celerio (Sphingidae)	2(2)	1 (0–3)	34.5 ± 3.5
		Nephele comma (Sphingidae)	1(1)	1	40
		Syngrapha circumflexa (Noctuidae)	5(5)	Not recorded	18.6 ± 0.5
		Unidentified noctuid 1	2(2)	0	17.5 ± 0.7
		Unidentified noctuid 2	1(1)	0	13
S. macrophyllum	Umgeni	*Philoliche aethiopica* (Tabanidae)	6(6)	0.3 (0–1)	15.9 ± 0.3
	Wahroonga	*Prosoeca* sp. (Nemestrinidae)	5(3)	3.3 (2–5)	24.7 ± 3.1
		Stenobasipteron sp. (Nemestrinidae)	1(1)	3	21.7 ± 0.0
S. monadenum	Nyika	Unidentified sunbird (Nectariniidae)	1	–	–
S. neglectum	Mt Aux Sources	*Prosoeca ganglbaueri* (Nemestrinidae)	>20(5)	1.5 (1–2)	19.8 ± 2.4
	Sani Pass	*Acraea horta* (Nymphalidae)	16(16)	3.8 (3–5)	9.30 ± 1.06
		Colias electo (Pieridae)	11(11)	3.7 (1–11)	9.97 ± 1.14
S. parviflorum	Witsieshoek	Unidentified moths (Noctuidae)	c. 10 (0)	ca. 5	–
	Grahamstown	*Agrotis cincithorax* (Noctuidae)	1(1)	2	6.5 ± 0.0
		Tycomarptes inferior (Noctuidae)	1(1)	8	9.4 ± 0.0
		Agrotis ipsilon (Noctuidae)	1(1)	8	8.0 ± 0.0
		Vietteania torrentium (Noctuidae)	34(34)	4.3 (0–18)	6.6 ± 0.9
		Helicoverpa armigera (Noctuidae)	19(19)	3.8 (0–12)	9.2 ± 1.1
		Vietteania sp. (Noctuidae)	1(1)	4	6.0 ± 0.0
		Craterestra definiens (Noctuidae)	6(6)	4.2 (0–13)	9.1 ± 1.4
		Hadena bulgeri (Noctuidae)	5(5)	7.6 (0–16)	9.0 ± 0.4
		Agrotis longidentifera (Noctuidae)	6(6)	5.2 (1–2)	7.8 ± 0.9

Table 1 continued

Satyrium species	Study site	Flower visitor species (family)	Number observed (captured)	Pollinaria per captured individual, $\bar{x}$ (range)	Mouthpart length (mm) $\bar{x} \pm$ SD
S. princeae	Nyika	Unidentified butterflies (4 skippers, 3 pierids, and 1 other)	8 (2)	ca. 4	–
S. sceptrum	Nyika	Unidentified sunbird (Nectariniidae)	2 (1)	>5	19.0 ± 0.0
		Malachite sunbird (Nectariniidae)	1 (1)	>5	
S. sphaerocarpum	Quachas Nek	*Amegilla spilostoma* (Anthophoridae)	10 (2♂)	1.5 (0–3)	4.1 ± 0.0
	Naudes Nek	Unidentified bee (Anthophoridae)	>20 (1)	1	–
	Wild Coast	Unidentified bee (Anthophoridae)	1 (1)	–	–
S. trinerve	Entabeni	*Atrichelaphinus tigrina* (Cetonidae)	9 (7)	6.6 (0–16)	1.4 ± 0.1
		Spilostethus sp. (Lygaeidae)	3 (3)	1.6 (1–3)	4.3 ± 1.0
		Lycus cf. *melanurus* (Lycidae)	1 (1)	9	2.9

The sex of captured insects is provided only for some bees where this information was obtainable

in the case of species pollinated by day-flying animals and at dusk in the case of moth-pollinated species. The sugar concentration in these samples was determined using a hand-held refractometer for a 0–50% range (Bellingham and Stanley model 45–81, Tunbridge Wells, UK). Samples of nectar were also spotted on Whatman no. 1 filter paper for later analysis of sugar composition using HPLC, as described by van Wyk et al. (1993).

Pollination success

For each species, we estimated male and female components of pollination success by recording the frequency of removal of pollinaria from flowers and the frequency of stigmas that had received pollen massulae, respectively.

Results

Pollinator observations

We observed over 350 animal visitors on flowers of the 10 *Satyrium* species and captured 168 individuals, 126 of which carried pollinaria (Table 1). Brief descriptions of the pollinator observations made for the study species (in alphabetical order) are given below.

S. crassicaule has pink flowers with medium sized (ca. 16 mm) spurs and viscidia laterally positioned on the rostellum. Plants observed in dembos (seasonally wet marshes) in Malawi were visited almost exclusively by the day-flying hawkmoth *Macroglossum trochilus* (Table 1). Most of these moths carried large loads of pollinaria on their proboscides, although pollinaria were subsequently dislodged from the bodies of all but one of the captured individuals and thus could not be counted precisely.

S. cristatum has white flowers with conspicuous red markings and short spurs (ca. 10 mm). Plants observed in montane grasslands of South Africa were visited exclusively by anthophorid bees, especially various *Amegilla* species (Table 1, Fig. 1d). Flowers of *S. cristatum* var. *cristatum* have terminal, plate-like viscidia on bifid rostellum arms. These viscidia become attached to the upper proboscis of bee visitors as they probe the spurs.

Flowers of *S. cristatum* var. *longilabiatum* have bifid rostellum arms with terminal globose viscidia. These are placed on the frons of bee visitors as they probe the spurs, which are slightly longer than those of subsp. *cristatum*.

S. longicauda has sweetly scented white flowers with relatively long spurs (ca. 40 mm) and a three-lobed rostellum with lateral viscidia. Plants in grassland habitats in South Africa were visited mainly by hawkmoths (particularly *Basiothia schenkii*) at the Mt. Gilboa, Wahroonga, and Granny Mouse sites (Table 1, Fig. 1g). Activity of these insects took place almost exclusively in a short period of ca. 15 min after dusk. Hawkmoths also pollinated plants at Entabeni, but the most abundant visitor at this site was the noctuid moth *Empusada hutchisoni* which was active for several hours after dusk. Pollinaria of *S. longicauda* are attached to the proboscis of visiting moths.

S. macrophyllum has pink flowers with relatively long (ca. 33 mm) downward curved spurs and a bifid rostellum with terminal, plate-like viscidia. Plants observed in tall unburnt grasslands in South Africa were visited exclusively by long-proboscid flies belonging to the families Nemestrinidae (tanglewing flies) and Tabanidae (horseflies). Pollinaria are placed on the proboscis (Table 1, Fig. 1i).

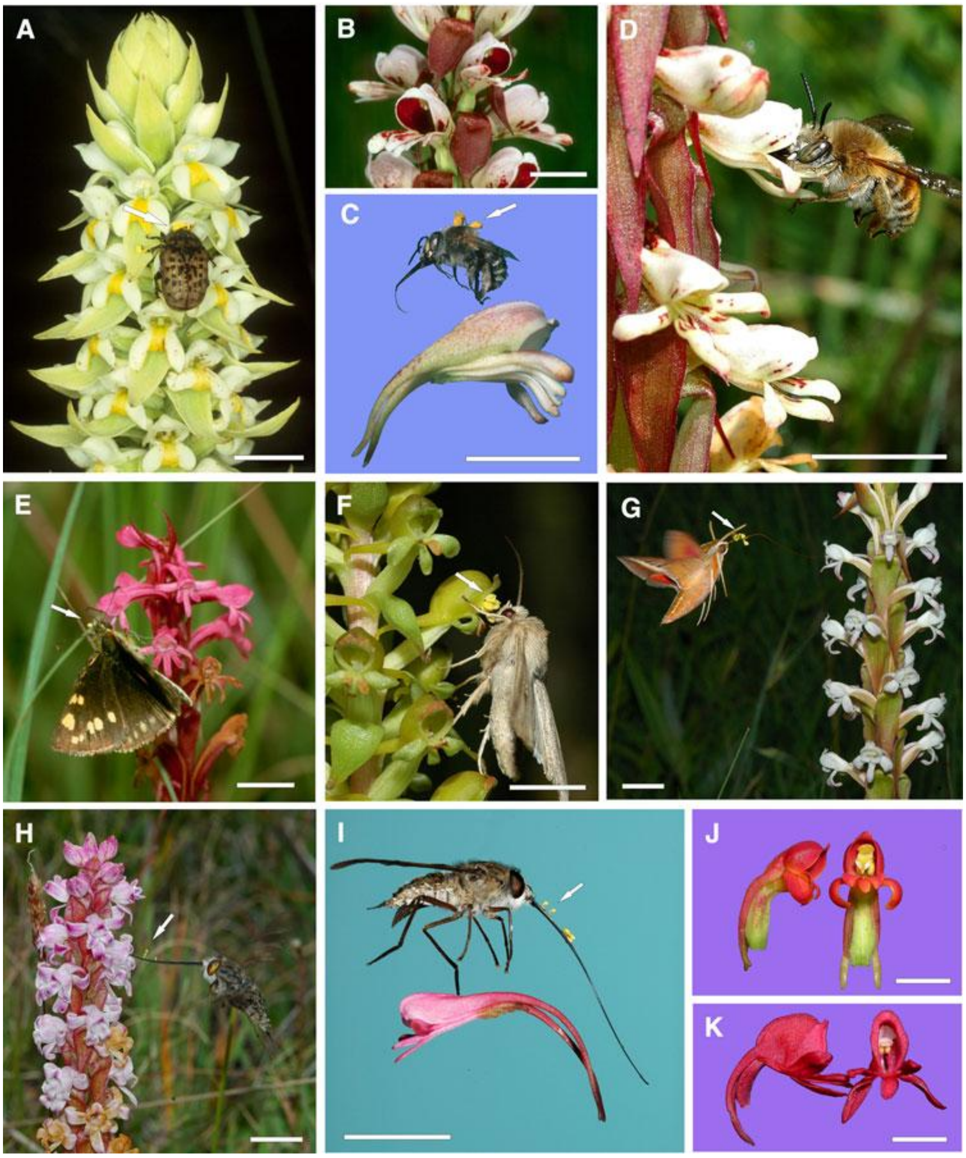

Fig. 1 Flowers and pollinators of grassland *Satyrium* species. Arrows indicate the placement of pollinaria on insect visitors. a *Satyrium* trinerve visited by the beetle *Atrichelaphinus tigrina* (Cetonidae). b Flowers of *S. sphaerocarpum*. c Flower of *S. sphaerocarpum* posed next to the bee pollinator *Amegilla spilostoma*. d *S. cristatum* var. *cristatum* visited by the bee *Amegilla natalensis*. e *S. princeae* visited by an unidentified skipper butterfly. f *S. parviflorum* visited by an unidentified noctuid moth. g *S. longicauda* visited by the hawkmoth *Basiothia schenkii*. h The high altitude form of *S. neglectum* visited by the long proboscid fly *Prosoeca ganglbaueri*. i *S. macrophyllum* posed next to its pollinator, the long proboscid fly *P. ganglbaueri*. j Flowers of *S. sceptrum*. k. Flowers of *S. monadenum*. Scale bars 10 mm. Photo credits a Lourens Grobler, b–k Steve Johnson

S. monadenum has carmine red flowers with long (ca. 38 mm) spurs and a single large, terminal, globose viscidium. Plants observed in grasslands in Malawi by Dave Foot (Nyika Safari, personal communication, February 2003) were visited by unidentified sunbirds.

S. neglectum is a highly variable taxon that consists of at least two phylogenetically distinct forms (van der Niet and Linder 2008). Our observations suggest that these forms also differ in their pollination systems. The most common form has small pink flowers on dense, tall inflorescences with relatively short (16 mm) straight spurs, a three-lobed rostellum with a broad apex, and lateral viscidia. Plants of this form in the foothills of the Drakensberg mountains were observed to be pollinated by butterflies (Table 1). The "high altitude form" has pale white flowers tinged with pink markings, relatively short (ca. 13 mm) curved spurs, and a three-lobed rostellum with a narrow, finger-like apex, and lateral, plate-like viscidia. Plants of this form on the summit of the Drakensberg mountains were observed to be pollinated by long-proboscid flies (Table 1, Fig. 1h).

S. parviflorum has small green flowers with a narrow galea entrance, short spurs (ca. 12 mm) and minute laterally placed visicidia. We captured a large number of small noctuid moths on flowers of this species during the evening (Table 1, Fig. 1f). Most carried pollinaria that were placed on the basal part of the proboscis.

S. princeae has pink flowers with medium-sized spurs, and a bifid rostellum with terminal plate-like viscidia. Plants observed in wet montane grassland in Malawi were visited by hesperid and pierid butterflies that carried pollinaria on their proboscis (Table 1, Fig. 1e).

Table 2 Morphology, nectar traits, and pollination success in flowers of grassland *Satyrium* species

Species	Site	Spur length (mm), x ± SD (n)	Viscidium placement and shape	Nectar sugars (Fru:Glu:Suc)	Nectar volume (mm), x ± SD (n)	Nectar sugar concentration (g/100 g), x ± SD (n)	Flowers pollinated (%) (n)	Flowers with pollinaria removed (%)
S. crassicaule	Nyika	16.4 ± 1.4 (12)	Lateral, platelike	16:15:69	0.14 ± 0.13 (10)	23.3 ± 0.6 (3)	–	–
S. cristatum var. *cristatum*	Tarn cave	10.6 ± 1.3 (10) 11.6 ± 1.1	Terminal, platelike	17:21:62	0.28 ± 0.20 (9)	27.5 ± 7.8 (8)	60 (20)	63 (11)
	Ramas gate	12.2 ± 0.8 (10)	Terminal, platelike	–	1.0 ± 0.0 (7)	32.1 ± 7.2 (10)	50.0 (8)	64 (8)
S. cristatum var. *longilabiatum*	Entabeni	18.01 ± 1.52 (9)	Terminal, globose	–	0.12 ± 0.07 (11)	35.1 ± 6.2 (4)	86 (50)	74 (50)
S. longicauda	Wahroonga	46.8 ± 2.2 (6)	Lateral, platelike	10:10:80	1.41 ± 0.92 (16)	31.2 ± 2.0 (10)	–	–
	Entabeni	38.2 ± 2.57 (19)	Lateral, platelike		0.66 ± 0.52 (16)	24.4 ± 7.92 (16)	39.5 (43)	32.5 (43)
S. macrophyllum	Wahroonga	33.0 ± 2.9 (25)	Terminal, platelike	0:0:100	0.65 ± 0.43 (10)	27.9 ± 3.1 (10)	88 (25)	88 (25)
S. monadenum	Nyika	38.3 ± 1.6 (11)	Terminal, globose	–	1.93 ± 1.55 (11)	16.4 ± 1.1 (11)	36.3 (11)	72.7 (11)
S. neglectum	Sani pass	16.4 ± 3.3, (31)	Lateral, platelike	19:18:63	0.2 ± 0.1 (28)	31.3 ± 8.9 (16)	89 (18)	67 (18)
	Mt Aux sources	11.8 ± 0.8 (8)	Lateral, platelike	–	–	–	–	–
S. parviflorum	Grahamstown	5.6 ± 0.6 (29)	Lateral, platelike	15:19:66	0.72 ± 0.1 (23)	15.7 ± 0.7 (23)	53.6 (56)	58.9 (56)
S. sceptrum	Nyika	37.9 ± 9.4 (19)	Lateral, platelike	6:6:88	0.76 ± 0.66 (19)	18.7 ± 3.2 (16)	23.8 (19)	94.4 (18)
S. sphaerocarpum	Quachas Nek	13.3 ± 7.2 (7)	Terminal, globose	–	–	–	100.0 (8)	100 (8)
	Naudes nek	25.8 ± 1.8 (16)	Terminal, globose	0:0:100	0.50 ± 0.33 (15)	24.6 ± 3.5 (10)	76 (29)	79 (29)
S. trinerve	Entabeni	5.96 ± 0.53 (6)	Terminal, globose	–	0.36 ± 0.31 (12)	l12.4 ± 33 (10)	53.3 (30)	73.3 (31)

Fru fructose, Glu glucose, Suc sucrose

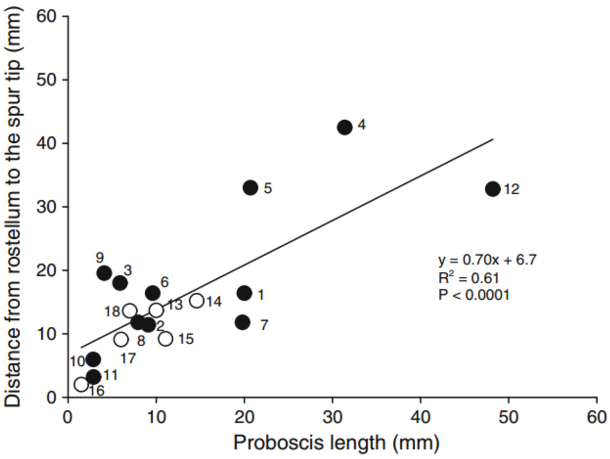

Fig. 2 The relationship between pollinator proboscis length and floral spur length in insect-pollinated *Satyrium* species from the African grasslands (closed symbols) and Cape shrublands (open symbols). Key to taxa (and pollinators): 1 *S. crassicaule* (butterflies), 2 *S. cristatum* var. *cristatum* (bees), 3 *S. cristatum* var. *longilabiatum* (bees), 4 *S. longicauda* (hawkmoths), 5 *S. macrophyllum* (beetles), 6 *S. neglectum* (butterflies), 7 *S. neglectum* high altitude form (long-proboscid flies), 8 *S. parviflorum* (noctuid moths), 9 *S. sphaerocarpum* (bees), 10 *S. trinerve* (beetles), 11 *S. microrrynchum* (beetles), 12 *S. hallackii* subsp. *ocellatum* (hawkmoths), 13 *S. stenopetalum* (noctuid moths), 14 *S. bicorne* (moths), 15 *S. ligulatum* (butterflies), 16 *S. bracteatum* (carrion flies), 17 *S. erectum* (bees), 18 *S. hallackii* subsp. *hallackii* (bees). Data sources 1–10 (this study); 11 (Johnson and Nicolson 2008); 12, 18 (Johnson 1997b); 13–17 (Johnson 1997a)

S. sceptrum has bright yellow-orange flowers with long (ca. 38 mm) spurs, a three-lobed rostellum with large, plate-like viscidia. Plants in tall vegetation on the Nyika Plateau, Malawi, were visited exclusively by sunbirds (Table 1). We observed three foraging bouts by sunbirds (tentatively identified at a distance as greater-double collared sunbirds), and in two of these instances large clumps of pollinaria were clearly visible on the upper mandible of the beaks of the birds. Each foraging bout consisted of visits to four to seven plants, each of which had three to six flowers probed by birds.

S. sphaerocarpum has large white flowers with prominent red markings and medium-length spurs (ca. 13 mm). Plants in the southern Drakensberg mountains were visited by large anthophorid bees, which carried pollinaria on the upper surface of the thorax (Table 1, Fig. 1b, c). The bees crawl into the wide-mouthed flowers, and contact occurs between the prominent globose viscidia that are in a terminal position of the overarching rostellum and the dorsal surface of their thorax when they are deep within the labellum chamber. We also observed a few visits by the long-proboscid fly *Prosoeca ganglbaueri* (Nemestrinidae), but these insects did not remove pollinaria from the flowers.

S. trinerve has white flowers with bright yellow lateral petals, and long, spreading whitish bracts and very short saccate spurs. It has bifid rostellum arms with terminal, globose viscidia. Plants were visited mainly by the large cetoniid beetle *Atrichelaphinus tigrina* (Table 1, Fig. 1a). These beetles carry pollinaria of *S. trinerve* on the upper suface of their heads (Fig. 1a).

Nectar measurements

Nectar of those species examined was dominated by sucrose (60%), with small proportions of fructose and glucose making up the balance (Table 2). The volume of the standing crop of nectar in flowers was generally very small (<2 μl) in the study species (Table 2). The mean sugar concentration of nectar ranged from 18 to 35% in the study species and was highest in the bee-pollinated species *S. cristatum* and lowest in the bird-pollinated species *S. sceptrum* (Table 2).

Pollination success

The percentage of flowers that had received pollen massulae ranged from 23 to 100% among the study species (median = 60%), while pollinaria removal occurred in 33 to 100% of flowers (median = 73%; Table 2).

Discussion

The observations reported in this study indicate that grassland *Satyrium* species possess a diverse range of specialized pollination systems. Eight distinct pollination systems (involving bees, long-proboscid flies, beetles, day-flying hawkmoths, noctuid moths, nocturnal hawkmoths, butterflies and sunbirds, respectively) were recorded among the 11 study species (Table 1, Fig. 1). These data allow the complex floral structures of these species to be interpreted for the first time.

There is a strong positive relationship between the average pollinator proboscis length and average floral spur length among insect-pollinated *Satyrium* species (Fig. 2). This is consistent with a recent study showing that spur length is a major contributor to overall variation among *Satyrium* flowers pollinated by different vectors (van der Niet et al. 2010). The longest floral spurs in the genus are found among grassland taxa, reflecting adaptations for pollination by hawkmoths, an insect group that is scarce or absent in Cape shrublands (Johnson 1997b). The shortest spurs are found in species pollinated by flies and beetles (Fig. 2).

Satyrium species pollinated by bees have conspicuous floral markings that may function as "nectar guides" and spurs of intermediate length (ca. 10–15 mm). Pollen placement is either via platelike viscidia attached to the proboscis or globose viscidia attached to the frons or upper part of the thorax of bees. The anthophorid bee genus *Amegilla* appears to be particularly important for the pollination of grassland orchids (Johnson 1994) and irises (Goldblatt et al. 1998).

Pollination by long-proboscid flies in *Satyrium* is reported here for the first time (Table 1). These findings are corroborated by earlier unpublished observations of John Manning (South African Biodiversity Institute, personal communication) who recorded pollination of *S. macrophyllum* by *Prosoeca umbrosa* (Nemestrinidae) in the Karkloof mountains on 28 March 1982 and pollination of the high altitude form of *S. neglectum* by *Prosoeca variegata* (Nemestrinidae) on the Sentinel peak on 3 February 1982. Vouchers of these flies are deposited in the Natal Museum, Pietermaritzburg. *S. macrophyllum* and the high altitude form of *S. neglectum* conform to the general long-proboscid fly floral syndrome of long-tubed unscented pink or cream flowers (Goldblatt and Manning 2000). Other southern African orchid genera in which this pollination system has been recorded include *Disa* (Johnson and Steiner 1995, 1997; Johnson 2000, 2006) and *Brownleea* (Johnson and Steiner 1995; Larsen et al. 2008).

The beetle-pollination system reported here for *S. trinerve* involves the same cetoniine species, *Atrichelaphinus tigrina*, that has been reported as a pollinator of *S. microrrhynchum* (Johnson et al. 2007) as well as some South African *Eulophia* orchids (Peter and Johnson 2009) and milkweeds (Ollerton et al. 2003; Shuttleworth and Johnson 2009). This is thus a generalist insect that appears to be attracted by fruity odors and that feeds on dilute exposed nectar (Johnson et al. 2007).

Day-flying hawkmoths are highly opportunistic foragers, but there is evidence that some plants are pollinated specifically by these insects (Herrera 1990). Our case for a specialized system of pollination by dayflying hawkmoths in *S. crassicaule* is strengthened by observations carried out at several sites over two nonconsecutive years and by the anecdotal observations of la Croix (1991), who also recorded day-flying hawkmoths on this species.

Pollination by moths is associated with a very clear syndrome of floral traits in *Satyrium*, including pale white or green flowers, platelike viscidia in lateral rostellum notches, long spurs, and production of evening scent. Pollination by noctuids has now been recorded in several *Satyrium* species with medium length (10–20 mm) spurs (Johnson 1997a, this study) and by hawkmoths in two taxa with long (<30 mm) spurs: *S. hallackii* subsp. *hallackii* (Johnson 1997b) and *S. longicauda* (Harder and Johnson 2005; Jersakova and Johnson 2007; Ellis and Johnson 2010; this study). Interestingly, some populations of *S. longicauda* are pollinated primarily by the noctuid moth *Empusada hutchison*, which has an unusually long proboscis (ca. 33 mm) giving it access to the nectar in flowers otherwise adapted for hawkmoths.

Pollination by birds in *Satyrium* was first documented in three Cape species by Johnson (1996). Our new observations of grassland species are more tentative, involving only three sightings of birds visiting flowers and carrying pollinaria of *S. sceptrum* in Malawi and a personal communication of birds visiting flowers of *S. monadenum* from a bird-watcher in Malawi. However, these two grassland species have floral traits that are strikingly convergent with those of bird-pollinated *Satyrium* species in the Cape flora. In particular, *S. sceptrum* and *S. monadenum* have very large viscidia and bright red or carmine pink unscented flowers (Fig. 1j, k) much like those of Cape bird-pollinated *Satyrium* species. On the basis of these features, we also predict that several other grassland *Satyrium* taxa, including *S. rhodanthum* and *S. neglectum* ssp. *woodii*, are bird-pollinated. Spur length in bird-pollinated *Satyrium* species does not reflect the bill length of the birds, as the spur accommodates the extended tongue of birds rather than their bills (Johnson 1996).

Data from this study and earlier ones dealing with Cape *Satyrium* species (Johnson 1996, 1997a) indicate that nectar in *Satyrium* tends to be dominated by sucrose. This is consistent with evidence for strong phylogenetic conservatism in nectar sugar composition in other genera (van Wyk et al. 1993). It was previously thought that flowers pollinated by sunbirds would have low sucrose content because of the dogma that all passerine birds preferred hexose sugars. It was, however, recently shown that nectar properties of flowers pollinated by sunbirds are similar to those pollinated by hummingbirds (Johnson and Nicolson 2008). Thus, sucrose-dominated nectar in *Satyrium* would not have been an impediment to the evolution of pollination by sunbirds.

Overall, our results suggest that grassland *Satyrium* species have pollination systems that are at least as specialized (in the sense of pollination by a particular functional group) and diverse as those previously reported for members of the genus in the Cape fynbos (Johnson 1996, 1997a). This implies that similar processes of pollinator-driven evolution have shaped floral diversity in the two regions and is consistent with a recent study showing that the frequency of evolutionary shifts in ecological and morphological traits did not differ significantly between plant species pairs in the Cape and those in the rest of southern Africa (Van der Niet and Johnson 2009). Together, these studies are suggestive of the importance of diversification in pollination systems for the radiation of southern African plants as a whole and not just those in the Cape region.

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