Floral biology of *Romulea* (Iridaceae: Crocoideae): a progression from a generalist to a specialist pollination system

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ABSTRACT
Field observations, floral dissections, and pollen load analyses of insects captured on 32 species of *Romulea*, including all the main flower types in the genus, show that flowers of this African and Eurasian genus of c. 90 species centered in the winter-rainfall zone of southern Africa are cross pollinated by a relatively narrow range of insects. Observations indicate that there are four modes of floral presentation in the southern African members of the genus. The *Romulea flava* group is typically pollinated largely by female bees representing four families of native Apoidea. In contrast, the *Romulea monadelpha* group is pollinated exclusively by hopliine beetles (Scarabaeidae). The *Romulea eximia* group combines morphological and pigmentation characters found in the other two groups and is pollinated by bees or hopliine beetles alone or in combination. Species in the *Romulea hantamensis* group have elongated floral tubes and are pollinated by long-proboscid flies (Nemestrinidae). Pollination systems within the genus are comparatively fewer than in other irid genera of similar size (e.g., *Ixia, Lapeirousia*). The relatively low level of adaptive radiation in *Romulea* appears to be a consequence of both a conservative floral phenology and floral architecture. Outgroup comparison strongly suggests that long-tongued fly pollination and exclusive beetle pollination represent relatively recent syndromes derived from pollination primarily by bees or a more generalist condition of combined bees and hopliines.

KEY WORDS
Pollination biology,
Iridaceae,
Romulea,
Hopliini,
Apidae,
Andrenidae,
Halictidae,
Nemestrinidae

RÉSUMÉ

Biologie florale des Romulea (Iridaceae : Crocoideae) : vers un système de pollinisation spécialisé.

Des observations de terrain, des dissections florales et des analyses des pelotes polliniques d'insectes capturés sur 32 espèces de *Romulea* comprenant tous les principaux types floraux du genre, montrent que les fleurs de ce genre africain et eurasien, qui renferme env. 90 espèces centrées dans la zone à pluie hivernale du sud de l'Afrique, sont à pollinisation croisée, par une gamme relativement limitée d'insectes. Ces observations indiquent qu'il existe quatre types de présentation florale parmi les membres sud-africains du genre. Le groupe de *Romulea flava* est essentiellement pollinisé par des abeilles femelles appartenant à quatre familles d'Apoidea indigènes. Par contre, le groupe de *Romulea monadelpha* est pollinisé exclusivement par des Coléoptères Hopliines (Scarabaeidae). Les espèces du groupe de *Romulea eximia* présentent des caractères morphologiques et de pigmentation rencontrés dans les deux autres groupes et sont pollinisées par des abeilles ou des Coléoptères Hopliines, seuls ou en association. Les représentants du groupe de *Romulea hantamensis* possèdent des longs tubes floraux et sont pollinisés par des mouches à longue trompe (Nemestrinidae). Les modes de pollinisation des *Romulea* sont, comparativement, moins abondants que chez d'autres genres d'Iridaceae de même importance (e.g. *Ixia*, *Lapeirousia*). Le niveau de radiation adaptative relativement faible chez *Romulea* est une conséquence de la conservation de caractères de phénologie et de l'architecture florale. Une comparaison avec des extra-groupes semble indiquer clairement que la pollinisation par les mouches à longue trompe et celle réalisée exclusivement par les Coléoptères représentent un syndrome relativement récent dérivé d'une pollinisation principalement par les abeilles ou, de manière moins spécialisée, par l'association abeilles-Hopliines.

MOTS CLÉS
Biologie pollinique,
Iridaceae,
Romulea,
Hopliini,
Apidae,
Andrenidae,
Halictidae,
Nemestrinidae

INTRODUCTION

The radiation and diversification of the African Iridaceae has depended to a great extent on the plasticity of pollination mechanisms. Most of the larger genera exhibit a wide range of floral adaptations and correlated sets of insect or avian pollinators (BERNHARDT & GOLDBLATT 2000). For example, *Lapeirousia* consists of 40 species pollinated by long-proboscid flies, or bees and butterflies, or night-flying moths (GOLDBLATT et al. 1995). The majority of the 165 species of southern African *Gladiolus* appear to be pollinated primarily by nectar-feeding anthophorine bees (GOLDBLATT et al. 1998b) but some red-flowered species are pollinated by the large butterfly, *Aeropetes* (JOHNSON & BOND 1994), while others are dependent on andrenid bees, a combination of these bees and hopliine beetles (GOLDBLATT et al. 1998a), or long-proboscid flies, moths, or birds (GOLDBLATT & MANNING 1999, 2000; GOLDBLATT et al. 2001). Consequently, adaptive radiation of pollination-related floral characters appears to have played a prominent role in the evolution and speciation of the sub-Saharan African Iridaceae. Unlike these genera, the African and Mediterranean genus *Romulea* appears to be highly conservative in its mode of floral presentation and the approximately 90 species (MANNING & GOLDBLATT 2001) differ largely in such vegetative features as the corm morphology, leaf anatomy, and the size, shape, and texture of the floral bracts. Flower shape is remarkably similar across the genus, with some notable exceptions, but perianth color and patterning is diverse. Field studies of the pollination

systems of a range of *Romulea* species were undertaken to define and compare intrageneric trends in the evolution of pollination mechanisms and the function(s) of floral traits.

METHODS

Inflorescence phenology and floral life span

Direct observations are presented on 48 populations of *Romulea* representing 32 species made in the field from 1993 to 2001 (Table 1) and supplemented by living collections at Kirstenbosch Botanic Gardens, Cape Town. The range of species studied includes representatives of all the main flower types in the genus. Study sites were selected in the southern African winter-rainfall zone, including the southwestern Cape and western Karoo, where the genus is most diverse, and in Namaqualand to the north. The area has a Mediterranean-type climate with wet winters and dry summers. Other species of *Romulea* occur in Lesotho, the eastern Cape and Drakensberg escarpment of South Africa, the tropical African highlands, Somalia, Arabia, and Socotra, and in North Africa, southern Europe, the Canary Islands, and Near East (DE VOS 1972; MANNING & GOLDBLATT 2001). Observation of insect foraging covered a period of 4-10 hours per plant species and included recording of floral attractants (pigmentation patterns, scent), the mode and timing of anthesis (opening of individual buds), daily phenology, anther dehiscence, expansion of stigmatic lobes, the behavior of insects on the flower, and the taxonomic diversity of floral foragers. Floral scent was noted in the field and in cultivated plants. Scents too weak to be discerned by the human nose were recorded after individual flowers were picked and placed in clean, lidded glass jars and stored in a warm place. The contents of each jar were smelled after a minimum of 60 minutes (BUCHMANN 1983).

Nectar analysis

Nectar volume measurements were taken primarily from unbagged flowers in the field, reflecting both rates of secretion and depletion. Nectar sugar chemistry and concentration are unlikely to be affected significantly, if at all, using this method as opposed to sampling bagged flowers. Studies on nectar characteristics of *Lapeirousia* (another southern African genus of Iridaceae-Crocoideae) indicate that nectar concentration is not affected using unbagged flowers versus those examined in the laboratory where insects were excluded (GOLDBLATT et al. 1995). Nectar volume may be lower in unbagged versus bagged flowers but sampling of nectar of unbagged flowers in populations being visited by pollinators reflects a realistic situation that confronts a particular pollinator at a given time. To collect nectar, whole flowers were picked and nectar was withdrawn from the base of the perianth tube with 3 µl capillary tubes after separating the ovary from the perianth base. The percentage of sucrose equivalents in fresh nectar was measured in the field using a Bellingham and Stanley hand-held refractometer (0-50%) from five or more individuals per population, unless fewer individuals were available. Additional nectar samples were dried on Whatman no. 1 filter paper and sent to B.-E. VAN WYK, Rand Afrikaans University, Johannesburg, for HPLC nectar sugar chemistry analysis.

Insect observation and pollen load analyses

Observations were made on whether insects visiting flowers of *Romulea* contacted anthers and stigmas while foraging. Only insects observed probing the floral tube or brushing the anthers or stigmas were captured and killed in a jar using ethyl acetate vapor. Pollen was removed from insects after specimens were pinned. To prevent contamination of the body of an insect with pollen carried by another in the same jar, each insect was wrapped in tissue as soon as it was immobilized. Body and proboscis length were recorded from captured specimens. At some sites bee and hopliine floral visitors were especially common and we captured and killed only representative samples (n = 5-10) of these insects for identification and pollen load analysis. Removal of pollen from insects involved gently scraping

TABLE 1. — Study sites and voucher information for ***Romulea*** species studied. Vouchers are deposited at MO (GOLDBLATT & MANNING) or at NBG (other collectors). All study sites are in South Africa.

Species	Study site	Voucher
R. alba J.C. Manning & Goldblatt	Northern Cape, near Middelpos	*Goldblatt & Manning 10367*
R. amoena Schltr. ex Bég., site 1	Northern Cape, Lokenburg	*Goldblatt & Manning 10941*
site 2	Northern Cape, near Lokenburg	*Goldblatt 11396A*
R. aquatica G.J. Lewis	Western Cape, near Hopefield	*Goldblatt & Manning 10694*
R. atrandra G.J. Lewis, site 1	Northern Cape, Roggeveld near Blomfontein farm	*Goldblatt & Manning 10944*
site 2	Western Cape, Swartberg Pass	*Goldlbatt & Porter 11844*
R. barkerae M.P. de Vos	Western Cape, Cape Columbine	*Goldblatt & Nänni 1113*
R. citrina Baker site 1	Northern Cape, near Kamieskroon	*Goldblatt & Manning 11534*
site 2	Northern Cape, top of Kamiesberg Pass	*Goldblatt & Porter 11726*
R. cruciata (Jacq.) Baker, site 1	Western Cape, Darling Reserve	*Goldblatt & Nänni 11098*
site 2	Western Cape, S of Malmesbury	*Goldblatt 11386*
R. eximia M.P. de Vos, site 1	Western Cape, Mamre hills	*Goldblatt s.n.* no voucher
site 2	Western Cape, Darling Reserve	*Goldblatt & Nänni 11169*
site 3	Western Cape, Langebaan	*Goldblatt & Manning 10976*
site 4	Western Cape, Vredenburg granite rocks	*Goldblatt & Manning 11083*
R. flava (Lam.) de Vos, site 1	Western Cape, Signal Hill	*Goldblatt 10241*
site 2	Western Cape, Potsdam	*Manning s.n.* no voucher
site 3	Western Cape, Malmesbury	*Goldblatt & Nänni 11380*
R. hantamensis (Diels) Goldblatt	Northern Cape, Hantamsberg	*Goldblatt 9175*
R. hirsuta (Eckl. ex Klatt) Baker		
site 1	Western Cape, Lions Head	*Esterhuysen 15759*
site 2	Western Cape, Darling	*Goldblatt 11097*
R. hirta Schltr., site 1	Northern Cape, Glenlyon Farm	*Goldblatt s.n.* no voucher
site 2	Northern Cape, Nieuwoudtville trekpath	*Goldblatt 11399*
R. kamisensis M.P. de Vos	Northern Cape, Kamiesberg Farm Outuin	*Goldblatt & Porter 11726*
R. komsbergensis M.P. de Vos	Northern Cape, Roggeveld	*Goldblatt & Manning 10946*
R. luteoflora (M.P. de Vos) M.P. de Vos, site 1	Northern Cape, Roggeveld near Middelpos	*Goldblatt & Nänni 11405*
site 2	Western Cape, Cold Bokkeveld	*Goldblatt 11468*
R. monadelpha (Sweet) Baker,		
site 1	Northern Cape, Glenlyon Farm	*Goldblatt 4036*
site 2	Northern Cape, near Calvinia	*Goldblatt & Manning s.n.* no voucher
R. montana Schltr. ex Bég.	Northern Cape, Nieuwoudtville Trekpath	*Goldblatt 11397*
R. monticola M.P. de Vos	Northern Cape, Nieuwoudtville Cloudskraal road	*Goldblatt 11402*
R. namaquensis M.P. de Vos	Northern Cape, Kamiesberg	*Goldblatt & Manning 10987*
R. obscura M.P. de Vos	Western Cape, Waylands, Darling	*Goldblatt & Manning 10316*
R. rosea (L.) Eckl., site 1	Western Cape, Fairfield, Caledon	*Kemper IPC174*
site 2	Western Cape, Buffeljagsrivier	*Goldblatt 11435*
R. sabulosa Schltr. ex Bég., site 1	Northern Cape, S of Nieuwoudtville	*Lewis 5831*
site 2	Northern Cape, Nieuwoudtville trekpath	*Goldblatt 11398*
R. saldanhensis M.P. de Vos	Western Cape, near Paternoster	*Goldblatt 11164*
R. schlechteri Bég.	Western Cape, Mamre Nature Reserve	*Goldblatt & Nänni 11099*
R. setifolia N.E. Br.	Western Cape, near Worcester	*Goldblatt & Manning s.n.* no voucher
R. sladenii M.P. de Vos	Western Cape, Gifberg plateau	*Goldblatt 11446*
R. subfistulosa M.P. de Vos	Northern Cape, Roggeveld, near Fransplaas	*Goldblatt & Manning 10305*
R. sulphurea Bég.	Western Cape, Pakhuis Pass	*Goldblatt & Manning 11076*
R. syringodeflora M.P. de Vos	Northern Cape, near Sutherland	*Goldblatt & Nänni 11192*
R. tortuosa (Licht. ex Roem. & Schult.) Baker, site 1	Northern Cape, Middelpos road	*Goldblatt & Manning 10943*
site 2	Northern Cape, near Nieuwoudtville	*Perry 2116*
site 3	Northern Cape, Komsberg Pass	*Manning s.n.* no voucher
R. toximontana M.P. de Vos	Western Cape, Gifberg	*Goldblatt & Manning 10709*
R. triflora (Burm.f.) N.E. Br.	Western Cape, S of Malmesbury	*Goldblatt 11387*

pollen off the body and scopae or corbiculae of bees with a dissecting needle (see GOLDBLATT et al. 1998a, 1998b, 2000b). The residue from needle probes was collected on glass slides and mounted in 1-2 drops of Calberla's fluid (OGDEN et al. 1974), a combined stain and rehydrating medium. In the case of long-proboscid flies, which are comparatively large insects, sites of pollen deposition are usually quite discrete for a particular plant visited and pollen species can usually be identified without recourse to microscopic examination, due to pollen coloration and position. Pollen grains were identified microscopically by comparison with a reference set of pollen grain preparations made from plants flowering at study sites. *Romulea* pollen grains are recognizable by their large size, oblong shape, perforate-scabrate exine, and monosulcate aperture with a prominent 2-banded operculum (GOLDBLATT et al. 1991).

Insect specimens were identified by R.W. BROOKS (Andrenidae, Apidae, Halictidae), University of Kansas, H. Dombrow, Worms, Germany (Scarabaeidae), J.C. MANNING (Diptera, Lepidoptera), and K.E. STEINER (Melittidae), National Botanical Institute, Kirstenbosch, South Africa. Voucher specimens are deposited at the University of Kansas Museum, Lawrence, Kansas.

RESULTS

Inflorescence phenology and floral life span

Species of *Romulea* are acaulescent, semi-acaulescent or shortly caulescent, low-growing geophytes (Fig. 1) bearing leaves in a basal tuft and flowers at or close to ground level on short pedicel-like branches (DE VOS 1972, 1983; MANNING & GOLDBLATT 2001). Flowers rarely reach more than 8 cm above the ground, but in a few species the flowering stem may reach up to 20 cm. Individuals produce a branched inflorescence, and each branch carries a single flower (Fig. 1). The floral axis is subterranean in many species, or emergent. Flowering is closely synchronized within a population, which may be quite dense and thus produce mass displays at flowering time. Flowering periods subdivide into a winter-spring season (mainly July to September) in the southern African winter-rainfall zone or a summer-autumn season (December to April) in the southern African summer-rainfall zone and tropical Africa (Table 2). This coincides with two periods of optimal plant growth, during or soon after the main rainy season within the region.

In all species studied a mature bud expands at a specific time of day, usually mid-morning, and closes in the late afternoon, always before sunset. A flower typically lasts three to four days, regardless of species, but maintains the pattern of opening and closing at specific times of day. Flower buds on the same inflorescence open sequentially, usually one to three days apart, hence there may one to three flowers open at any time on an inflorescence, depending on the number of branches on an individual. When flowers close the tepals cloak the anthers and stigmas completely. Ambient temperature influences anthesis and on cold (< 15°C), overcast, or misty days flowers may not open completely the entire day or the normal time of opening may be delayed until conditions are more favorable.

The perianth is cup-shaped in most species (Fig. 1A-B) and the tepals are united below in a short funnel-shaped tube. In species of section *Bicarinatae* (sensu DE VOS 1972), which includes *Romulea monadelpha, R. sabulosa,* and *R. subfistulosa*, the tube forms a shallow disc and the floral cup is correspondingly somewhat wider than in species with funnel-shaped tubes. In a few species the perianth tube is elongate and cylindric (Table 2). Perianth coloration is varied and almost every color is encountered among the species (Table 2). Species with wide ranges often have more than one color morph (DE VOS 1972). Cup-shaped flowers typically have tepals with the lower part erect and forming the sides of the floral cup, while the upper part spreads horizontally. The floral cup is usually yellow, sometimes cream to pale greenish, or in a few species blackish (*R. monadelpha* and *R. sabulosa*), and the edges of the cup may bear a blotch of dark pigmentation, often brown in species with yellow tepals, or red to blackish in species with pink to reddish tepals. Flowers of a few species are scented (see *R. flava* group below).

TABLE 2. — Floral characteristics of ***Romulea*** species arranged according to flower type. Characteristics are for study populations and may not reflect the full range for the entire species. + = presence, (–) = absence, tr = trace amount too little to measure volumetrically.

Species	Perianth shape	Flower tepal limbs	Pigmentation floral cup	Pollen color	Tube length mm	Scent	Nectar present	Flowering-time
***Romulea sulphurea* group**								
R. aquatica	cup	white	yellow	yellow	c. 4	yes	tr	Aug.-Sep.
R. barkerae	cup	white	brown and yellow	yellow	c. 5	no	tr	July-Aug.
R. citrina	cup	yellow	yellow	yellow	c. 5	no	tr	Aug.-Sep.
R. flava	cup	yellow or white	yellow	yellow	c. 4	often	tr	July-Aug.
R. hirta	cup	pale yellow	darker yellow	yellow	4-5	no	tr	July-early Sep.
R. namaquensis	cup	pink to copper	yellow	yellow	c. 6	no	tr	Aug.
R. saldanhensis	cup	deep yellow	yellow	yellow	c. 4	no	tr	mainly Sep.
R. schlechteri	cup	white	yellow	yellow	c. 6	yes	tr	mainly Aug.
R. setifolia	cup	yellow	yellow often with dark marks at edges	yellow	c. 5	no	tr	July-early Aug.
R. sladenii	cup	white	cream	white	c. 5	no	tr	Aug.-Sep.
R. sulphurea	cup	deep yellow	yellow	yellow	c. 4	yes	tr	July-early Aug.
R. tortuosa	cup	deep yellow	pale yellow	yellow	c. 4	yes	tr	July-early Aug.
R. toximontana	cup	white	cream	pale yellow	4-5	no	tr	mainly Aug.
R. triflora	cup	yellow	yellow	pale yellow	c. 4	no	tr	mainly Aug.
***Romulea eximia* group**								
R. atrandra	cup	magenta to pale pink	uniformly pale or with dark lines and edged dark purple	brown or yellow	c. 6	no	tr	Aug.-Sep.
R. cruciata	cup	pink	cream edged with dark blotches	yellow	3-5	no	tr	Aug.-Sep.
R. eximia	cup	brick red	yellow edged with dark red	yellow	5-8	no	tr	Aug.-early Sep.
R. hirsuta	cup	red	yellow edged with dark red	yellow	c. 4	no	tr	mainly Aug.
R. luteoflora	cup	yellow	yellow edged with dark brown	yellow	c. 5	no	tr	Sep.
R. montana	cup	yellow	yellow edged with dark brown	yellow	c. 5	no	tr	Aug.-Sep.
R. monticola	cup	yellow	yellow sometimes edged with black	yellow	c. 5	no	no	Aug.-Sep.
R. rosea	cup	purple to mauve-pink	pale yellow	yellow	c. 4	yes/no	tr	Aug.-Sep.
R. subfistulosa	cup	pink	yellow edged with dark blotches	yellow	3-5.5	no	(–)	Aug.–early Sep.
***Romulea monadelpha* group**								
R. amoena	cup	red	cream edged with blackish marks	yellow	5-7	no	(–)	Aug.
R. komsbergensis	cup	purple	pale with a dark streaks, edged with dark blotches	brown	3-4	no	tr	Aug.-Sep.

Species	Perianth shape	Flower tepal limbs	Pigmentation floral cup	Pollen color	Tube length mm	Scent	Nectar present	Flowering-time
R. monadelpha	cup	red	pale with a brown center edged with purple-black blotches	yellow	c. 5	no	(–)	Aug.-Sep.
R. obscura	cup	red	whitish with black streaks, edged dark red	yellow	c. 4	no	?	Sep.
R. sabulosa	cup	red	blackish with tepals edged with cream	yellow	c. 5	no	(–)	mainly Aug.
***Romulea hantamensis* group**								
R. albiflora	tube-salver	n/a	white	yellow	20-33	no	+	Oct.
R. hantamensis	tube-salver	n/a	purple with dark veins	brown	35-60	no	+	Sep.-Oct.
R. kamisensis	tube-salver	n/a	magenta or purple with dark veins	yellow	17-24	no	+	Aug.-early Sep.
R. syringodeoflora	tube-salver	n/a	pink, tepal bases darkly marked, throat yellow	brown	18-22	no	+	Sep.-Oct.
Unplaced species								
R. stellata	tube-salver	n/a	pale violet, throat yellow	mauve	11-17	no	?	June-July

The style is held in the center of the flower and is surrounded by the three symmetrically disposed stamens, the filaments of which are typically contiguous and usually papillate or hairy below (Fig. 1A-B). In *Romulea monadelpha* the filaments are united into a smooth column. Species with cylindric perianth tubes also have smooth filaments. The anthers are usually parallel and contiguous, but divergent in *R. aquatica, R. komsbergensis*, and a few other species not included in our study (e.g., including *R. diversiformis*). Anthers are extrorse with loculicidal dehiscence. Pollen adheres to the dehisced anther locules until removed as a result of activity of insect visitors. Pollen is usually yellow or whitish, but several species (Table 2) have dark brown pollen.

The style typically divides opposite the anther apices or barely above them into three short branches, each forked for half its length and ciliate and stigmatic along the forked part. In the rare *R. multifida* (not studied), the style branches are multifid (DE VOS 1972). When the style diverges below the anther apices, the paired arms of the style branches extend between the anthers (Fig. 1A-B). In a few species, including *R. diversiformis* M.P. de Vos, the variant of *R. komsbergensis* studied here, and a genotype of *R. tortuosa,* sporadic in the Calvinia district (not studied), the style is elongated and it diverges well above the anthers. In a few other species the style divides opposite the bases of the anthers, including in *R. aquatica*, and the typical form of *R. komsbergensis*.

Flowers of *Romulea* species are weakly protandrous according to DE VOS (1972), which we have confirmed for selected species. The anthers dehisce longitudinally one to four hours after the tepals first unfold, and depending to some extent on ambient temperature and humidity (see

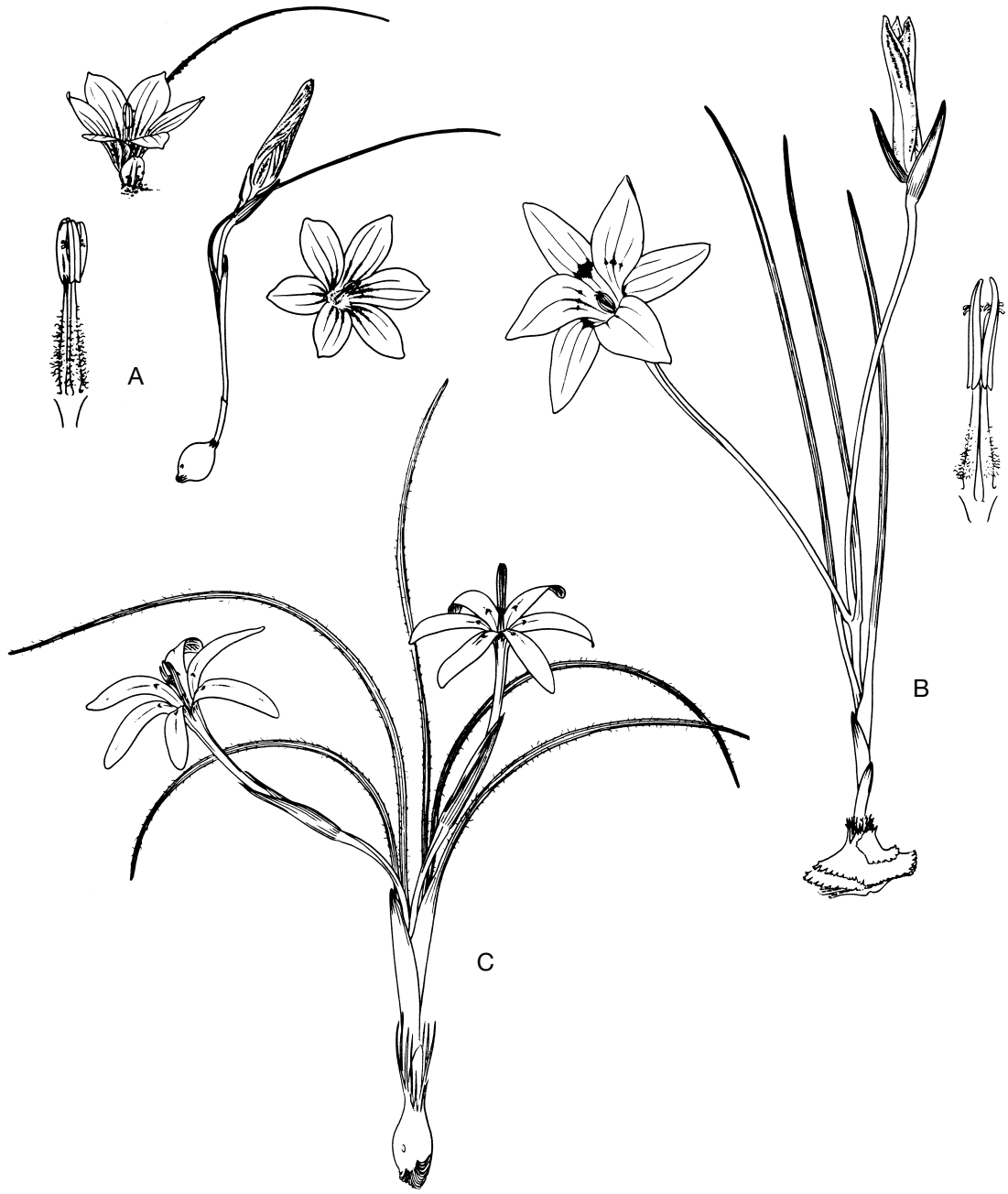

Fig. 1. — Vegetative and floral morphology of ***Romulea***: **A**, ***R. lilacina***, acaulescent habit with bell-shaped flower, detail of stamens with style branches emerging from between the anthers and papillate-hairy filaments; **B**, ***R. discifera***, caulescent habit with bell-shaped flowers, the stamens enclosed in the floral cup and detail of stamens showing papillate-hairy filaments; **C**, ***R. alba***, subacaulescent habit and tubular flowers with patent tepals and stamens fully exserted from the tube.

TABLE 3. — Nectar properties of ***Romulea*** species that produce measurable quantities of nectar (Table 2). SD = standard deviation, (n) = number or individuals sampled, Fru = fructose, Glu = glucose, n/a = not assessed.

Species	Volume µl (n)	Mean % sugar (SD)	Fructose	Glucose	Sucrose	Mean Sucrose/ Glu + Fru (n)
R. albiflora	1.8-2.3(10)	25.7(3.8)	17-18	20-22	61-62	1.61(3)
R. hantamensis	3.7-5.2(3)	20(n/a)	23	27	50	1(1)
R. kamisensis	0.8-2.3(10)	23.3(3.4)	n/a	n/a	n/a	n/a
R. syringodeoflora	0.7-1.2(10)	23.5(2.4)	n/a	n/a	n/a	n/a

above) dehiscence may occur later in the day under wet-cool conditions. The stigmas are receptive on the second or third day of anthesis (DE VOS 1972). Male and female phases of the flower are thus separated by one or two days. DE VOS (1972) also reported that species of *Romulea* are self-compatible but can only be crossed with a few closely related species within the same taxonomic section. Interspecific and infraspecific compatibility was not investigated for this study.

Flower types in the genus can be subdivided into four major groups based on overall shape, width of the floral cup or shape of the perianth tube, pigmentation, and pollen coloration (Table 2). These groups more or less correlate with the range of insects that comprise the main visitors to the flowers.

Only one other flower type occurs in the genus. *Romulea stellata* M.P. de VOS, which flowers in June and July, has a small mauve flower with a narrow, cylindrical perianth tube 11-17 mm long and horizontally extended tepals 7-11 mm long. We were unable to determine whether the flowers contain nectar and have no observations on the insect visitors to the species. The flowers are by far the smallest in the genus and stand out because of their narrow tube.

Nectar

Nectar when present (Table 2) is produced by septal nectaries (DE VOS 1972), as they are in the entire subfamily Crocoideae (syn. Ixioideae) (GOLDBLATT 1990, 1991). Nectar is secreted from three minute pores at the top of the ovary (one per chamber) directly into the base of the perianth tube, where accumulated fluid is retained until removed by a foraging insect. In most species traces of nectar can be detected by removing the perianth from the ovary and brushing the base of the tube across the investigator's tongue, but quantities are usually too small to measure for volume or concentration. Measurable amounts of nectar are produced in those species whose perianth forms an elongate, cylindrical tube, and volume correlates with tube length (Table 3). The long-tubed *Romulea hantamensis* produced the most nectar, up to 5.2 µl, in flowers undisturbed by insects (the perianth had not fully expanded when nectar was measured). Sugar analyses indicate that species of *Romulea* offer nectars that are sucrose-rich with sucrose: hexose ratios of between 1 and 1.6 (Table 3).

Floral Presentation and pollination systems

Four pollination systems can be recognized in *Romulea*, although three of them overlap to some extent, and only one is quite discrete. Comparative floral traits of the four systems are summarized in Tables 2 and 4. The pollination systems and associated floral traits are not always correlated with phylogenetic relationships. No explicit phylogeny of the genus has yet been proposed, and so we cannot make detailed comments on species relationships.

The Romulea flava group (Table 2). — In this group, of which *R. aquatica, R. flava,* and *R. saldanhensis* are typical examples, the perianth is cup-shaped (Fig. 2A) with a short, funnel-shaped perianth tube. The cup is formed by the tepal claws and is relatively deep (8-12 mm), while the tepal limbs extend horizontally. With few exceptions floral color is cream to yellow, as expressed by the perianth cup, the pollen, and to a lesser

TABLE 4. — Comparison of the critical floral features of the ***Romulea flava, R. monadelpha*** and ***R. eximia*** groups, pollinated by bees alone, beetles alone, or bees and beetles together.

Character	*R. flava*	*R. monadelpha*	*R. eximia*
filaments	hairy/papillate	smooth or minutely papillate	hairy/papillate
tepal limbs color	yellow, cream or white	red or orange	red, purple, or yellow
pollen color	yellow	yellow or brown	yellow
floral cup color	yellow	red, black or cream	yellow
nectar	trace	none	trace
beetle marks	none	present	occasionally present
scent	sometimes present	absent	occasionally present

extent the tepal-limbs (Table 2). The edges of the cup are sometimes lightly streaked with dark color or have a small dark spot on each tepal. Flowers of *R. barkerae* are exceptional in this group in having a large dark brown blotch on the lower half of each tepal edged in yellow, rendering the cup mostly a dark color. The stamens, and usually the entire style, are included in the floral cup and form a prominent central column (Fig. 1A). The floral tube consists of a short narrow cylindrical part, 1-2 mm long, and a flared upper portion, mostly about 3 mm long, at the base of which the filaments are inserted. The cylindrical portion of the tube is hollow but the interior space is virtually filled by the style. Only trace amounts of nectar are present, confined to the base of the wider part of the tube. Filaments are loosely held against one another and are invariably lightly hairy to papillate in the lower half. Some species of this group have scented flowers (Table 2), the fragrance usually sweet and reminiscent of honey (*R. aquatica*, *R. schlechteri*), sweet pea (*R. sulphurea*), or narcissus (*R. tortuosa*). *Romulea flava* is reported to have populations with scented flowers (DE VOS 1972) but those we studied did not. Flowering time for species with this flower type shows a marked trend for early flowering, mostly blooming from mid July to late August, and flowering seldom lasts much later than early September (Table 2).

Species in this group are pollinated by a range of large bees, 10-15 mm long, in the families Andrenidae (*Andrena*), Apidae (*Anthophora, Apis*) (classification of Apidae after ROIG-ALSINA & MICHENER 1991, which includes Anthophoridae in Apidae), and Melittidae (*Rediviva*), and medium-sized bees, 8-10 mm long, in the family Halictidae (*Patelapis*). After alighting on a flower *Apis mellifera* workers typically crawled over the tepals and into the floral cup, with their heads directed toward the base of the filaments, where nectar is located. Then bees would climb onto the staminal column and scrape pollen from anthers onto their legs. This pattern of behavior resulted in pollen being brushed onto the dorsal thorax of a bee in addition to the ventral accumulation that occurs during active scraping of the anthers. Active collection of pollen without probing the floral cup was observed in worker honey bees visiting flowers of *R. montana.*

Behavior of other bee taxa was different in that most *Andrena*, *Rediviva*, and halictid bees landed directly on the staminal column and scraped the anthers with their legs to remove pollen. The bees are polylectic foragers (Table 5) and individuals at different study sites were found to carry the pollen of co-blooming species of *Bulbinella* (Asphodelaceae), *Lachenalia* (Hyacinthaceae), *Hermannia* (Malvaceae), and other *Iridaceae* (including *Gladiolus* and *Moraea*) in their scopae or corbiculae and/or on various parts of their bodies. Frequently, however, pollen loads in scopae or corbiculae contained a high proportion of *Romulea* pollen, and in the case of *Apis* it was evident from direct observation of foraging behavior and the presence of pure corbicular loads of *Romulea* pollen (e.g., in *R. barkerae*, *R. sulphurea, R. tortuosa*) that *Apis* workers are typically flower constant, and that various species of *Romulea* constitute a significant pollen resource for them (Table 5).

We also observed large-bodied *Anthophora* species (Apidae) in several study sites but noted that with few exceptions they ignored *Romulea* flowers. These large-bodied bees forage for nectar most frequently on bilabliate flowers (*Babiana, Gladiolus, Lachenalia, Sparaxis*), and also on eudicotyledons, especially species of *Lobostemon* and *Hermannia*, carrying quantities of pollen of these genera. This seems highly significant, as large-bodied anthophorines are intrinsic to the pollination of other genera of Iridaceae, especially *Babiana* (GOLDBLATT et al., in prep.), *Gladiolus* (GOLDBLATT et al. 1998b), *Nivenia* (GOLDBLATT & BERNHARDT 1990), and *Sparaxis* (GOLDBLATT et al. 2000a).

Visits of hopliine beetles to species of this group was not consistent, but *Romulea tortuosa*, which has sweetly scented flowers, and *R. hirta* were seen at some study sites being visited by *Lepithrix forsteri*, though flowers of these two species appear to be more consistently visited by *Apis mellifera* or other bees such as Halictidae (Table 5). These flowers lack the usual dark markings, sometimes called beetle marks, often associated with hopliine pollination. Whether the visits by hopliines to flowers of this group were opportunistic is impossible to determine, but casual visits are often observed when beetle populations are large or flowers more suited to their needs are scarce or absent. Clearly beetles are able to accomplish pollen transfer from one individual to another. Hopliine foraging behavior is explained more fully below. One surprise for us was the presence of bees alone visiting and pollinating *R. barkerae,* because the flowers of this species have large dark brown blotches on the tepals, often associated with hopliine pollination. Nevertheless, no hopliines were encountered on this rare, local endemic of limestone outcrops along the western Cape coast.

Neither divergent anthers, found in *Romulea aquatica*, nor the level at which the style branches diverged relative to the anthers, seemed to be associated with a particular pollinator or with a particular pollinator behavior.

The Romulea monadelpha group. — This second group includes species confined to the western Cape and western Karoo. Flower structure (Fig. 2B) is broadly similar to that found in the *R. flava* group with the important exception that the perianth tube is shallowly disk-shaped rather than funnel-shaped in *R. sabulosa* and *R. monadelpha*. In direct contrast to the *R. flava* group, perianth color is dominated by reds and purples, with prominent dark botches (beetle marks) at the mouth of the floral cup. These blotches are usually outlined with a lighter rim or halo (white to yellow or bluish), usually considered indicative of a true beetle mark (BERNHARDT 2000). In some species (e.g., *R. monadelpha, R. sabulosa*) the center of the dark blotch may have a contrasting white to cream "bulls-eye", which sometimes resembles in outline the body of a hopliine beetle and appear to be an important part of the floral presentation. The shape and size of the dark blotches is variable within and between populations. Perhaps merely the presence of a dark blotch is significant, not its exact shape and size. The filaments and anthers may also be darkly colored, but pollen is usually yellow, while in *R. komsbergensis* grains are reddish brown. The floral cup in *R. amoena* is whitish to greenish cream but streaked with longitudinal lines. Flowers of species in the *R. monadelpha* group are unscented and we were unable to detect the presence of nectar. Unlike the *R. flava* group the filaments are only sparsely papillate or smooth and in *R. monadelpha* they are fused together. Flowering is mostly from mid August to mid September.

Species in this group are pollinated exclusively by hopliine scarab beetles. In fair weather, individual flowers invariably have one to five beetles crawling, resting, or copulating in the floral cup, or crawling over the tepals. Four species of beetles were captured in flowers of *Romulea sabulosa* and sometimes individuals of two species were captured within a single flower. In contrast, *Clania glenlyonensis* was the only hopliine species captured on *R. monadelpha* in two separate and isolated populations of the species. *Anisonyx ignitus* was captured on *R. komsbergensis* in 1998 and *A. inornatus* in 1999. Clearly there is not a one to one relationship between most *Romulea* species and their beetle visitors.

Pollen load analyses of beetles indicate that they are polyphagic foragers, visiting large co-

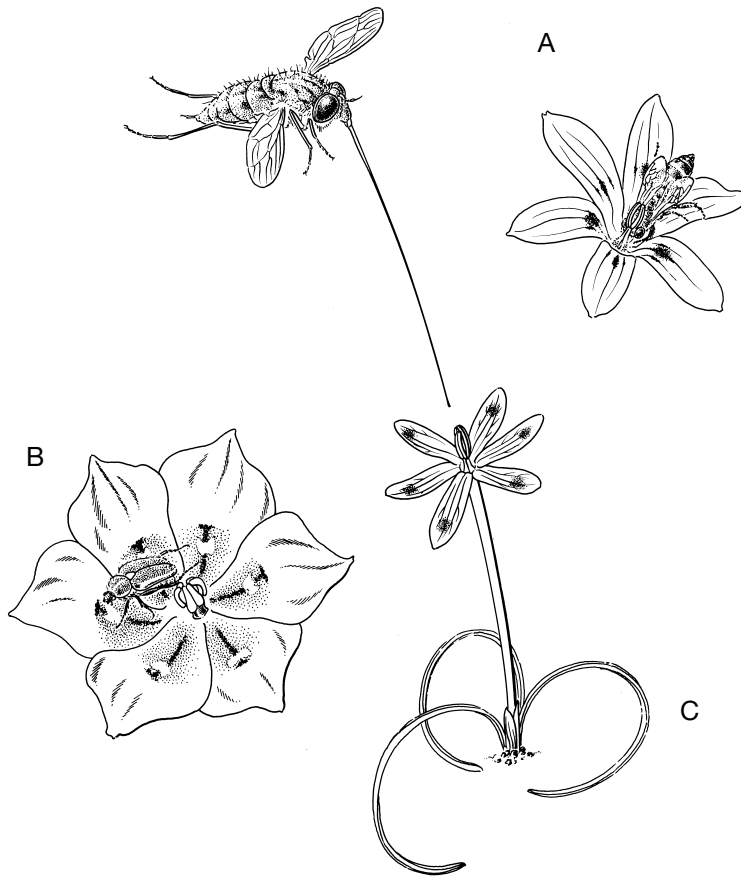

Fig. 2. — Principal flower types in ***Romulea*** and their pollinators: **A**, flower of ***R. tortuosa*** with a visiting honey bee, *Apis mellifera*; **B**, ***R. monadelpha*** with the hopliine beetle *Clania glenlyonensis*; **C**, plant and flowers of ***R. hantamensis*** with the long proboscid fly *Prosoeca* sp. 1.

blooming flowers and inflorescences of a variety of different families, including Asteraceae, Hypoxidaceae, and other Iridaceae. Individual beetles usually spend at least several minutes in a flower, either crawling about or at rest with their head pointed toward the center. Whatever their behavior, they invariably become covered with pollen of the host flower within moments of landing. Visits last from a few seconds to more than 15 minutes (no beetle visit was timed for longer than this). Beetles were sometimes seen to feed on pollen, but more often crawled around the floral cup before either leaving the flower or assuming a resting position with its head directed toward the center of the cup. Microscopic examination consistently confirmed the presence of host flower pollen and beetles usually also carried pollen of one or more other plant species (Table 5), most commonly a member of Asteraceae, or sometimes another genus of Iridaceae, Hypoxidaceae, or even Droseraceae (GOLDBLATT et al. 1998a). For example, *Clania glenlyonensis* beetles captured on *R. monadelpha* at our study site near Nieuwoudtville carried pollen of *Hesperantha vaginata* (Sweet) Goldblatt (Iridaceae), and *Arctotis acaulis* L. (Asteraceae).

The Romulea eximia group. — This group of species, of which *R. eximia* and *R. hirsuta* are typical examples, is typified by floral characters

intermediate between the two previous groups. Consequently pollination systems may incorporate bee and beetle pollinators (see below). Species of the *R. eximia* group have a cup-shaped perianth in which yellow pigmentation is usually confined to the center of the floral cup while the tepal limbs are often glossy pink, purple or red. Dark blotches form a narrow band between the yellow center and the tepal limb (Table 2). The flowers in this group are typically unscented, as in the *R. monadelpha* group, but several species secrete trace amounts of nectar, as do species of the *R. flava* group. The filaments are hairy to papillate below. Flowering in the *R. eximia* group is primarily from mid August through September.

Species in the *Romulea eximia* group are pollinated by a combination of hopliine beetles and bees representing four families (Table 5). Bee behavior appears to be the same as described for flowers in the *R. flava* group. Foraging bees are polylectic, combining the pollen of co-blooming *Bulbinella* (Asphodelaceae), *Lachenalia* (Hyacinthaceae), *Hermannia* (Malvaceae), and *Iridaceae* (including *Gladiolus*, *Moraea*) in their scopae or corbiculae and/or on various parts of their bodies.

Beetle behavior on flowers of species in the *Romulea eximia* group follows the same pattern as described for the *R. monadepha* group: beetles use the flowers as sites of assembly, competitive agonistic activity, and mating (STEINER 1998; GOLDBLATT et al. 1998a). Beetles bearing more than one type of pollen appear to forage on different flowers of different species with similar or very different color patterns. For example, *Lepithrix ornatella* captured on the red-flowered *R. eximia* near Darling in the western Cape coast also visited the large creamy yellow flowers of *Ixia lutea* Eckl., which have a dark central blotch, and the white flowers of *Ornithogalum thyrsiflora* Jacq. This same beetle species has also been captured on the bright orange flowers of *Ixia maculata* L. and *I. tenuifolia* Vent. or the golden yellow flowers of *I. aurea* Goldblatt & J.C. Manning at different sites in the western Cape (GOLDBLATT et al. 1998a, 2000b). At Waylands Reserve, Darling, *Lepisia rupicola* individuals captured on *R. obscura* carried pollen of *Spiloxene capensis* (L.) Garside (Hypoxidaceae), *Ixia maculata* L. (Iridaceae), and *Drosera cistiflora* L. (Droseraceae). Additional individuals of the same beetle species were also captured on *Gladiolus meliusculus* (G.J. Lewis) Goldblatt & J.C. Manning which has flowers of nearly identical coloration to those of *R. obscura*. More examples of plants exploited by hopliine beetles at specific sites are listed by GOLDBLATT et al. (1998a).

While flowers in the *Romulea eximia* group are visited by both beetles and bees, the density of bee versus beetle pollinators may vary seasonally. In some years and at some sites bees or hopliines may not be encountered in a particular species in the *R. exima* group, although this may represent inadequate sampling as well as seasonal fluctuations in local insect populations. At three of four study sites (Table 5) bees were found actively visiting flowers of *R. eximia* and pollen loads containing high proportions of *Romulea* pollen suggest that this is a significant food resource for the insects. The ratio and diversity of beetles and bees varies at different sites for *R. eximia* (Table 5).

At many sites species using hopliine beetles for their pollination appear to form guilds, the members of which resemble one another to a greater or lesser extent. Sometimes flowers (or flower heads) are similar to one another in general form, or in having dark blotches, or in their nearly identical pigmentation and patterning. Flowers of some co-blooming species of *Romulea* can sometimes be so similar that only by examining vegetative features can they be distinguished. Striking examples of this convergence for floral pigmentation are *R. eximia, R. hirsuta,* and some forms of *R. obscura* (GOLDBLATT et al. 1998a).

The Romulea hantamensis group. — Restricted to the western Karoo and Namaqualand, species of this group are easily distinguished from members of the preceding groups by having flowers with a hollow, elongate perianth tube 17-60 mm long. The tepals are 12-15 mm long, spread horizontally, and are shorter than the tube (Figs. 1C, 2C). The flowers are always odorless and are colored pink to purple, or rarely white (*R. albiflora*). The tube is relatively wide, about 2 mm in diameter, and contains large quantities of nectar (Table 3). The stamens and style lobes of these species are fully exserted from the flower, except in *R. kamisensis* which has the stamens included

TABLE 5. — Pollen load analysis of captured insects on ***Romulea*** species. Coleoptera: Scarabaeidae: *Anisochelus, Anosonyx, Anisothrix, Lepisia, Lepithrix, Pachycnema, Stigmatioplia.* Hymenoptera: Andrenidae: *Andrena.* Apidae: *Anthophora*, *Apis*. Halictidae: *Patellapis*. Melittidae: *Rediviva.* Diptera: Nemestrinidae: *Prosoeca.*

Plant and [insect] taxon	Number of insects carrying pollen load(s)		
	Host flr only	Host flr + other sp.	other species or no pollen
R. amoena (site 1)			
Stigmatioplia nicolaji	2	0	0
Lepithrix steineri	3	12	2
R. aquatica			
Apis mellifera	3	2	0
R. atrandra (site 1)			
Anisonyx hilaris	1	2	0
Apis mellifera	2	0	0
Lepithrix forsteri (site 2)	2	0	0
Patellapis sp. 4 ♀	2	2	0
R. barkerae			
Apis mellifera	4	0	0
R. citrina (site 1)			
Lepisia ornatissima (site 2)	1	4	0
hopliine	3	0	0
Apis mellifera	1	1	0
R. cruciata (site 1)			
Anisonyx ursus	2	3	0
Rediviva aurata ♀ (site 2)	0	2	0
Apis mellifera	2	4	0
R. eximia (site 1)			
*Lepisia rupicola**	0	4	0
Lepithrix ornatella (site 2)	0	2	0
Andrena sp. ♀	0	4	0
Lepisia rupicola (site 3)	0	3	0
Rediviva aurata ♀	0	2	0
Patellapis sp. 2 ♀	0	2	0
Heterochelus detritus (site 4)	1	0	0
Patellapis sp. ♀	2	2	0
Apis mellifera	1	0	0
R. flava (site 1)			
Apis mellifera (site 2)	1	4	
Apis mellifera (site 3)	4	1	0
Apis mellifera	2	0	0
Anisonyx ursus	0	1	0
R. hantamensis			
Prosoeca sp. 1	0	2	0
R. hirsuta (site 1)			
Andrena sp.	1	2	0
Rediviva aurata 5 ♀ (site 2)	0	5	0
Apis mellifera	2	1	0
R. hirta (site 1)			
Apis mellifera	2	3	0
Lepithrix steineri (site 2)	3	2	0

Plant and [insect] taxon	Number of insects carrying pollen load(s)		
	Host flr only	Host flr + other sp.	other species or no pollen
Lepithrix steineri	4	0	0
halictid bees 2 ♀	2	0	0
R. kamisensis			
Proeoeca peringueyi	1	0	0
(a second *P. peringueyi* seen visiting flowers evaded capture)			
R. komsbergensis			
Anisonyx ignitus	0	5	0
A. inornatus	2	2	0
Apis mellifera	2	0	0
halictid bees 2 ♀	3	0	0
R. luteoflora (site 1)			
Anisonyx hilaris	0	2	0
Apis mellifera	not captured		
Lepithrix cf. *forsteri* (site 2)	0	3	0
Lepithrix sp.	1	0	0
R. monadelpha (site 1)			
Clania glenlyonensis [1, 3] (site 2)	2	6	0
Clania glenlyonensis [1, 3]	4	2	0
R. montana (site 1)			
Lepithrix forsteri (site 2)	2	3	0
Lepithrix cf. *forsteri*	0	0	0
Apis mellifera	1	0	0
Anthophora cf. *diversipes* 2 ♀	2	0	0
R. monticola			
Lepithrix cf. *forsteri*	2	3	0
Apis mellifera	1	0	0
halictid bees 2 ♀	2	0	0
Anthophora cf. *diversipes* ♀	0	1	0
R. namaquensis			
Apis mellifera	0	3	0
R. obscura[2]			
Lepisia rupicola	0	4	0
Pachycnema crassipes	0	4	0
R. rosea (site 1)			
Apis mellifera (site 2)	0	1	0
Andrena sp.	1	0	0
R. sabulosa (site 1)			
Anisochelus inornatus	3	3	0
Lepithrix stigma	0	5	1
Heterochelus sp. (site 2)	3	0	0
Pachycnema calviniana	5	0	1
R. saldanhensis			
Apis mellifera [3]	4	0	0
R. schlechteri			
Apis mellifera [3]	2	0	0
R. setifolia			
Apis mellifera	0	2	0
R. sladenii			
Apis mellifera [3]	5	0	0
R. subfistulosa			
Clania steineri [3]	0	7	0
Patellapis sp.[3] 2 ♀	0	2	0
Anisonyx hilaris	0	3	0

Plant and [insect] taxon	Number of insects carrying pollen load(s)		
	Host flr only	Host flr + other sp.	other species or no pollen
R. sulphurea			
Apis mellifera [3]	4	2	0
R. syringodeoflora			
?*Prosoeca* sp.		not captured	
R. tortuosa (site 1)			
Apis mellifera	2	0	0
(site 2)			
Apis mellifera	4	1	0
Lepithrix forsteri	2	3	2
halictid bee	1	1	1
(site 3)			
Apis mellifera	3	1	0
R. toximontana			
Apis mellifera	2	2	0
Andrena sp. ♀	0	1	0
Anthophora diversipes ♀	0	1	0
R. triflora			
Apis mellifera	2	3	0
bibionid fly	0	2	0
Total	118	150	7

[1] = *Lepisia* sp. of GOLDBLATT et al. (1998a)
[2] = published as *Romulea eximia* in GOLDBLATT et al. (1998a)
[3] = represents a fraction of the total number observed but not collected

within the upper part of the perianth tube. The filaments are smooth and the anthers and pollen are dark brown in *R. hantamensis* and *R. syringodeoflora*. *Romulea kamisensis* flowers from early August to early September, while *R. hantamensis* and *R. syringodeoflora* bloom in mid to late September and flowering in *R. albiflora* lasts into October. At least one species in this group may be found in flower at all times from August to October.

Flowers of *Romulea hantamensis* are visited and pollinated exclusively by the long-proboscid fly *Prosoeca* sp. 1 (MANNING & GOLDBLATT 1996a). Our observations on *R. kamisensis* showed that the only floral visitors are a second long-proboscid fly, *Prosoeca peringueyi,* which is a pollen vector. We also observed a long-proboscid fly visiting flowers of *R. syringodeoflora* but we failed to capture any individuals and cannot identify the fly. The proboscis of *Prosoeca* sp. 1 is typically slightly longer than the length of the perianth tube of *R. hantamensis*. Proboscis length in *P. peringueyi* is extremely variable and ranged from 15.5 to 37.5 mm (n = 5) at our study site, thus may be shorter than or substantially exceed the perianth tube length of *R. kamisensis*, 17-24 mm long. We have no pollinator observations for *R. albiflora*, which appears to belong a small guild of plant species of the western Karoo with long-tubed, white to cream flowers, evidently adapted for pollination by an as yet undiscovered long-proboscid fly. Other species that may belong to this long-proboscid fly guild in the region include *Disa karooica* H.P. Linder (Orchidaceae) and *Babiana spathacea* (L.f.) Ker Gawl. *Disa karooica* is pollinated in other parts of its range by the fly *Philoliche rostrata* (Tabanidae) (JOHNSON & STEINER 1997), which is not known to occur in the western Karoo.

The foraging behavior of *Prosoeca* flies has been described in detail by MANNING & GOLDBLATT (1996a, 1997) and GOLDBLATT & MANNING (2000). The fly grasps the tepals with its tarsi and probes the floral tube for nectar while continuing to vibrate its wings. As the fly inserts its proboscis into the floral tube the frons and dorsal part of its head contact the anthers and stigmas. Field observations and pollen load analyses show that fly species visit open flowers of other species dur-

ing foraging bouts, most of which have morphologically convergent flowers that may be regarded as belonging to specific pollination guilds. Thus, *Prosoeca* sp. 1 visits *Babiana flabellifolia* Harv. ex Klatt and probably *Hesperantha oligantha* (Diels) Goldblatt (both Iridaceae) at our study site. These two species have flowers very similar in shape and color to those of *R. hantamensis*. The formation of guilds in plant communities where long-proboscid fly-pollinated plants occur has been described in more detail elsewhere (MANNING & GOLDBLATT 1996a). It is unusual for less than three long-proboscid fly-pollinated plant species to occur at any site where flies are active (GOLDBLATT et al. 1995; MANNING & GOLDBLATT 1996a; GOLDBLATT & MANNING, 2000).

DISCUSSION

Until now the pollination of *Romulea* species has received little critical attention. SCOTT ELLIOT (1891) noted that halictid and anthophorine bees (*Halictus* sp., and *Allodape* sp.) visit the flowers of plants he identified as *R. hirsuta* (visited by Halictidae) and *R. rosea* (perhaps by another species) and noted the presence of nectar in the flower that he thought was secreted from the base of the filaments. He also noted one unusual feature of the flowers, the fine hairs or papillae on the lower half of the filaments that cover the mouth of the perianth tube, which he suggested might protect the nectar from rain. VOGEL (1954) considered *Romulea* species to be pollinated by bees attracted to the nectar in the short tube but made no observations himself of pollen vectors. VOGEL also drew attention to the crown of hairs at the base of the filaments of many species. DE VOS (1972), in her detailed systematic monograph of *Romulea*, did not mention pollination at all. Our analysis and comparison of the flowers of a representative range of species of *Romulea* indicates that the genus largely depends on three groups of insects (female or worker bees, hopliine beetles, and nemestrinid flies) for pollen transport. Pollination by these three groups is not unique to the genus and is well expressed in other genera of African Iridaceae and other families of African herbs (BERNHARDT & GOLDBLATT 2000; JOHNSON & STEINER 2000). Co-adaptation between *Romulea* species and their prospective pollen vectors appears to be sufficiently old for pollination systems to have diverged into three distinct modes of floral presentation. Pollination by long-proboscid flies in the *Romulea hantamensis* group converges with the nemestrinid pollination syndrome described in several other genera of Iridaceae in southern Africa, including *Babiana*, *Geissorhiza, Gladiolus*, *Hesperantha, Ixia, Lapeirousia*, *Sparaxis*, and *Tritonia* (GOLDBLATT et al. 1995, 2000a, 2000b; MANNING & GOLDBLATT 1996a; GOLDBLATT & MANNING 1999; BERNHARDT & GOLDBLATT 2000).

Likewise, pollination by hopliine scarab beetles has been described in eight other genera of the family and may be found in ten other angiosperm families (see review by BERNHARDT 2000). In particular, pollination by hopliine beetles in red- or purple-flowered *Romulea* species exploits the same set of floral characters described by GOLDBLATT et al. (1998a, 2000a, 2000b) in *Ixia, Moraea*, *Sparaxis* and *Tritonia*. It is common for hopliine pollinated species to have two or more species of hopliine visitors, and the fact that a single beetle is associated with *R. monadelpha* at both study sites is unusual. This may reflect the absence locally of other hopliines. *Romulea monadelpha* is restricted to a specific soil type, heavy red clay, and its beetle pollinator may also be similarly restricted in its distribution.

Bee pollination is so common in southern Africa that it has been described in 14 out of the 20 genera of Iridaceae occurring there. However, BERNHARDT & GOLDBLATT (2000) noted that bee pollination is not a homogenous system in the family and the diversity of bee pollinators depends at least in part on variation in the physical size, floral symmetry, and nectar secretion of the flower. For example, within the genus *Gladiolus* bee pollination is subdivided into two systems. The majority of bee pollinated *Gladiolus* species are nectariferous, offer a large landing platform, have concealed anthers, and are pollinated primarily by large bodied anthophorines in the genera *Amegilla* and *Anthophora*. In contrast, a much smaller group of *Gladiolus* species are usual in that they are nectarless, show radial symmetry, have prominently displayed anthers, and

are pollinated primarily by smaller bees collecting pollen for their offspring (GOLDBLATT et al. 1998b). Bee pollination in the *Romulea flava* group follows this second mode. Large-bodied anthophorines are rarely collected on any *Romulea* species (Table 5).

Romulea species pollinated in part or exclusively by bees share an interesting character noted in many bee pollinated flowers of other angiosperm families, hairs or papillae on the staminal filaments. Within *Romulea*, this feature is found only in the bee pollinated species. There is no evidence in the literature that floral hairs confined to the interior of a flower actually encourage bee foraging. Rather, botanists in the 19th century suggested that floral hairs discouraged the participation of insects that were not the appropriate size, and lacked manual dexterity and specialized mouthparts of bees (HENSLOW 1893). That is, flowers with furry interiors cloak or shield nectar from small-bodied "robbers", including flies, moths and butterflies. For example, BERNHARDT (1990) concurred with TRELEASE (1882, 1888), who made the original suggestion that these staminal hairs excluded flies and small lepidopterans from the nectaries of *Oxalis violacea* L. BERNHARDT & DAFNI (2000) observed that bees were the only foragers in flowers of *Mandragora officinarum* L. capable of reaching nectar by penetrating the dense beards of staminal hairs occluding the large nectar glands on the receptacle.

Pollination systems in *Romulea* intergrade. We interpret species placed in the *R. eximia* group as taxa that "hedge their bets" by exploiting both bees and hopliine beetles. Intergradation of floral characters in this group is striking, particularly in regard to pigmentation patterns. Specifically the distinctive beetle marks seen in these flowers are confined to a band between the yellow floral cup and the glossy, red, purple or yellow tepals. While staminal hairs persist, the flowers appear to be odorless and nectarless, indicative of systems encouraging visitation by beetles (GOLDBLATT & BERNHARDT 1998a). We must emphasize though that intergradation of pollination systems is not unique to *Romulea* and has been described in other southern African genera of Iridaceae. For example, as floral characters intergrade in some *Lapeirousia* species, the flowers are pollinated by a combination of bees and diurnal or crepuscular Lepidoptera (GOLDBLATT et al. 1995). More important to this study, the bee/beetle pollination system in the *R. eximia* group has its parallel in some *Gladiolus* and *Moraea* species (BERNHARDT & GOLDBLATT 2000; GOLDBLATT & BERNHARDT 1999). Whether anthers diverge or are held erect seems to have no relationship to pollination strategy, nor does the level at which the style branches diverge. *Romulea aquatica*, whose style branches diverge at the base of the anthers, and *R. saldanhensis,* whose style exceeds the anthers are both pollinated by *Apis mellifera*. The level at which the style is held is more likely to be related to the degree of outcrossing versus selfing in a species, since self-compatibility is the rule in the genus (DE VOS 1972).

BERNHARDT & GOLDBLATT (2000) noted that the adaptive radiation of pollination systems in an irid lineage correlated positively with species diversity. That is, the more species in a genus, the greater the number of pollination systems. Thus, the estimated 166 *Gladiolus* species in southern Africa have seven modes of pollination, whereas there are only 10 *Nivenia* species and only two modes of pollination (apid bee or long-proboscid nemestrinid fly pollination) (GOLDBLATT & BERNHARDT 1990). *Romulea*, with 75 of its 90 species restricted to southern Africa, should have more pollination systems than *Lapeirousia* with only 40 species, or *Ixia* with only 50. This is, however, not the case. Both *Ixia* and *Lapeirousia* have five distinct pollination systems while *Romulea* has only three (four if we treat the *R. eximia* group as a unique generalist system). Furthermore, *Sparaxis*, a relatively small genus of only 15 species, exhibit 5 pollination systems, with members being pollinated either exclusively by apid bees, hopliine beetles, long-proboscid nemestrinid flies, or by a combination of bees and beetles, or tabanid flies with short probosces and beetles (GOLDBLATT et al. 2000).

We conclude that the adaptive radiation of pollination systems in *Romulea* remains conservative compared to other lineages of comparative size in African Iridaceae. Why are so few pollination systems expressed in such a relatively large genus? There are two possible reasons. First, most *Romulea* species flower in late winter to mid spring, giving

them less access to more warm-weather pollinators such as sphinx moths, sunbirds, large-bodied anthophorines, acrocerid flies, and true butterflies. Second, and more important, variation in the pollination systems of *Romulea* appears to have remained conservative due to genetic constraints in floral presentation. Specifically, while floral evolution in the genus has incorporated modification of floral tube length, staminal hairiness, pigmentation patterns, scent, and nectar production, other characters remain uniform. Unlike the genera *Gladiolus* and *Lapeirousia*, floral symmetry in *Romulea* species is consistently actinomorphic. And unlike the genus *Moraea*, there has been no trend towards the evolution of a meranthium (GOLDBLATT & BERNHARDT 1999), where a single flower functions as three separate bilabiate reproductive units. Consequently, morphological conservatism in both the perianth and the sexual organs of *Romulea* seems to have limited the evolution of novel pollination systems regardless of the number species.

Convergence for long-proboscid fly pollination

Perhaps the most remarkable flowers in *Romulea* are those with a hollow, elongate cylindrical tube and outspread tepals. The first *Romulea* to be described with this flower type, *R. hantamensis*, was placed in another genus, *Lapeirousia*, when first named. It is perhaps even more notable that the species sharing this flower structure do not form a clade: *R. kamisensis* is a member of *Romulea* subg. *Romulea* sect. *Ciliatae*; and *R. hantamensis* and *R. syringodeoflora* belong in subgenus *Spathalanthus* (MANNING & GOLDBLATT 2001). These two species have different chromosome numbers and corm types that suggest each belongs to a different section (MANNING & GOLDBLATT 2001). They were previously placed in their own section and subgenus by DE VOS (1972), a decision based on their unique flower type. One more species with this flower type, *R. albiflora,* is evidently closely related to *R. syringodeoflora*. Thus long-proboscid fly pollinated flowers most likely evolved independently three times in the genus, in *R. kamisensis,* in *R. hantamensis*, and in *R. syringodeoflora*. The scattered distribution of the flower type across the genus makes it clear their pollination strategies are derived.

Trends in floral evolution

Outgroup comparison (MANNING & GOLDBLATT 2001) strongly suggests that species of *Romulea* belonging to sections *Romulea* and *Ciliatae* are ancestral in the genus as they have corms of the same or similar type as those in the related genera *Hesperantha*, *Geissorhiza,* and *Syringodea*. With few exceptions *Romulea* species in these sections are pollinated either by bees alone, or by a combination of bees and beetles. In contrast, *Romulea* species pollinated exclusively by hopliine beetles and long-proboscid flies have derived corms. Furthermore, *R. monadelpha* and *R. sabulosa* have specialized bracts and floral tubes (DE VOS 1972) that mark them as highly specialized species. Consequently beetle pollination and long-proboscid fly pollination are inferred to be derived syndromes that probably evolved from ancestors pollinated primarily by bees or a combination of beetles and bees. As noted above, pollination systems within the genus are comparatively less diverse than in other irid genera of comparative size (e.g., *Ixia, Lapeirousia*). We interpret the comparative lack of adaptive radiation in *Romulea* as a consequence of both a conservative floral phenology and floral architecture.

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