

The pollination of *Tritoniopsis parviflora* (Iridaceae) by the oil-collecting bee *Rediviva gigas* (Hymenoptera: Melittidae): the first record of oil-secretion in African Iridaceae

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The Western Cape geophyte *Tritoniopsis parviflora* (Iridaceae: Crocoideae) has been found to secrete floral oils as well as nectar. The oils are secreted from epithelial elaiophores over much of the proximal parts of the perianth. This is the first report of oil-secretion in the subfamily Crocoideae and the first record of oil-secretion in the Old World representatives of the Iridaceae. The species is pollinated by the large oil-collecting bee

Rediviva gigas (Hymenoptera: Melittidae) and is part of a guild of yellow-flowered, often fragrant species that flower in late spring and early summer, usually only after a fire the previous season. *Tritoniopsis parviflora* will not self-pollinate and the provision of both oil and nectar may be a strategy for ensuring pollination in populations in areas where *R. gigas* is not present

Introduction

The production of floral oils as a pollinator reward is an important alternative to the secretion of nectar in many plant species. Estimates of the number of plant species involved range from 1 800 (Steiner and Whitehead 1991) to over 2 400 species, in at least 12 angiosperm families of diverse taxonomic affinity (Buchmann 1987, Vogel 1988). This strategy represents a highly specialised pollination system based on a relatively small pool of potential pollinators as only a limited number of bee species can utilise floral oil (Buchmann 1987). The oil is secreted either from patches of glandular trichomes (trichome elaiophores) or from specialised patches of the epidermis itself (epithelial elaiophores). Although most abundant in tropical American savannas and forests, oil flowers are also known from the north-temperate region and the palaeotropics (Vogel 1974, 1976). Their discovery in southern Africa is relatively recent (Whitehead *et al.* 1984, Manning and Brothers 1986) but it has since become apparent that oil-collecting bees are the main or sole pollinators of some 140 species in 13 genera of temperate southern African Orchidaceae and Scrophulariaceae (Steiner and Whitehead 1988, Johnson and Steiner 1999). Other families in which oil-secretion is an important pollination strategy include Malpighiaceae, Gesneriaceae and the neotropical members of Iridaceae. Most neotropical genera of Iridaceae secrete floral oils, typically from conspicuous trichomes on either the tepals (*Ainea*, *Alophia*, *Cardenanthus*, *Cipura*, *Cypella*, *Ennealophus*, *Gelasine*, *Herbertia*, *Kelissa*, *Neomarica*, *Onira*, *Tigridia* and *Trimezia*) or staminal column

(*Sisyrinchium*) (Goldblatt *et al.* 1998a). Until now oil-secretion in Iridaceae was thought to be restricted to these genera of subfamily Iridoideae. Our observations here, however, represent the first report of oil-secretion in subfamily Crocoideae and the first record of oil-secretion in the Old World representatives of the family.

Materials and Methods

The plant

Tritoniopsis parviflora (Jacq.) G.J. Lewis is a seasonal geophyte that is widely distributed through the western parts of the Cape Floral Region from Citrusdal in the north to Agulhas in the south (Lewis 1959). It is restricted to sandstone slopes, flowering prolifically in the season following a fire and poorly or not at all in subsequent years. The flowers are borne in a dense spike 15–40cm tall and although distichous in bud the spike twists before anthesis so that the flowers are spirally arranged on the spike axis. The leaves are produced well before the flowers and are dry and withered or more usually burnt off by the time flowering takes place. The flowers are strongly bilabiate and ca. 20mm long and have a short floral tube 4–5mm long. All the tepals are more or less clawed with the dorsal tepal largest and arching forwards, the upper lateral tepals recurving and the lower three tepals projecting downwards. The lower tepals are fused at the base for ca. 3mm to form a short palate (Figure

1). The upper tepals are uniformly lemon yellow, although the upper laterals soon flush pale brownish orange, and the lower tepals are yellow with dark purple blotches in the midline in bud but at anthesis are flushed almost completely with purplish maroon. The palate is translucent white with maroon streaks and bands and is covered with prominent glistening papillae. In some populations, including the study population, the palate is strongly pouched inwards at the insertion of the median stamen to form a small mound at the base of the filament that largely obstructs the mouth of the floral tube. The unilateral filaments and style arch beneath the dorsal (adaxial) tepal. The flowers produce a strong sweetish/acrid scent resembling the scent of several South African oil-producing orchids in the genera *Disperis* and *Pterygodium*. Flowering takes place during the dry summer months from November to January.

Study site

The observations were made in November 2000 on a large population located in the Kogelberg Biosphere Reserve near Kleinmond (34°19'7"S, 18°58'7"E). The area had been burnt one year earlier, in the summer of 1999, and hundreds of plants were in flower.

Pollinator observations

The population was observed for floral visitors on two days from 10:00 to 14:00. Vouchers are deposited in the South African Museum (SAM).

Nectar measurement

Nectar volume measurements were made from both unbagged flowers in the field and from spikes that had been picked and kept in water overnight. Nectar was withdrawn from the base of the floral tube of picked flowers with 3 µl capillary tubes after removing the ovary. This technique is suitable in Iridaceae where the potential for cell sap produced by removing the perianth to dilute the nectar is insignificant. The percentage of sucrose equivalents in fresh nectar was measured using a Bellingham & Stanley handheld refractometer (0–50%).

Oil localisation

Potential sites of oil secretion were located by visual inspection. The presence of oil was tested by placing a few crystals of Sudan IV on to any observed secretions.

Fragrance analysis

Floral fragrance was sampled onto glass capillary tubes packed with Poropack by drawing air through the tubes with a vacuum pump from glass chambers containing open flowers. Floral fragrance chemistry was analysed by R Kaiser, Givaudan-Roure Research Ltd., Switzerland, by gas chromatography using a DB-Wax Capillary column (Kaiser 1993).

Results

Floral phenology

Flowers are protandrous and markedly herkogamous (Figure 1). On day 1 tepals expand fully and the previously yellow tips of the lower tepals flush maroon. On day 2 upper lateral tepals flush pale brown, and the median filament arches downward such that the anther descends lower than the lateral anthers and dehisces. On day 3 median filament straightens such that the anther ascends to immediately below the dorsal tepal, well above the style, and the lateral anthers descend and dehisce. On day 4 lateral filaments straighten such that the anthers ascend to immediately below the dorsal tepal, the style descends and the stigmatic arms partially diverge and are then assumed to be receptive. On day 5 perianth withers and the stigmatic arms open further. In some flowers the male stage is extended for a further day and the flower life is thus prolonged to six days. The flowers remain open day and night throughout their life. The marked herkogamy prevents self-pollination.

Fragrance

Flowers have a strong, bittersweet, somewhat acrid fragrance that is released throughout the day and night. Analysis of the fragrance indicates that it is dominated by 3,5-dimethoxy toluene (65%), with co-dominants benzyl benzoate (16%), caryophyllene (7%) and methyl salicylate (5.5%). Trace concentrations of several other compounds are present.

Floral rewards

Small quantities of nectar (0.1–0.5 µl \bar{x} =0.3 µl, N=4) with a sugar concentration between 30–35% (\bar{x} =32%, N=4) accumulate in the floral tube. The nectar is secreted from septal nectaries (unpublished data) and rises to the mouth of the tube where it is accessible to insects with very short mouthparts. Visual inspection of the flowers revealed the presence of a glistening, colourless surface film over parts of the perianth. This film covered most of the outer surface of the perianth tube, and both the upper and lower surfaces of the claws of all the tepals as well as the base of the limbs of the lower lateral tepals. It was also present on the papillae in the palate and mouth of the tube, and on the lower portions of the filaments (Figure 2A–D). Tests with crystals of Sudan IV indicate that this film is an oil and the absence of glandular trichomes indicates that it is secreted by epithelial elaiophores.

Pollinator observations

No floral visitors were observed except during the period 12:30–13:15 when visits by two female individuals of the oil-collecting bee *Rediviva gigas* (Hymenoptera: Melittidae) (Figure 2E, F) were observed. The bees flew swiftly through the population, alighted on an inflorescence and proceeded to work several flowers on each of a number of plants in the immediate vicinity before moving off. Bees contacted either the anthers or stigmatic surfaces of the style branches when

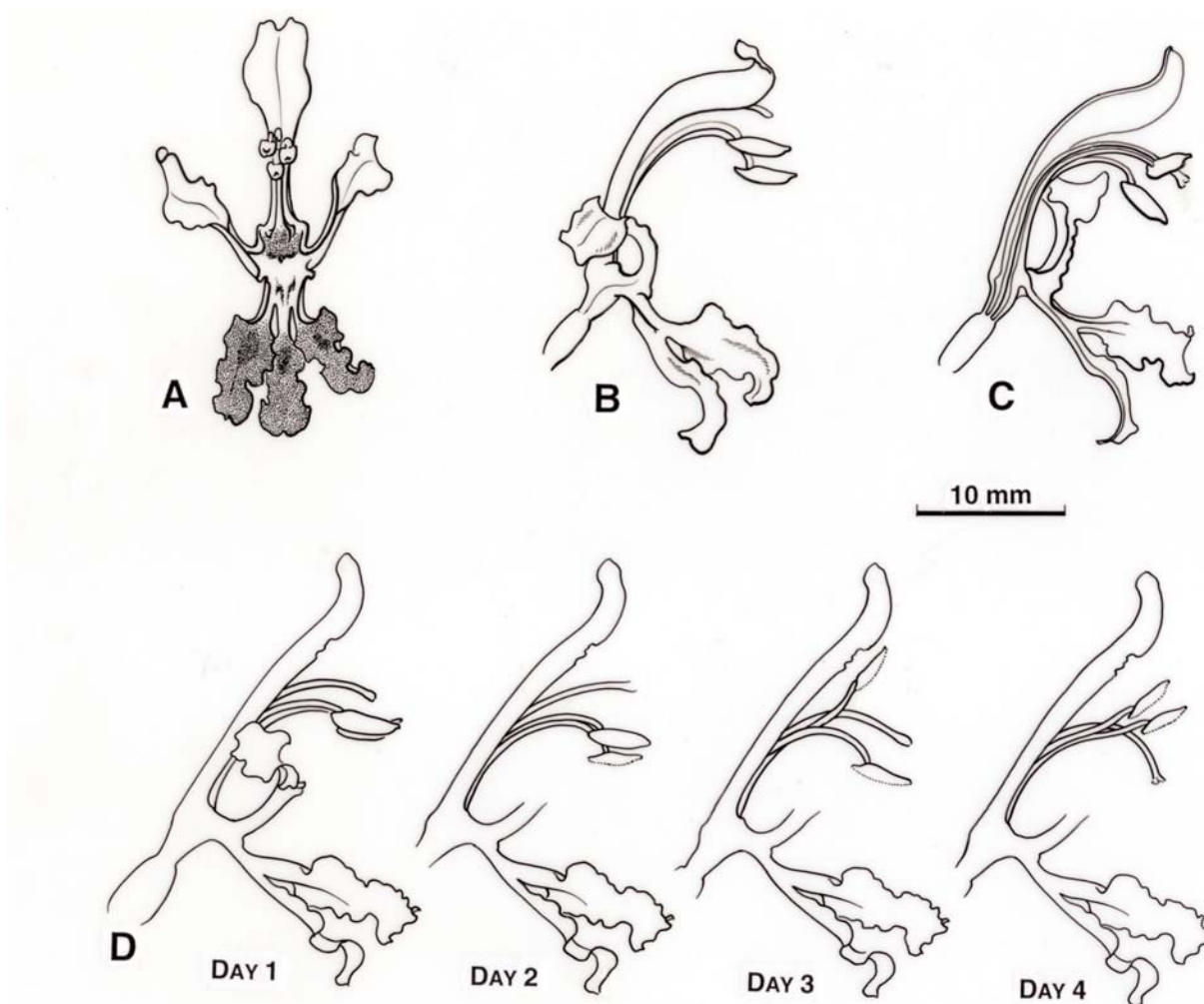


Figure 1: Floral morphology and phenology in *Tritoniopsis parviflora*; (A) front view; (B) side view; (C) half flower; (D) side view of flower on days 1, 2, 3 and 4

alighting on a flower (Figure 2E). Captured insects were examined for presence of pollen and the site of deposition was confirmed to be the anterior portion of dorsum of the thorax and the vertex of the head. One of the insects had in addition several pollinia of an orchid, probably *Pterygodium acutifolium*, attached to both midlegs.

Discussion

The Cape Region is a major centre of diversity for the family Iridaceae (Goldblatt 1991, Goldblatt and Manning 2000a). Furthermore, the Cape members of the family are characterised by a range of pollination strategies absent or only weakly evident elsewhere across its range (Goldblatt and Manning 1998). Shifts in pollination strategy among closely related species are frequent in most Cape genera of Iridaceae, including *Gladiolus*, *Ixia*, *Lapeirousia*, *Sparaxis* and *Watsonia* (Goldblatt and Manning 1998, Goldblatt *et al.* 2000). Among the pollination strategies that are unusually well represented among Cape Iridaceae are pollination by monkey beetles (Sacarabaeidae: Rutelinae: Hopliini)

(Goldblatt *et al.* 1998b), long-proboscid flies in the families Nemestrinidae and Tabanidae (Goldblatt and Manning 2000b) and the butterfly *Aeropetes tulbaghia* (Satyridae) (Johnson and Bond 1994). Pollination by oil-collecting bees in the genus *Rediviva* (Melittidae) is an important system in several Cape genera of Scrophulariaceae and Orchidaceae (Steiner and Whitehead 1988, Johnson and Steiner 1999). Until now, however, no African species of the Iridaceae has been known to secrete floral oil as a pollinator reward. The observation that *Tritoniopsis parviflora* is adapted to pollination by the oil-collecting bee *Rediviva gigas* extends the known diversity of pollination strategies in the African Iridaceae.

Rediviva gigas is a large species in the genus that is on the wing in November and December, unlike the other Cape species of *Rediviva*, which are active in August and September (Whitehead and Steiner 1993). It has been found to collect oil from several species that flower during these months. Until now these included only the terrestrial orchids *Ceratandra atrata* (L.) T. Durand & Schinz, *C. bicolor* Sond. ex Bolus, *C. harveyana* Lindl., *Evetella rubiginosa* (Sond. ex

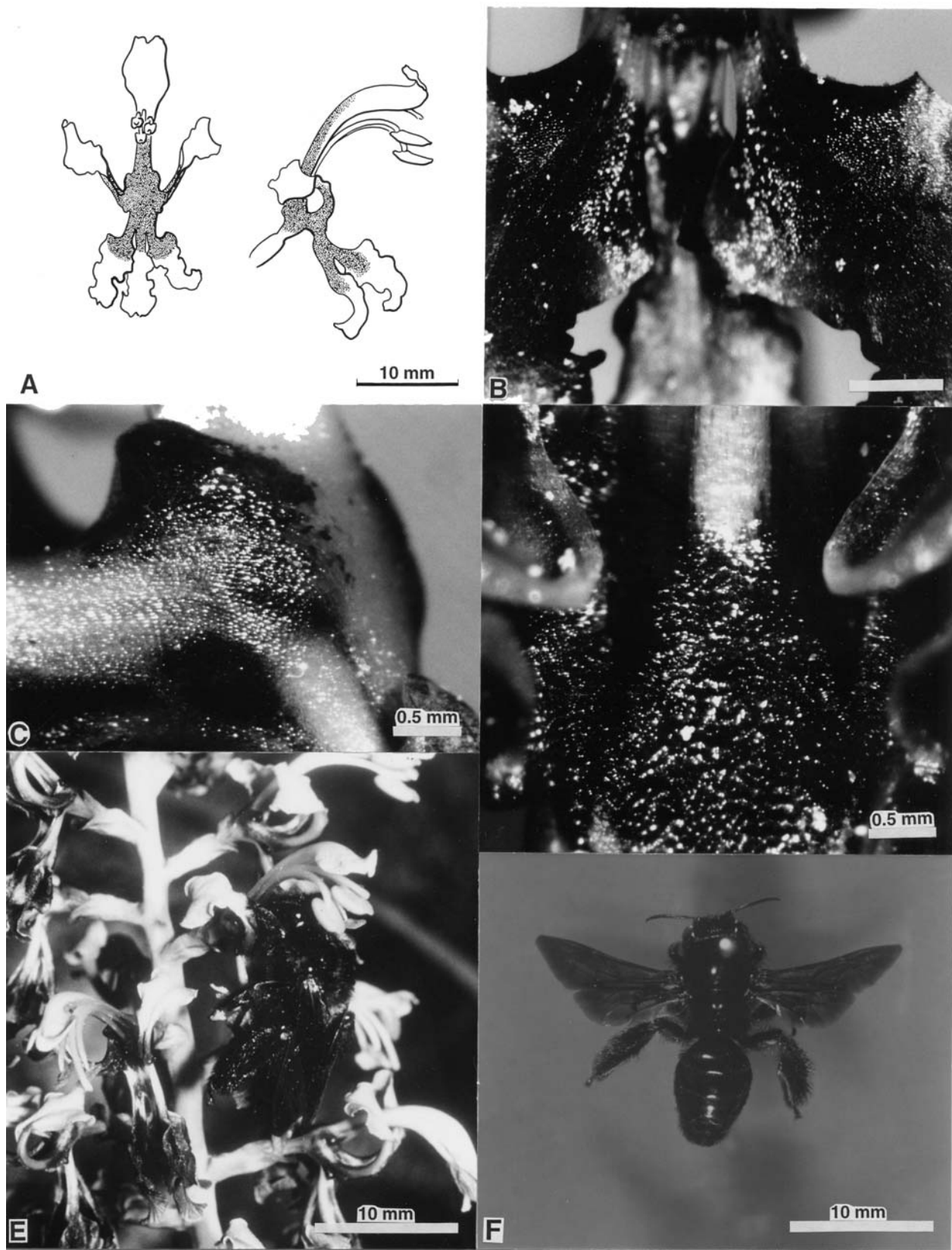


Figure 2: Oil secretion and pollination in *Tritoniopsis parviflora*; (A) sites of oil secretion; (B) oil droplets on the lower lateral tepal limbs; (C) oily film on the outer surface of the floral tube at the base of the tepals; (D) oil droplets on the papillae at the base of the median filament; (E) female of *Rediviva gigas* posed at a flower; (F) female of *Rediviva gigas*

Bolus) Kurzweil & H.P. Linder, *Pterygodium acutifolium* Lindl. and the shrub *Ixianthes retzioides* E. Mey. ex Benth. (Scrophulariaceae) (Whitehead and Steiner 1993, Steiner and Whitehead 1996). In addition, *R. gigas* is reported to collect nectar from *Moraea ramosissima* (L.f.) Druce (Iridaceae) (Whitehead and Steiner 1993). All of these species have medium-sized yellowish or rarely maroon flowers that are often strongly scented. With the exception of *Ixianthes*, flowering in all of the food plants is strongly fire-dependent, with the species only flowering in profusion in the late spring and early summer following a fire the previous summer or autumn. *Tritoniopsis parviflora* agrees with these species in all of these respects but is the only member of this guild that secretes both oil and nectar. The floral tube of *T. parviflora* is the shortest in the genus and the nectar is consequently available to insects with extremely short mouthparts, including *R. gigas*, the mouthparts of which are only 2–3mm long. The other recorded nectar plants of *R. gigas*, *Moraea ramosissima* and *M. sp.* (Whitehead pers. com.) lack a floral tube and secrete the nectar into shallow pockets or expose it directly on the surface of the tepals. Both bees captured on *T. parviflora* are female. It is assumed that floral oils are collected by female *Rediviva* bees for use in nest provisioning (Steiner and Whitehead 1988), and oil-collection is therefore restricted to nest-building females. We therefore infer that females of *R. gigas* visit the flowers of *T. parviflora* for oil but it is possible that they also consume nectar.

The dominant chemical in the fragrance suite produced by *Tritoniopsis parviflora*, 3,5-dimethoxy toluene, is also a major component of the fragrance of the oil-producing orchid *Corycium orobanchoides* (L.f.) Sw. (Kaiser pers. com.). This orchid has a somewhat wider distribution than *T. parviflora* and although it bears a superficial resemblance to it in the dense spike of yellow and brown flowers it is in flower somewhat earlier, mainly during September and October. It is pollinated by other, early-flying species of *Rediviva* (Johnson and Steiner 1999). The distinctive fragrance of *T. parviflora* is not known in other species of this genus, and related species which also have partly yellow flowers, including *T. bicolor* J.C. Manning & Goldblatt, *T. flava* J.C. Manning & Goldblatt and *T. nemorosa* (E. Mey. ex Klatt) G.J. Lewis, produce different floral scents and only nectar (unpublished data). Pollination in these species is by species of *Amegilla* (Apidae: Anthophorini) that do not collect oil (unpublished data).

Rediviva gigas occupies an isolated position within its genus and is characterised by a number of plesiomorphies that suggests that it predates the radiation of the other Cape species of *Rediviva* (Steiner and Whitehead 1996). These authors speculate that the relationship between *R. gigas* and *Ixianthes* may thus predate the evolution of the oil-secreting orchids, which comprise a guild that probably evolved alongside *Ixianthes* in order to take advantage of the pre-existing relationship between it and the late-flying *R. gigas* (Steiner and Whitehead 1996). *Tritoniopsis parviflora* is unusual in the genus in several floral features, especially flower shape, colouring, fragrance and the secretion of oil, but all of these features are part of the syndrome of the Cape oil-secreting orchids that are visited by *R. gigas*. This sug-

gests that *T. parviflora* is a relatively recent entrant into the *R. gigas* pollination-guild, capitalising on the characteristics of the Cape orchids that form part of the guild. *Tritoniopsis parviflora* has a similarly marked post-fire flowering strategy to these orchids, several of which co-occur with it at the Kogelberg site although in much lower numbers, notably *Ceratandra atrata*, *C. harveyana*, *E. rubiginosa* and *Pterygodium acutifolium*.

Tritoniopsis is a genus comprising just over 20 species (Goldblatt and Manning 2000a) nearly restricted to the Cape Region. The phylogeny and species' relationships in the genus are unknown but the great diversity of floral form evident reflects the diversity of pollination strategies present in the genus, including pollination by sunbirds, long-proboscid flies, the satyrid butterfly, *Aeropetes*, anthophorine bees, and probably night flying moths (unpublished data). The range of flower forms is similar to that encountered in several other genera of Iridaceae that have undergone a similar diversification of pollination systems in the Cape, including *Gladiolus* and *Sparaxis*. The species of *Tritoniopsis* differ from most Cape genera of Iridaceae, however, in their late flowering, with most species in flower in the summer and autumn. The specialisation of *T. parviflora* for pollination by the late-flying *R. gigas* through the unique evolution of oil-secretion is further evidence of strong selection for prezygotic isolation within the genus.

Tritoniopsis parviflora occurs throughout the known range of *R. gigas* and is probably an important source of oil for the bees because it is often common after burns. In some localities, however, particularly on the Cape Peninsula, the species may rely on visits by other insects because *R. gigas* has not been recorded there. *Tritoniopsis parviflora* does not set seed in the absence of active pollination, illustrating the potential dangers of specialist pollination systems in which the pollinator becomes locally extinct or the host plant expands beyond the range of its pollinator. Similarly, seed-set in populations of *Ixianthes* that are not visited by *R. gigas* is extremely low (Steiner and Whitehead 1996). A shift to alternative pollinators in part of the range is one evolutionary solution and such a shift from *R. gigas* to beetles in *Ceratandra bicolor* has already been recorded (Steiner 1994). Another solution may be the provision of alternative rewards. *Tritoniopsis parviflora* is unique among oil-producing flowers in providing both oil and nectar. The provision of both floral rewards may, therefore, ensure that the flowers are attractive to nectar-feeding bees other than *R. gigas*. In this way some seed set may be ensured in most populations.

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