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Reproductive biology of three co-occurring, primarily small-mammal pollinated *Protea* species (Proteaceae)

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ABSTRACT

This study investigated the role of vertebrates in the reproductive biology of a community of three co-occurring *Protea* species (*Protea canaliculata*, *Protea sulphurea* and *Protea humiflora*, Proteaceae) at Kraggashoek in the Western Cape, South Africa. Live-trapping, measures of facial and faecal pollen loads, and footage from remote cameras facing the inflorescences confirmed that all three are visited by small mammals and specialist nectarivorous birds. These plant species have bowl-shaped inflorescences that produce highly concentrated (34.1–42.9%) sucrose-rich nectar and a "yeasty" scent, typical traits of small-mammal pollinated plants. Camera footage indicated that 140 visits were made by eight small mammal species to an accumulative 19 inflorescences of the three *Protea* species. Small mammals thus made up 69% of total visits by all vertebrate species. Camera footage revealed that *Micaelamys namaquensis* visited flowers more frequently than any other vertebrate visitor (41% of total visitations by birds and mammals) but *Elephantulus edwardii* (10% of total visitations by birds and mammals) spent the longest time foraging on inflorescences (~28 s per inflorescence). The 'trap shy' species, *Graphiurus ocularis*, was observed for the first time on camera, visiting *Protea humiflora* and *Protea sulphurea* inflorescences. *Galerella pulverulenta* (Cape gray mongoose) also visited inflorescences of *P. canaliculata*. The remaining 31% of total vertebrate visits, primarily to *Protea humiflora*, were by three species of specialist nectarivorous birds (the Cape sugarbird *Promerops cafer*, and the shorter-billed sunbirds, *Anthobaphes violacea* and *Cinnyris chalybeus*). Breeding system experiments with *Protea sulphurea* revealed that pollinators are needed for seed set, although exclusion of vertebrate pollinators only marginally lowered seed set of this partially self-incompatible species. These three *Protea* species had asynchronous flowering periods, supporting a wide range and relative abundance (overall live mammal trapping success rate of 22.7% nocturnal and 5.7% diurnal species) of vertebrate pollinators over a three month period in winter. That these *Protea* species co-occur suggests there is a community of vertebrate flower visitor species and primarily small mammal-pollinated plant species. The partitioning of nectar resources over a long period has conservation implications for both the plants and animals.

1. Introduction

The sedentary nature of plants promotes the need for external pollination agents to transfer pollen to stigma. Abiotic agents include wind and water and biotic agents include insects, birds and mammals. The pollination syndrome concept suggests that convergent floral evolution in angiosperms is driven by adaptation to the most effective agent of pollination where floral traits tend to converge for plant species that share the same pollinator guild (Fenster et al., 2004; Rosas-Guerrero et al., 2014). *Protea* species (family Proteaceae) that bear large, terminal, red inflorescences with long pollen presenters are typically suited to bird pollinators such as sunbirds and sugarbirds, while species that produce geoflorous inflorescences with wiry, flexible styles, a shorter nectar presentation to stigma distance and a yeasty scent are usually visited by small mammal pollinators (Rourke and Wiens, 1977; Wiens et al., 1983; Rebelo et al., 1984; Collins and Rebelo, 1987). The floral traits, however, of primarily small mammal pollinated (SMP) *Protea* species do not preclude visits by other pollinator guilds, such as sunbirds and insects, that will opportunistically visit inflorescences to exploit nectar and pollen rewards (e.g. sunbirds visiting the geoflorous *Protea scabra*; Zoeller et al., 2017).

Apart from Wiens et al.'s (1983) initial review, most SMP *Protea* studies have focussed on a single species at a site at any one time (e.g. *Protea foliosa* and *Protea nana*; Biccard and Midgley, 2009; Melidonis and Peter, 2015). Our study aimed to describe the reproductive biology of three co-occurring, winter-flowering *Protea* species that conform to an SMP syndrome (*Protea canaliculata* Andrews, *Protea humiflora* Andrews and *Protea sulphurea* Phillips). We propose that

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sequential flowering has maintained the co-existence of these *Protea* species at the study site and that the plants provide an important source of sugar to a community of vertebrate floral visitors during cold, wet winter periods. Overall we determined 1) which animal species were visiting these plants; 2) the characteristics of floral rewards; 3) whether there was a separation in the flowering time of the SMP *Protea* species at this site; and lastly, 4) the contribution of vertebrates to plant reproduction in *P. sulphurea* using pollinator exclusion experiments.

2. Materials and methods

2.1. Study site and species

Apart from nectar measurements for two species sampled elsewhere for comparison, this study was conducted from May to December 2013 on Kraggashoek farm (33° 20′ 13″ S, 20° 23′ 35″ E) in the northern central part of the Western Cape bordering the central Karoo. This is a high-lying region with an elevation of 1175–1200 m above sea level, experiencing hot, dry summers and very cold (frequently falling below freezing to −2 °C at night in June–July), wet winters (estimated range of 200–500 mm mean annual precipitation, as inferred from data for the nearby Anysberg Nature Reserve; BirdLife, 2015). This site is one of a few patches of *Protea*-dominated fynbos in a Renosterbos (*Elytropappus*)-dominated landscape. This site was chosen as it has two putative SMP *Protea* species that have been poorly studied (*Protea canaliculata* and *Protea sulphurea*; Fig. 1). The SMP species, *P. humiflora*, and bird pollinated *P. laurifolia* were also present in the community (Fig. 1). Representative specimens of the main study species have been submitted to the Bews Herbarium at the University of KwaZulu-Natal. Unless otherwise stated, data for determining floral traits and visitors for the three ground/shrub proteas were collected at Kraggashoek. Available data on nectar (volume and sugar concentration) for *P. humiflora* were included for a population from Jonaskop (Nicolson and Fleming, 2003; Balmer, 2013), and for *P. laurifolia* from a population from Drie Kuilen Private Nature Reserve (standing crop and a few morphological measurements conducted in August 2014; −33°35′52.0″ S 20°02′59.2″ E, 991 m a.s.l.). The effect of excluding visitors to inflorescences and of hand pollinations on seed set was also investigated for *P. sulphurea* at Kraggashoek.

As it has been hypothesised that successful small mammal pollination is inversely related with plant height (Nicolson and Fleming, 2003, but see Biccard and Midgley, 2009), plant height was measured and averaged for 25 randomly selected individuals of each of the *P. sulphurea* and *P. canaliculata* populations, and 10 individuals of the *P. humiflora* population. Floral morphological traits including inflorescence traits (length, diameter, the number of florets contained in each inflorescence) and individual floret traits that mediate pollinator interactions (floret length, style length, pollen presenter length, and the distance between: nectar presentation to nectar production; nectar presentation to the stigma; and nectar presentation to the base of the floret) were measured according to methods described by Steenhuisen and Johnson (2012a; see labelled photograph within), and compared across species (N = 5 inflorescences for *P. canaliculata*; N = 6 for *P. sulphurea*; N = 10 for *P. humiflora*; N = 12 for *P. laurifolia*) to investigate differences in morphology of small mammal and bird pollinated *Protea* species (Supplementary Table 1). Due to

Fig. 1. Gross morphology of inflorescences of a) *Protea canaliculata*, b) *Protea sulphurea*, c) *Protea humiflora*, d) *Protea sulphurea x canaliculata*, and e) *Protea laurifolia*. Still images from video footage are shown of f) *Rhabdomys pumilio* visiting *Protea sulphurea*, g) *Elephantulus edwardii* visiting *Protea canaliculata*, h) *Graphiurus ocularis* visiting *Protea humiflora* and i) *Anthobaphes violacea* visiting *Protea humiflora*. Scale bar = 2 cm.