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Research article

Reward quality predicts effects of bird-pollinators on the reproduction of African *Protea* shrubs

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ABSTRACT

Pollination syndromes predict the principal animal pollinators of plant species from floral traits. However, the existence of discrete pollination syndromes and the relevance of these syndromes for the effects of animal pollinators on plant reproduction are controversial and a quantitative, trait-based approach may refine the concept of pollination syndromes. Thus far, however, very few studies have simultaneously analysed the importance of signalling, accessibility and reward traits of flowers for the effects of animal pollinators on plant reproduction of multiple species. The genus *Protea* is an ideal study system because the members of this genus exhibit remarkable variation in functional traits and *Protea* species are visited by several guilds of animal pollinators, such as birds and insects. We experimentally investigated seven *Protea* species from the South African fynbos to assess variability in reproduction (seed set, seed mass, germination) in response to pollinator exclusion (full-exclusion, bird-exclusion) and hand-pollination (self-pollen, cross-pollen). To generalise the findings of the experiment, we conducted a meta-analysis of the effects of bird-exclusion on seed set of 14 *Protea* species, associating effect sizes of bird-pollination with signalling (inflorescence length and diameter), accessibility (inflorescence and style length) and reward traits (length of pollen presenter, nectar volume and sugar concentration). All *Protea* species were self-compatible, but only two species were to a small degree capable of autonomous selfing. *Protea* species varied in their dependence on animal pollinators, including species depending exclusively on birds, insects, or both for seed production. *Protea* species exhibited continuous variation in the effects of bird-pollinators on reproduction, challenging the accuracy of categorical pollination syndromes for predicting pollinator effects on plant reproduction. The meta-analysis showed that the quality of floral rewards, especially nectar sugar concentration, predicts the dependence of *Protea* reproduction on bird pollinators, whereas the signalling and accessibility traits tested in this study were not important. Beyond revealing new insights into the pollination biology of *Protea* species, our findings underscore the usefulness of experimental approaches on multiple plant species to test the effects of pollinator guilds on plant reproduction and contribute to a trait-based understanding of the functional determinants of plant–pollinator interactions.

Introduction

Many plant species have evolved floral traits adapted to specific groups of animal pollinators (Faegri and Van der Pijl, 1966; Schiestl and Johnson, 2013). Suites of floral traits might thus enable the prediction of guilds of animal pollinators that are associated

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with particular plant species, as formulated by the pollination syndrome hypothesis (Faegri and Van der Pijl, 1966). Even though the pollination syndrome hypothesis has received much support over the past decades (Fenster et al., 2004; Johnson, 2013; Rosas-Guerrero et al., 2014), the accuracy of this hypothesis for predicting pollinator effects on plant reproduction is controversial (Johnson and Steiner, 2000; Ollerton et al., 2009). While the pollination syndrome hypothesis proposes a qualitative and nomenclatural approach to classify plant–pollinator interactions, a quantitative, trait-based approach that accounts for the phylogenetic relatedness among plant species may help to refine and revitalise the classical concept of pollination syndromes (Rosas-Guerrero et al., 2014).

Because flower visitors greatly vary in their efficiency to pollinate (King et al., 2013), the pollination syndrome hypothesis proposes that flowers should adapt their traits to the most frequent and efficient pollinators (Stebbins, 1970) that contribute most to plant reproduction. Rather than quantifying the number of floral visits, it is therefore crucial to quantify pollinator effects on plant reproduction, notably on seed set and seed quality (King et al., 2013). In cases where pollinator guilds differ in body size or activity periods, their effects on plant reproduction can be estimated experimentally by selective exclusion (Kearns and Inouye, 1993). Exclusion experiments on multiple plant species with differences in functional traits, thus, allows to test which traits mediate the effects of particular pollinators on plant reproduction. However, studies with exclusion experiments on multiple species have mostly been qualitative or have only used categorical measurements of traits (Hargreaves et al., 2012; Lange et al., 2000; Liu and Huang, 2013; Steenhuisen et al., 2012b). It is therefore timely to quantify the relationship between continuous floral traits and pollinator effects on plant reproduction, i.e. seed set, seed mass and germination, across an array of related species with diverse floral traits.

Pollination biologists face the challenge to identify plant traits that mediate the effects of animal pollinators on plant reproduction. For instance, the pollination syndrome hypothesis predicts that plant species that exhibit vivid floral displays, emit little scent and contain large quantities of diluted nectar are primarily bird-pollinated (Brown et al., 2011; Cronk and Ojeda, 2008; Johnson and Nicolson, 2008), whereas flowers that produce easily accessible, odour-rich nectar and generous pollen rewards are primarily insect-pollinated (e.g. Steenhuisen and Johnson, 2012a). The attraction of particular pollinators and the exclusion of others occur in two stages that are mediated by different sets of plant traits. The first array of traits is associated with the approach of pollinators to floral resources. These traits include *signalling traits* to attract animal pollinators, like the size of floral display (Nattero et al., 2011), colour (Hopkins and Rausher, 2012), and scent (Wright and Schiestl, 2009). This array of traits also includes morphological *accessibility traits* that determine the ease of pollinator access to the floral resources, such as the depth to the nectaries (Castellanos et al., 2004; Stang et al., 2009; Thompson et al., 2013). The second array of traits relates to plant rewards for animal pollinators. *Reward traits*, especially pollen and nectar, positively influence visitation rates and duration of pollinator visits (Cresswell, 1999; Kudo and Harder, 2005; Leiss and Klinkhamer, 2005), increase pollen deposition by pollinators and may ultimately increase plant reproduction (Bolstad et al., 2010; Hodges, 1995; Thomson and Plowright, 1980).

In this study, we investigated the importance of animal pollination (birds and insects) in *Protea* species (Family Proteaceae) and experimentally tested the association between floral traits and the effects of avian pollinators on plant reproduction. In the Cape Floristic Region in southern Africa, the genus *Protea* includes many ecologically and economically important species (Schurr et al., 2012). It is an ideal study system because *Protea* species exhibit remarkable variation in floral traits and are visited by different guilds of animal pollinators, such as birds (Hargreaves et al., 2004; Wright et al., 1991), insects (Coetzee and Giliomee, 1985; Johnson et al., 2012; Steenhuisen et al., 2012b) and mammals (Biccard and Midgley, 2009; Wiens and Rourke, 1978). Nectarivorous birds are frequent visitors on tall-growing *Protea* shrubs producing large, scent-less inflorescences offering diluted nectar (Collins and Rebelo, 1987; Hargreaves et al., 2004; Wiens et al., 1983). While birds are generally considered to be the main pollinators of these species (Steenhuisen et al., 2012a), some *Protea* species produced seeds when bird-pollinators were excluded from inflorescences (Coetzee and Giliomee, 1985; Wright et al., 1991), questioning the general importance of bird pollinators for *Protea* reproduction (Steenhuisen and Johnson, 2012a, 2012b).

In order to identify floral traits that drive the effect of bird pollinators on *Protea* reproduction, we selectively excluded bird-pollinators from inflorescences of seven *Protea* species and quantified effects of bird pollinators on plant reproduction (seed set, seed mass, germination). Additional self- and outcross-treatments served to measure how plant reproduction depends on pollen transfer by animal pollinators. To generalise our findings, we conducted a meta-analysis comprising a total of 14 *Protea* species to test whether the effect of bird-pollinators on reproduction is primarily associated with signalling, accessibility or reward traits.

Methods

Study system

The genus *Protea* has a centre of diversity in the Cape Floristic Region (CFR) in South Africa. We selected seven overstorey *Protea* species present in the CFR (*P. laurifolia*, *P. magnifica*, *P. eximia*, *P. compacta*, *P. repens*, *P. punctata*, *P. longifolia*). All species are thus far assumed to be primarily bird-pollinated (Steenhuisen et al., 2012a), but require a reassessment of their breeding system (see Steenhuisen and Johnson, 2012b and references therein). Flowers of *Protea* species are grouped in large inflorescences surrounded by involucral bracts (Rebelo, 2001). Flowers are proteandrous: when the anthers dehisce, pollen is deposited on the upper part of the stigma (the area designated as pollen presenter) and the stigma tip becomes receptive to pollen after two to three days (Steenhuisen and Johnson, 2012b). Within an inflorescence, flowers open sequentially from the outer to the inner circles and thus flowers in male phase (pollen donor) and female phase (receptive to pollen) co-occur within an inflorescence. The abundant nectar produced often form a pool of nectar at the bottom of the inflorescence (Steenhuisen and Johnson, 2012a). Each flower can develop a single hard-coated seed and the presence of endosperm distinguishes fertilised from unfertilised seeds.

Our study area extended from Bainskloof Pass (33°37′ S, 19°06′ E) in the north-west to Gansbaai (34°36′ S, 19°30′ E) in the south-east of the Western Cape region of South Africa. The multispecies experiments were conducted from May to September 2011 on 15 study sites with different *Protea* communities. During the flowering season, we mostly observed two pollinating bird species on the study sites: Cape sugarbird (*Promerops cafer*) and orange-breasted sunbird (*Anthobaphes violacea*). In addition to nectarivorous birds (Rebelo, 1987; Skead, 1967), several insect guilds have been reported to visit or reside in inflorescences of the studied *Protea* species, including bees (e.g. Cape honey bee *Apis mellifera capensis*), butterflies (e.g. mountain pride butterfly *Aeropetes tulbaghia*), flies (e.g. nemestrinid fly *Prosoeca longipennis*), ants (Collins