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Do floral syndromes predict specialization in plant pollination systems? An experimental test in an “ornithophilous” African *Protea*

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Abstract We investigated whether the “ornithophilous” floral syndrome exhibited in an African sugarbush, *Protea roupelliae* (Proteaceae), reflects ecological specialization for bird-pollination. A breeding system experiment established that the species is self-compatible, but dependent on visits by pollinators for seed set. The cup-shaped inflorescences were visited by a wide range of insect and bird species; however inflorescences from which birds, but not insects, were excluded by wire cages set few seeds relative to open-pollinated controls. One species, the malachite sunbird (*Nectarinia famosa*), accounted for more than 80% of all birds captured in *P. roupelliae* stands and carried the largest protea pollen loads. A single visit by this sunbird species was enough to increase seed set considerably over unvisited, bagged inflorescences. Our results show that *P. roupelliae* is largely dependent on birds for pollination, and thus confirm the utility of floral syndromes for generating hypotheses about the ecology of pollination systems.

Keywords Floral syndrome · Ornithophily · Pollen-limitation · Pollination efficiency · Proteaceae

Introduction

The remarkable diversity of angiosperm flowers has been attributed largely to selective forces imposed on plants by their pollinators (Stebbins 1970). Animal-pollinated flowers are generally believed to possess convergent suites of features including colour, scent and structure, which reflect the foraging preferences and morphology of their

pollinators (Faegri and van der Pijl 1979). It is widely accepted that these “pollination syndromes” arise from specialization by plants for pollination by particular animals, possibly driven, inter alia, by selective pressures for efficient transfer of pollen between conspecific flowers (Stebbins 1970; Keeton and Gould 1993).

Both the reliability of pollination syndromes in predicting primary pollinators and the assumption of specialization in pollination systems have been questioned recently (Waser et al. 1996; Ollerton 1998; Hingston and McQuillan 2000; Johnson and Steiner 2000). Many pollination systems are more generalized than previously thought (Waser et al. 1996) and in some cases the primary pollinators predicted by pollination syndromes play only minor roles in the reproductive success of the plant (cf. Coetzee and Giliomee 1985). The relative importance of various flower visitors as pollinators depends not only on the frequency of their visits, but also on their ability to transfer pollen successfully between flowers of the same species (Carthew 1993). This, in turn, depends on several factors, including the amount of pollen picked up and carried by the pollinator and the composition of the pollen load, as this may reflect the likelihood that stigmas will become clogged with pollen from other plant species. The level of outcross-pollen provided may also be an important qualitative difference between pollinators (Wright 1994; Mayfield et al. 2001).

The suite of floral adaptations to bird pollinators, widely referred to as “ornithophily” (cf. Faegri and van der Pijl 1979), has traditionally been viewed as one of the most clear-cut of the floral syndromes. However, a number of studies have shown that the ecological importance of birds as pollinators of a plant species is not necessarily predicted by its floral traits (Coetzee and Giliomee 1985; Wright et al. 1991; Ollerton 1998; Mayfield et al. 2001; Muchhala 2003; Wolff et al. 2003).

Protea is an ideal genus for testing exclusiveness in pollination systems, as species have evolved to use a diverse array of pollinators, including beetles, small mammals and birds (Collins and Rebelo 1987; Wright et al. 1991). *Protea* species with large, red-coloured terminal

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inflorescences, long pollen presenters and copious amounts of nectar are often assumed to be primarily or exclusively bird-pollinated (Rebello et al. 1984; Vogts 1984). However, studies have shown that insects play a larger than expected role in pollination of various “ornithophilous” species such as *P. repens* (Coetzee and Giliomee 1985), *P. cynaroides* and *P. nitida* (Wright et al. 1991).

Protea roupelliae Meisn, is one of the most common *Protea* species in eastern South Africa, and exhibits several features associated with ornithophily, including dark pink flower bracts, diurnal flowering, lack of scent, and large quantities of dilute nectar (Faegri and van der Pijl 1979; Beard 1993). Explosive flower opening, characteristic of many *Protea* species, may also be an adaptation to maximize pollen removal by birds, which may have lower removal but higher deposition efficiency than insect pollinators (Thompson et al. 2000). Despite the species’ wide range and abundance, the reproductive ecology of *P. roupelliae* has been virtually unstudied. The assumption that it is bird pollinated has been based solely on floral traits and observations of frequent visits by nectarivorous birds, namely malachite sunbirds (*Nectarinia famosa*) and Gurney’s sugarbirds (*Promerops gurneyi*) (De Swardt 1993). However, inflorescences of *P. roupelliae* are also visited by a wide array of insects, including two beetle species, *Atrichelaphinus tigrina* and *Trichostetha fascicularis* (Scarabaeidae: Cetoniinae), that pollinate several other *Protea* species (S.L. Steenhuisen and S.D. Johnson, unpublished data) as well as insectivorous birds, which may act as incidental pollinators.

In this study we sought to determine the importance of bird pollinators to the reproduction of *P. roupelliae*. Specifically, we investigated (1) whether *P. roupelliae* depends on animal vectors for seed production, (2) whether exclusion of birds would affect seed production, and (3) which bird species are the most important pollinators.

Materials and methods

Study site and species

This study was carried out in a grassland *Protea* community on Mt Gilboa (29°19’S, 30°17’E, 1,700 m), in the Karkloof mountain range of eastern South Africa. Three *Protea* species (*P. roupelliae*, *P. caffra*, and *P. simplex*), *Aloe boylei* (Asphodelaceae) and a wide array of other flowering plants are present at this site. *Protea roupelliae* ssp. *roupelliae* occurs in fairly diverse habitats throughout eastern South Africa (Rourke 1980). The species grows as a small tree 3–5 m in height, with flowers arranged in cup-shaped terminal inflorescences (Beard 1993). Flowers are protandrous, opening from the inflorescence edge inward. In the male phase pollen is presented on a terminal stigmatic pollen presenter, after which flowers become receptive to pollen deposited in or near a terminal stigmatic groove.

Breeding system

Breeding system experiments were used to establish the compatibility system and dependency of *P. roupelliae* on animal vectors for seed production. Three inflorescence buds on each of 12 *P. roupelliae* trees were enclosed in mesh bags placed around wire frames to exclude all pollinators. Once flowers had begun to open, pollen presenters of either another *P. roupelliae* tree (out-crossed treatment) or a different inflorescence from the same tree (self-pollinated treatment) were rubbed against the stigmatic grooves of open flowers in the study inflorescence. The third bagged inflorescence was left unpollinated as a control to test for autogamy (autonomous self-pollination). Hand pollination was repeated at least three times for each inflorescence. Inflorescences were collected after the end of flowering to determine seed set.

Pollinator effectiveness

To evaluate the relative importance of bird and insect pollinators, bird visitors were excluded from some inflorescences using wire mesh cages painted matte-green to blend in with background vegetation. A single replication consisted of four treatments on five inflorescences: one large-mesh cage (30 mm diameter) to exclude only birds, one small-mesh cage (15 mm diameter) to exclude birds and possibly a species of large beetle, *Trichostetha fascicularis* (Cetoniinae), two uncaged controls to estimate natural pollination levels, and one hand-pollinated inflorescence to establish whether seed production in control inflorescences was pollen-limited. Hand pollination was conducted using a pollen-bearing stigma from another *P. roupelliae* tree collected that day to ensure fresh pollen and high pollen viability (Collins and Rebello 1987). Thirty replications were carried out on 29 randomly selected *P. roupelliae* trees, from December 2001 to April 2002. To control for variation in environmental and genetic factors between trees, each replication was carried out on a single tree in all but one instance where a set of controls were placed on an adjacent tree due to lack of inflorescences, and two cases where two replicates were placed on a single tree. These deviations from the experimental design did not have any significant effect on the overall results. To verify that cages did not impede insect visitors, insects were surveyed on each inflorescence throughout the flowering season. Insects were categorized as large beetles (*Trichostetha fascicularis*), medium-sized beetles (*Atrichelaphinus tigrina*), honeybees (*Apis mellifera adansonii*), ants and ‘all’ insects. Total numbers of insects in each category over the entire flowering time were then compared between treatments.

To examine the effectiveness of malachite sunbirds as pollinators, we tested whether a single visit from one of these birds was enough to effect seed set in *P. roupelliae*. Fine-mesh bags supported by simple wire frames were placed around 12 inflorescence buds on a single *P. roupelliae* tree to exclude all pollinators. Once anthesis had occurred, each inflorescence was unbagged and observed until it had received a single visit by a sunbird, and then rebagged for the rest of the flowering period. Sunbirds were rarely seen visiting inflorescences after 2/3 of the flowers had opened, so inflorescences were exposed when 1/3 to 1/2 of the flowers were open, at which point there were flowers in both male and female stages. Of the 12 inflorescences, 7 were visited by male malachite sunbirds, 4 were never exposed and 1 was exposed without being visited. Seed set in unvisited inflorescences was compared to that in sunbird-visited inflorescences, while the inflorescence that had been unbagged but not visited was excluded from the analysis.

Floral visitors and their pollen loads

To better evaluate the pollinating roles of specific bird species, the quantity and composition of the pollen loads carried by birds on Mt Gilboa were assessed and used to indicate their effectiveness as *Protea* pollen vectors. Birds were caught using mist-nets set up in *P.*

roupelliae stands on 7 days from 3 December 2001 to 9 February 2002. Pollen was collected from the heads and bills of each bird using fuchsin-stained gel, which was then melted onto microscope slides in the field to produce permanent slides (Beattie 1971). The pollen grains on each slide were identified to genus using reference samples and counted to estimate the pollen load. For sunbirds and sugarbirds pollen was collected separately from the forehead, throat, and bill, to test for spatial segregation of pollen originating from different plant species. Such segregation can reduce pollen loss to flowers of other species (Armbruster et al. 1994). Only *Protea* and *Aloe* pollen could be accurately identified when compared with reference samples, so pollen grains were categorised as 'protea', 'aloe', or 'other'. It was not possible to distinguish between pollen from the three *Protea* species on Mt Gilboa. Both *P. roupelliae* and *P. caffra* were flowering in December and were visited by malachite sunbirds, so pollen samples from this month were not used in determining *P. roupelliae* pollen loads and partitioning of protea pollen. Aside from a few very late inflorescences, *P. caffra* had finished flowering by the January sampling, and had finished entirely by February. While *P. simplex* was flowering in January, malachite sunbirds were almost never seen visiting this species on Mt Gilboa (S.L. Steenhuisen and S.D. Johnson, unpublished data). We thus assumed that *Protea* pollen collected from birds in January and February was almost entirely from *P. roupelliae*. As there was only one species of aloe blooming on Mt Gilboa, all aloe pollen could be identified as that of *Aloe boylei*, and pollen counts from all 3 months were included in analyses.

Observations of the foraging behaviour of malachite sunbirds at *P. roupelliae* inflorescences were made over 13 h in January and February 2002. Only instances where the sunbird made at least one probe at an inflorescence were considered visits. For each observed visit we counted the number of probes and timed the visit duration. To evaluate the level of outcrossing sunbirds might effect, we categorized the destination of sunbirds after each feeding event as either an inflorescence on the same tree, an inflorescence on a different *P. roupelliae* tree, or departure from the stand (i.e. flying out of sight).

Estimating seed production

Seed-set was used to estimate female pollination success in the breeding system, exclusion and single visit experiments. Each *P. roupelliae* flower has a single ovule and can thus set only one seed. Seeds in trial inflorescences could be detected with confidence 7 weeks after flowering was completed, so inflorescences were collected after this time and stored in mesh bags at room temperature. Within 2 days of collection we removed flowers from half the inflorescence and counted the number of ovaries with filled seeds. In older inflorescences seeds could be detected either by colour, as unsuccessful ovaries turned dark pink, or by feel, as ovaries with seeds were firm and plump while those without were shrivelled and hard or hollow-feeling. Where there was any doubt, ovaries were dissected under a light microscope to find embryos. We also attempted to quantify pollen germination and pollen tube growth using UV fluorescence microscopy (Fuss and Sedgley 1991; Van der Walt and Littlejohn 1996), but found that the style and

stigma were too woody for visualization of pollen tubes, even after extensive softening in acid.

Statistical analysis

All analyses were conducted using STATISTICA (StatSoft 1999), with the exception of the non-parametric Dunn multiple comparison test (Dunn 1964; Hollander and Wolfe 1973), which was computed according to Zar (1999). Due to the large number of inflorescences that set no seed in treatments from which birds were excluded, distributions of percent seed set were highly skewed even after various transformations, therefore nonparametric statistics were used for all analyses in these experiments.

Pollen load data were not normally distributed and were analysed using non-parametric statistics. To determine whether male and female malachite sunbirds differed in the quantity of pollen they carried, a Kruskal-Wallis test was conducted for each pollen type, with sex as the factor. To test for partitioning of aloe and protea pollen, pollen loads on the crowns, throats and bills of malachite sunbirds were ranked from smallest to largest. The resulting rank values were normally distributed, and were analysed using a randomised block design ANOVA with the individual bird as the blocking factor and location as the main factor. This was done separately for aloe and protea pollen. There were not enough Gurney's sugarbirds trapped to conduct similar tests for this species.

Results

Breeding system experiments

Control inflorescences that were bagged and not hand-pollinated throughout their flowering period set significantly fewer seeds than bagged hand-pollinated inflorescences (Table 1). However, there was no significant difference in seed set between bagged inflorescences that received self-pollen and those that received outcross-pollen (Table 1).

Pollinator effectiveness

Seed set in *Protea roupelliae* inflorescences was low, never exceeding 60% of florets, but varied significantly among treatments (Kruskal-Wallis test, $H_{3, 144}=51.77$, $P < 0.0001$; Fig. 1). Inflorescences from which birds were excluded set significantly fewer seeds than uncaged controls ($P < 0.001$), while seed set did not increase with pollen supplementation ($P > 0.5$). Inflorescences in large-mesh cages set slightly more seeds than those in small-

significant differences (Kruskal-Wallis test, Dunn's multiple comparison test, $\alpha=0.05$). Mean and 95% confidence intervals are given for comparison to literature values

Table 1 Seed set in bagged inflorescences that were either hand-pollinated (outcrossed or selfed) or left as a control to test for autogamy. Percent seed set refers to the percentage of flowers within the inflorescence that set seed. Dissimilar lower case letters indicate

Treatment	Percent of inflorescences that set seed	Percent seed set median (range)	Percent seed set mean (95% CI)	Number of inflorescences
Cross-pollinated	80.0	8.89 ^{ab} (0–26.79)	10.31 (3.38–17.24)	10
Self-pollinated	100.0	15.70 ^a (3.70–29.63)	17.34 (12.83–21.85)	12
Unmanipulated	54.5	1.59 ^b (0–9.09)	1.67 (0–3.34)	11

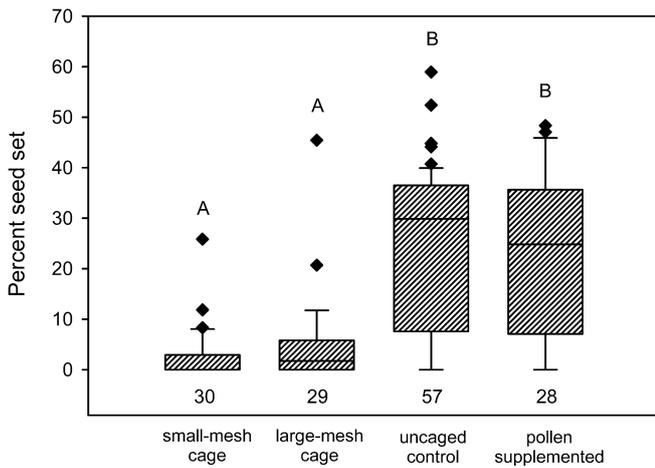


Fig. 1 Seed set in *Protea roupelliae* inflorescences on Mt Gilboa, South Africa, after bird exclusion and pollen-supplementation. Boxes show median, 25% and 75% quartiles, whiskers show 10th and 95th percentiles, and diamonds depict outliers. Differing letters above the boxes indicate significant differences between treatments (Kruskal-Wallis test, Dunn's test, $\alpha=0.05$). Numbers below boxes indicate sample sizes (number of inflorescences)

mesh cages, but the difference was not significant ($P > 0.05$). Three quarters of caged inflorescences had seed sets of 5% or less, while more than two-thirds of uncaged control inflorescences had seed sets of more than 5%.

Inflorescences visited once by a sunbird set significantly more seed (median = 5.7%, range = 1.0–11.7%, $n = 7$) than unvisited inflorescences (median = 1%, range = 0–9.5%, $n = 4$, Mann-Whitney $U = 1.00$, $P = 0.014$).

Floral visitors and pollen loads

Male malachite sunbirds were observed feeding at *P. roupelliae* inflorescences much more frequently ($n = 65$) than females ($n = 5$). Average visit duration was 49.2 s (SD = 43.8, $n = 68$), while the mean number of probes per visit was 15.5 (SD = 12.8, $n = 72$). After feeding at a *P. roupelliae* inflorescence, malachite sunbirds visited another inflorescence on the same tree in 25.9% of observed cases ($n = 54$), an inflorescence on a different *P. roupelliae*

tree in 38.9% of cases, and flew out of sight directly after feeding in 35.2% of cases. No other bird species were seen feeding at *P. roupelliae* trees during these observation periods.

The most common insect visitors to *P. roupelliae* inflorescences were ants and honeybees (Table 2). The large beetle *Trichostetha fascicularis* was seen less frequently. Caged treatments had significantly more insect visitors overall than uncaged inflorescences, although there were significantly more large beetles in open treatments. *T. fascicularis* beetles regularly stayed on an inflorescence for several hours and possibly days, often joined by a second and sometimes a third beetle. They were seen prying open unopened flowers, and appeared to be feeding on the pollen.

A total of 62 birds representing 11 species were captured from December to February on Mt Gilboa (41 birds from 6 species if December data are excluded; Table 3). Malachite sunbirds and Gurney's sugarbirds were the only nectarivores seen or caught on Mt Gilboa, with malachite sunbirds comprising >80% of all captures. More than 95% of pollen grains collected from birds were from either proteas or aloes. All birds caught carried some protea pollen, and the median and highest nectarivore pollen loads outnumbered those found on insectivores by one and three orders of magnitude respectively. Both sunbirds and sugarbirds fed at *P. roupelliae* inflorescences, while sunbirds fed at *P. caffra* and *Aloe boyei* inflorescences as well.

There was no significant difference in protea pollen loads carried by male and female Malachite Sunbirds (Mann-Whitney $U = 108.5$, $P = 0.654$, $n_{\text{male}} = 20$, $n_{\text{female}} = 12$), though females did carry significantly more aloe pollen than males (Mann-Whitney $U = 165.0$, $P = 0.0239$, $n_{\text{male}} = 29$, $n_{\text{female}} = 20$; Fig. 2). The number of pollen grains from other plant species carried by sunbirds was very low, and did not differ between males (median = 1, $n = 29$) and females (median = 1, $n = 20$; Mann-Whitney $U = 248.0$, $P = 0.636$). Sunbird sample size is lower for protea pollen as only birds caught in January and February are included, since protea pollen from these months can safely be considered that of *P. roupelliae*.

Table 2 Frequency of insect visitors to *Protea roupelliae* inflorescences in bird excluded (small- and large-mesh cages), open-pollinated (uncaged) and pollen-supplemented (uncaged) treatments. Each inflorescence was observed 8–10 times during its

flowering. Sample sizes were 30 inflorescences in each of the caged and pollen-supplemented treatments and 60 inflorescences in the uncaged control treatment

Insect group	No. of visitors per treatment median (range)				Kruskal-Wallis $H_{3,150}$	P
	Small-mesh cage	Large-mesh cage	Uncaged control	Uncaged, pollen-supplemented		
Ants	0 (0–18)	0 (0–47)	0 (0–12)	0 (0–8)	5.233	0.1555
<i>Apis mellifera adansonii</i>	0 (0–4)	0 (0–10)	0 (0–2)	0 (0–1)	17.893	0.0005
<i>Atrichelaphinus tigrina</i>	0 (0–1)	0 (0)	0 (0–3)	0 (0–1)	3.148	0.3694
<i>Trichostetha fascicularis</i>	0 (0)	0 (0–1)	0 (0–2)	0 (0–2)	17.605	0.0005
All insects	1 (0–20)	1.5 (0–48)	0 (1–15)	2.5 (0–12)	11.514	0.0093

Table 3 *Protea* pollen loads carried by birds at Mt Gilboa, collected from the crown, throat and bill. Birds were caught using mist nets in January and February 2003, when protea pollen would be almost exclusively from *Protea roupelliae*

Bird species	<i>n</i>	Protea pollen loads (no. of grains)		
		Median	Range	
<i>Nectarinia famosa</i>	Malachite sunbird	34	498.5	18–7,321
<i>Promerops gurneyi</i>	Gurney's sugarbird	1	574	-
<i>Cisticola lais</i>	Wailing cisticola	2	18	9–27
<i>Pycnonotus barbatus</i>	Black-eyed bulbul	2	3	2–4
<i>Pseudochloroptila symonsi</i>	Drankensberg siskin	1	7	-
<i>Cossypha caffra</i>	Cape robin	1	3	-

The size of pollen loads carried on the crown, throat and bills of malachite sunbirds varied significantly for both protea and aloe pollen (Fig. 3). Malachite sunbirds carried more protea pollen on their crowns than on their throats or bills, and the difference was significant ($F_{2, 62}=67.06$, $P < 0.001$). The pattern was reversed for aloe pollen, where pollen loads were significantly larger on the chin and bills of birds than on their crowns ($F_{2, 76}=24.70$, $P < 0.001$).

Discussion

Protea roupelliae was pollinated almost exclusively by birds in our study population, which is consistent with its “ornithophilous” floral syndrome. Malachite sunbirds, the most frequent visitors to *P. roupelliae* inflorescences, were the primary pollinator of this species on Mt Gilboa. The geographical distribution of this sunbird encompasses all of the known populations of *P. roupelliae*, with the exception of a few populations in the far north of South Africa (Harrison et al. 1997). Although Gurney's Sugarbirds carried large *Protea* pollen loads and have previously been implicated in the pollination of *P. roupelliae* (De Swardt 1993), they were unimportant pollinators at Mt Gilboa due to their low abundance. Nevertheless, Gurney's Sugarbirds also occur over most of the distribution range of *P. roupelliae* (Harrison et al. 1997), and we thus cannot exclude the possibility that these or other birds

play a larger role as pollinators in different years or in other populations.

That bird-excluded inflorescences had such low seed sets despite having frequent insect visits strongly supports the conclusion that insects do not play a major role in the pollination of *P. roupelliae*. Higher insect numbers in caged inflorescences were probably due to decreased predation and increased nectar and pollen resources resulting from excluding birds. It is reasonable to assume that those seeds which did occur in bird-excluded inflorescences were mostly the result of insect pollination, as almost no seeds were produced through autonomous pollination (Table 1). Inflorescences in which large beetles were found often appeared damaged, with many discoloured or broken flowers. While beetles might be big enough to effect pollination and seemed to come in contact with both pollen presenters and receptive stigmas, their foraging behaviour may damage flowers and thus reduce seed set. It remains unclear whether the large beetles help or hinder *P. roupelliae* reproduction.

Seed production in the study population was not limited by pollen receipt on stigmas, as evidenced by the non-significant effect of supplemental hand-pollination on seed set (Fig. 1). This finding is consistent with several other studies of Proteaceae (Pyke 1982; Whelan and Goldingay 1986; Collins and Rebelo 1987).

Birds are particularly effective pollinators at high altitudes where strong winds, rain, and cold temperatures often create poor flight conditions for insects (Cruden

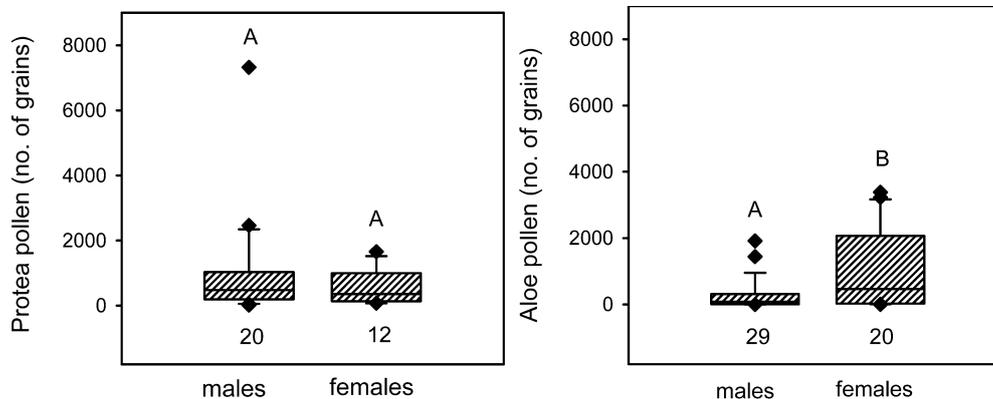
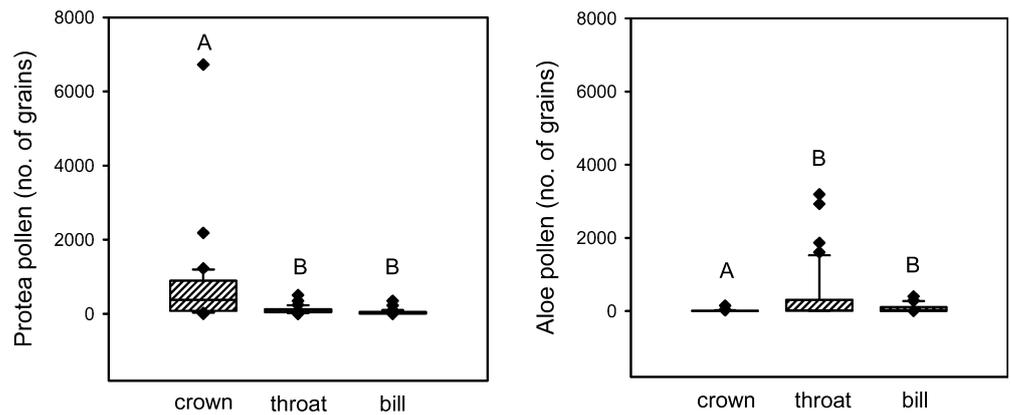


Fig. 2 Protea and aloe pollen loads carried on the heads and bills of male and female malachite sunbirds, December 2001 to February 2002. Boxes show median, 25% and 75% quartiles, bars show 10th and 90th percentiles, and diamonds represent outliers. Dissimilar capital letters denote significant differences between the sexes

(Mann-Whitney U test, $\alpha=0.05$). Numbers below boxes indicate sample sizes. Sample sizes for protea pollen counts are lower as only birds captured in January and February, when protea pollen is almost entirely from *Protea roupelliae*, are included

Fig. 3 Placement of *Protea* and *Aloe* pollen on malachite sunbirds, caught from December 2001 to February 2002. Boxes shown median, 25% and 75% quartiles, whiskers indicate 10th and 90th percentiles, and diamonds denote outliers (untransformed data). Dissimilar capital letters indicate significant differences, as determined using 95% confidence intervals for rank transformed data (randomised block design ANOVA; $n_{\text{protea}}=34$ sunbirds, $n_{\text{aloe}}=41$)



1972). The high proportion of bird movements that we observed between inflorescences on different trees, as opposed to on the same tree, underlines their effectiveness as outcrossing agents. Provision of outcross pollen and long-distance pollen transfer may be especially important roles of bird pollinators, which can generally fly greater distances than their insect counterparts (Wright 1994; Collins and Rebelo 1987, but see Mayfield et al. 2001). Efficient transfer of outcross pollen could be especially important for plants whose flowers are in dense, hermaphroditic inflorescences and are thus prone to self-pollination, such as those in the family Proteaceae.

Differences in the relative amounts of *Protea* and *Aloe* pollen carried by male and female malachite sunbirds may be explained by territoriality or by differing size and morphology. We often observed male sunbirds exhibiting territorial behaviour, calling from *P. roupelliae* trees and chasing other sunbirds (male and female) away from *P. roupelliae* inflorescences. Female malachite sunbirds are 17% smaller than males and were always displaced from inflorescences in interactions between the sexes. They may have thus been forced to forage more at *Aloe* inflorescences, which were not defended. The shorter bills of females may also enable them to make better use of the shorter *Aloe* flowers (see Stein 1992; Temeles et al. 2002).

The differing distribution of *Protea* and *Aloe* pollen on sunbird pollinators is easily explained by the shapes of their respective flowers, which are characteristic of the two genera. Newly opened *P. roupelliae* flowers present pollen on the side of the stigma facing the inflorescence centre. As sunbirds foraged from the centre of the inflorescence, the crowns of their heads would be more likely to pick up pollen than their throats. The short, tubular shape of *Aloe boylei* flowers would prevent the bird from inserting its head into the flower, thus reducing the amount of aloe pollen transferred to the crown feathers. While this study indicates partial segregation of pollen from *P. roupelliae* and *A. boylei*, most sunbirds carried pollen from both species on both their crowns and throats. Incomplete separation and the typical flower morphology of the *Protea* and *Aloe* species on Mt Gilboa suggest that pollen segregation is not due to an evolutionary mechanism to reduce pollen competition between the two species, but differences in general flower form between the genera.

Even low levels of pollen partitioning, however, may make the pollination systems of both species more efficient where they grow concurrently and so may promote plant assemblages that contain both *Protea* and *Aloe* species.

Although our findings show that floral syndromes can lead to accurate predictions about the effective pollinators of plant species, we do not advocate that syndromes be accepted as evidence that a plant is pollinated by particular animals. Rather than being viewed as fixed categories, as was often the case in the past, floral syndromes can be seen as patterns of convergent evolution that are useful for developing testable hypotheses about pollination systems.

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References

- Armbruster WS, Edwards ME, Debevec EM (1994) Floral character displacement generates assemblage structure of Western Australian triggerplants (*Stylidium*). *Ecology* 75:315–329
- Beard JS (1993) The Proteas of tropical Africa. Kangaroo, Hong Kong
- Beattie AJ (1971) A technique for the study of insect-borne pollen. *Pan-Pacific Entomol* 47:82
- Carthew SM (1993) An assessment of pollinator visitation to *Banksia spinulosa*. *Aust J Ecol* 18:257–268
- Coetzee JH, Giliomee JH (1985) Insects in association with the inflorescence of *Protea repens* (L.) (Proteaceae) and their role in pollination. *J Entomol Soc S Afr* 48:303–314
- Collins BG, Rebelo T (1987) Pollination biology of the Proteaceae in Australia and southern Africa. *Aust J Ecol* 12:387–421
- Cruden RW (1972) Pollinators in high-elevation ecosystems: relative effectiveness of birds and bees. *Science* 176:1439–1440
- De Swardt DH (1993) Factors affecting the densities of nectarivores in *Protea roupelliae* woodland. *Ostrich* 64:172–177
- Dunn OJ (1964) Multiple comparisons using rank sums. *Technometrics* 6:241–252
- Faegri K, van der Pijl L (1979) The principles of pollination ecology. Pergamon, Oxford

- Fuss AM, Sedgley M (1991) Pollen tube growth and seed set of *Banksia coccinea* R. Br. (Proteaceae). *Ann Bot* 68:377–384
- Harrison JA, Allan DG, Underhill LG, Herremans M, Tree SJ, Parker V, Brown CJ (1997). The atlas of southern African birds, vol 2. Passerines. Birdlife South Africa, Johannesburg.
- Hingston BA, McQuillan BP (2000) Are pollination syndromes useful predictors of floral visitors in Tasmania? *Aust J Ecol* 25:600–609
- Hollander M, Wolfe DA (1973) Nonparametric statistical methods. Wiley, New York
- Johnson SD, Steiner KE (2000) Generalization versus specialization in plant pollination systems. *Trends Ecol Evol* 15:140–143
- Keeton WT, Gould JL (1993) Biological science, V. W.W. Norton, New York
- Mayfield MM, Waser NM, Price MV (2001) Exploring the ‘Most Effective Pollinator Principle’ with complex flowers: bumble bees and *Ipomopsis aggregata*. *Ann Bot* 88:591–596
- Muchhala N (2003) Exploring the boundary between pollination syndromes: bats and hummingbirds as pollinators of *Burmeistera cyclostigmata* and *B. tenuiflora* (Campanulaceae). *Oecologia* 134:373–38
- Ollerton J (1998) Sunbird surprise for syndromes. *Nature* 394:726–727
- Pyke GS (1982) Fruit set in *Lambertia formosa* Sm. (Proteaceae). *Aust J Bot* 30:39–45
- Rebello AG, Siegfried WR, Crowe AA (1984) Avian pollinators and the pollination syndromes of selected mountain fynbos plants. *S Afr J Bot* 3:285–296
- Rourke JP (1980) The Proteas of southern Africa. Purcell, Cape Town
- Stebbins GL (1970) Adaptive radiation of reproductive characteristics in angiosperms. 1. Pollination mechanisms. *Annu Rev Ecol Syst* 1:307–326
- Stein BA (1992) Sicklebill hummingbirds, ants, and flowers. *Bioscience* 42:27–33
- Temeles ET, Linhart YB, Masonjones M, Masonjones HD (2002) The role of flower width in hummingbird bill length-flower length relationships. *Biotropica* 34:68–80
- Thompson JD, Wilson P, Valenzuela M, Malzone M (2000) Pollen presentation and pollination syndromes, with special reference to *Penstemon*. *Plant Species Biol* 15:11–29
- Van der Walt ID, Littlejohn GM (1996) Pollen morphology, male hybrid fertility and pollen tube pathways in *Protea*. *S Afr J Bot* 62:236–246
- Vogts M (1984) South Africa’s Proteaceae. Know them and grow them. Struik, Cape Town
- Waser NM, Chittka L, Price MV, Williams NM, Ollerton J (1996) Generalization in pollination systems, and why it matters. *Ecology* 77:1043–1060
- Whelan RJ, Goldingay RL (1986) Do pollinators influence seed-set in *Banksia paludosa* Sm. and *Banksia spinulosa* R. Br.? *Aust J Ecol* 11:181–186
- Wolff DM, Braun M, Liede S (2003). Nocturnal versus diurnal pollination success in *Iserfia laevis* (Rubiaceae): a sphingophilous plant visited by hummingbirds. *Plant Biol* 5: 71–78
- Wright MG (1994) Seed production by *Protea laurifolia* (Proteaceae) after insect versus insect and bird pollination: a quality difference? *S Afr J Sci* 90:199
- Wright MG, Visser D, Coetzee JH, Giliomee JH (1991) Insect and bird pollination of *Protea* species in the Western Cape: further data. *S Afr J Sci* 87:214–215
- Zar JH (1999) Biostatistical analysis, IV. Prentice Hall, New Jersey

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