



**Co-occurrence and phenological niche separation in rodent pollinated
*Proteaceae***



Submitted in partial fulfillment of the degree of Bachelor of Science (Honours)

Oliver Cowan

Supervisor: Prof. Jeremy Midgley

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ABSTRACT

Despite the numerous studies regarding rodent pollination in the Cape Floristic Region in the last few decades, little or no work has been done on patterns of co-occurrence and flowering phenology. The presence of three potentially rodent-pollinated *Protea* species at Fernkloof Nature Reserve, two of which were observed to co-occur, facilitated the following questions: i) are *P. cordata*, *P. scabra* and *P. angustata* therophilous? ii) do therophilous species co-exist at a fine scale? iii) do they have the same pollinator? iv) do they exhibit staggered flowering phenology? The floral characteristics of the study species suggest they were rodent pollinated and that the co-occurring species, *P. cordata* and *P. scabra*, would have staggered flowering phenologies. All three of the species' pollen was found in the faeces of *Acomys subspinosus*, the shared pollinator, while the phenological data provided the first empirical evidence of staggered flowering phenologies between fine scale, sympatric therophilous *Protea* species.

1. Introduction

Since the pioneering work on non-flying mammal pollination in the Cape Fynbos by Rourke and Wiens (1977) and Wiens et al. (1983), rodent pollination (therophily) has been relatively well known in the region, as well as in the adjacent Succulent Karoo Biome, with numerous studies undertaken (Wiens and Rourke, 1978; Johnson et al., 2001; Kleizen et al., 2008; Letten and Midgley, 2009; Biccard and Midgley, 2009).

Therophilous *Protea* species tend to occur in small localized populations, a feature common to the therophilous Australian genera *Dryandra* and *Banksia* (Wiens et al., 1983), although the reasons for this are unknown and there is no data on the issue in the literature (Rebelo and Breytenbach, 1987). It has been proposed that the restricted distribution, compared to the more widespread distribution of bird-pollinated species, has been a key aspect for the evolution of therophilous *Proteas* (Wiens et al., 1983). In addition, sympatric therophilous *Protea* species have been suggested to stagger their flowering seasons which contrasts with bird pollinated species' phenology where flowering generally overlaps (Wiens et al., 1983). Again, there is very little empirical data on the matter as most studies on therophilous *Proteas* have focused on single species and the question arises whether therophilous species tend to co-occur and whether they are phenologically niche differentiated?

There is a general absence of phenological data for therophilous species in the literature; however the issue of phenological separation within pollinator guilds has been addressed: Competition between sympatric species for space, resources and services provided by mutualistic animals, such as pollination, is reported to be critical for their reproduction and in their population dynamics (Morales and Traveset 2008). Pollinator-mediated competition occurs 1) if pollinators are limiting and flowering overlap 'dilutes' the number of visits due to an increased number of flowers (Caruso, 1999), or 2) if pollinators moving between plant species reduces intra-specific pollination (Flanagan et al., 2009). Conversely, co-flowering species may facilitate each other's pollination if pollinators are abundant and visitation increases with diversity and/or abundance of flowering plants (Ghazoul, 2006).

Pollinator-mediated competition can significantly structure plant communities and often lead to staggered flowering phenologies (Armbruster et al., 1994). Aizen and Rovere (2010) showed that staggered flowering phenologies in an assemblage of plant species in an Argentinean temperate forest were due largely to competition over their shared hummingbird pollinator. Stone et al. (1998) found that an *Acacia* community staggered its flowering throughout the day as a result of competition for pollinators. Beside a staggered flowering phenology, plant communities may respond to pollinator-mediated competition through evolutionary character displacement resulting in divergent floral morphology. Muchhala and Potts (2007) found that co-existing *Burmeistera* species, that share bats as their primary pollinators, exhibited different flower morphology which placed pollen on different parts of the bats' bodies increasing intra-specific pollination.

Models of competition involving inter-specific pollination, as a result of simultaneous flowering and shared pollinators, predict that the less common of the two competitors will go extinct unless they are separated along some dimension or niche axis (Morales and Taveset, 2008). It would thus appear that the staggered flowering phenology observed in therophilous *Proteas* (Wiens et al., 1983; Midgley, pers. comm.) is a potentially viable strategy to promote reproductive success if species have mutual primary pollinators, despite the fact that certain co-flowering species have been shown to facilitate each other's pollination (Ghazoul, 2006).

Three species of *Protea* described as potentially therophilous due to their floral characteristics (Wiens, et al., 1983; Rebelo and Breytenbach, 1987) were observed to occur at Fernkloof Nature Reserve. This facilitated the following questions: i) are *P. cordata*, *P. scabra* and *P. angustata* (see plate 1 for photos) therophilous? ii) do therophilous species co-exist at a fine scale? iii) do they have the same pollinator? iv) do they exhibit staggered flowering phenology?

P. cordata is a geoflorous shrublet with a woody base bearing several erect, unbranched stems with distinct heart-shaped leaves. Bract colour is rusty and flowers emit a yeasty odour. The species flowers in winter, and is found in isolated clumps of a few dozen plants although extensive stands may occur locally. (Rebelo, 1995; Rebelo and Breytenbach, 1987).

P. scabra is a geoflorous, rhizomatous shrublet with a turban-shaped flowerhead. Bracts are cream coloured with rusty, velvety hairs. Plants flower predominantly in late winter to early spring, usually 2 years after a fire; however unlike *P. cordata*, the flowers appear not to emit any odour (Rebelo, 1995, Rebelo and Breytenbach, 1987). Prior to the initialization of this study, *P. scabra* and *P. cordata* were observed growing on the same slope at Fernkloof Nature Reserve (J. Midgley, pers. comm.)

The final species of study, *P. angustata*, is a rhizomatous dwarf shrublet with a cup-shaped flowerhead. Bracts are yellow and although the species is geoflorous and can be considered cryptic, its inflorescence is held slightly off the ground and the peduncle is not very stout. Flowers with a distinctive yeasty odour are seen in late winter and early spring and occur almost exclusively following the first few years following a fire. The plant is restricted to several isolated localities of a few scattered plants and is considered vulnerable (Rebelo, 1995, Rebelo and Breytenbach, 1987).

Much is still unknown with regards to pollination in plants with therophilous traits. *P. cordata*, *P. scabra* and *P. angustata* are known to occur in Fernkloof Nature Reserve and consequently provided the ideal opportunity to investigate aspects of their ecology during the late winter of 2010. The primary objectives of this study are to test for therophily in all three of the species and to investigate the flowering phenology of *P. cordata* and *P. scabra*. Secondary objectives include identifying the pollinator/(s) and exploring the relationship between pollinator and plant. Due to the floral traits exhibited by all three species, I hypothesize that evidence of therophily will be found in each of the species of study. In addition, I hypothesize that the co-occurring *P. cordata* and *P. scabra* will stagger their flowering.

2. Study Site

Fernkloof Nature Reserve was formally created in 1957 and has increased in size from an initial 100 hectares to its current size of approximately 1500 hectares (www.fernkloof.com). Fernkloof is positioned to the North of, and borders, the coastal town of Hermanus, approximately 1.5 hours from Cape Town (**Figure 1**: inset). The reserve is situated in the Fynbos biome of South Africa but more specifically it is classified as Overberg Sandstone Fynbos (Mucina and Rutherford, 2006). The site experiences a typically Mediterranean climate with cool, moist winters and warm, dry summers (MAP=585mm; MAT=15.5°C) (www.weathersa.co.za). Acidic lithosol soils derived from Ordovician sandstones of the Table Mountain Group are distinguishing features of this vegetation type (Mucina and Rutherford, 2006). The reserve is particularly species rich with over 1600 plant species recorded. 130 bird species have been recorded in the reserve and mammalian fauna ranges from the small e.g. Cape Spiny Mouse (*Acomys subspinosus*), to the medium e.g. Chacma Baboon (*Papio ursinus*). The three species of study (*P. cordata*, *P. scabra* and *P. angustata*) are all present in the reserve (www.fernkloof.com).

Although both sites A and B are encompassed within the reserve they are discrete, approximately a kilometer apart (**Figure 1**). Site A is positioned on a steep, coast-facing slope and is dissected by a footpath. A small stream was present during the two visits to the site. Foliage cover was fairly dense but both *P. cordata* and *P. scabra* were present. The slope was fairly rocky and during the first trip *P. cordata* was the only *Protea* that was seen flowering. However, during the second trip, *P. scabra* was flowering concurrently with bird pollinated *Protea* species e.g. *P. lepidocarpodendron* and *P. repens*, although they were further than 100m away. I was unable to ascertain when the site was last burned, however it is likely that it was in 2001 when large veld fires swept through the area (www.fernkloof.com).

Site B is positioned on a flatter, sandy area directly below an inaccessible rocky slope. It was not frequented during the first visit to Fernkloof but on the second trip I witnessed several mats of *P. angustata* in flower. Foliage cover was considerably less dense compared with Site A and the site had burned in 2008 (Grant Forbes, Pers. Comm.).



Figure 1: A portion of Fernkloof Nature Reserve. Red Polygons denote study sites within the Reserve (inset: Hermanus’s position within the region) (Modified from Google Earth)

3. Materials and Methods

3.1. Rodent trapping:

Two periods of trapping were carried out. The first occurred on August the 6th and 7th when trapping was done solely at Site A while the second round of trapping was done on September the 8th to 10th and trapping occurred at Sites A and B.

Trapping was conducted using Sherman live traps baited with a mixture of peanut butter and rolled oats and lined with pieces of cotton wool to keep trapped animals warm during the night. At Site A during the first trapping episode traps (N=35) were laid out in the late afternoon.

Traps were spaced at approximately 5 meter intervals in transects approximately 2m off both sides of the footpath (n=20). Additionally, another transect of traps (n=15) was placed approximately 15m above the footpath. Traps were checked each morning at sunrise ($\pm 07:00$). If a trap contained a rodent, the rodent was identified, weighed and marked with a red permanent marker. Faeces from the traps were collected in vials containing 70% ethanol, labeled and stored. Successful traps were stringently cleaned to avoid contamination. Several traps were re-baited and left open during the day in an effort to catch diurnal rodents.

The second period of trapping followed the same protocol as the first period with a few exceptions: Due to the paucity of flowering plants at Site A, 10 traps were placed strategically in the vicinity of flowering *P. scabra*. At site B, 5 traps were placed in and around mats of *P. angustata*. As was the case during the first period of trapping, trap positions were designated by tying a black or red ribbon on a taller plant adjacent to the trap.

3.2. Phenological data collection:

During each of the visits phenological data was collected for *P. cordata* and *P. scabra* at site A. Four categories were created: 1) plants with no buds present 2) plants with immature buds present 3) plants with flowers present 4) plants with dead flowers present. As *P. scabra* was the less common of the two species at the site, 50 individuals were randomly selected, categorized and the distance between the plant and the nearest *P. cordata* was measured. The *P. cordata* was also categorized according to its flowering status.

3.3. Scat treatment and pollen counting:

Faeces stored within vials were mashed using a sterilized stainless steel spatula end. After each sample, the spatula was thoroughly cleaned and rinsed with 70% ethanol. Two drops of molten Fuchsin gelatin (Beattie, 1971) were pipetted into the vials containing the mashed faeces and the vials were centrifuged. Two drops of the suspension from each sample were pipetted onto

separate microscope slides. Two drops of Glycerol were pipetted onto each microscope slide to prevent desiccation. Pollen grains were counted for the full field of view at 40x magnification (0.45mm diameter) over three different scans of the entire coverslip length (22mm). This method of counting yielded an approximate 3% sample of the coverslip area. Total *Protea* pollen counts were recorded for each sample. Distinguishing between different *Protea* pollen types is possible but complicated to an untrained eye (Lynne Quick, pers. comm.) and was thus not done in this study. Other *Proteas* observed flowering during the visits (see Study Site) are known bird-pollinated species and were more than 100m away from the trapping site.



Plate 1: A- *Protea cordata* inflorescence
B- *Protea scabra* inflorescence
C- *Protea angustata* inflorescence
(source of photos: www.fernkloof.com)

4. Results

4.1. Rodent Captures:

In total 10 individual animals were caught during the first trapping period at Site A with 3 recaptures, totaling 13 captures with a trapping success of 19%. Three species were caught during this period: two rodent species (*Acomys subspinosus* and *Otomys irroratus*) and one species of shrew (*Myosorex varius*). *A. subspinosus* was the most captured species (8 individuals), while one individual of both *O. irroratus* and *M. varius* were caught (**table 1**).

Table 1: Composition of mammal captures at Site A during 6 & 7th August 2010

Species	No. of individuals	No. of Captures	Mean Body Mass (g) (\pm SD)
<i>Acomys subspinosus</i>	8	11	29 (6)
<i>Otomys irroratus</i>	1	1	>100 (na)
<i>Myosorex varius</i>	1	1	32 (na)

At site A during the second trapping period (**table 2**) a total of 7 individual rodents were caught with 2 recaptures, totaling 9 captures with a trapping success of 30%. As was the case during the first trapping period, *Acomys subspinosus* was the most caught rodent species (5 individuals), while one individual of *Otomys irroratus* was caught in the exact same spot as the *O. irroratus* during the first trapping period. The permanent marker used to mark the individual during the first trap would probably have worn off during the intervening month, thus I can only speculate that it is the same individual. A single *Aethomys namaquensis* was caught during the second trapping period

Table 2: Composition of mammal captures at Site A during 8-10th September 2010

Species	No. of individuals	No. of Captures	Mean Body Mass (g) (\pm SD)
<i>Acomys subspinosus</i>	5	7	29 (8)
<i>Otomys irroratus</i>	1	1	>100 (na)
<i>Aethomys namaquensis</i>	1	1	66 (na)

4 *Acomys subspinosus* individuals were caught at Site B during the second trapping period (table 3). There were no recaptures or other species caught and the trapping success was 27%. A lower mean body mass (15g) was recorded at Site B in comparison to the *A. subspinosus* mean body masses at Site A which was 29g during both trapping periods (tables 1 and 2).

Table 3: Composition of mammal captures at Site B during 8-10th September 2010

Species	No. of individuals	No. of Captures	Mean Body Mass (g) (\pm SD)
<i>Acomys subspinosus</i>	4	4	15 (5)

4.2. Pollen Counts:

The mean *Protea* pollen grains counted in the scats of the captured individuals from Site A during the August trapping period are illustrated below. The *O. irroratus* and *M. varius* individuals had only 5 and 8 pollen grains identified on their slides respectively. However, *A. subspinosus* had a mean *Protea* pollen count of 246 although the standard deviation was almost half of this value (SD=121) (Figure 2).

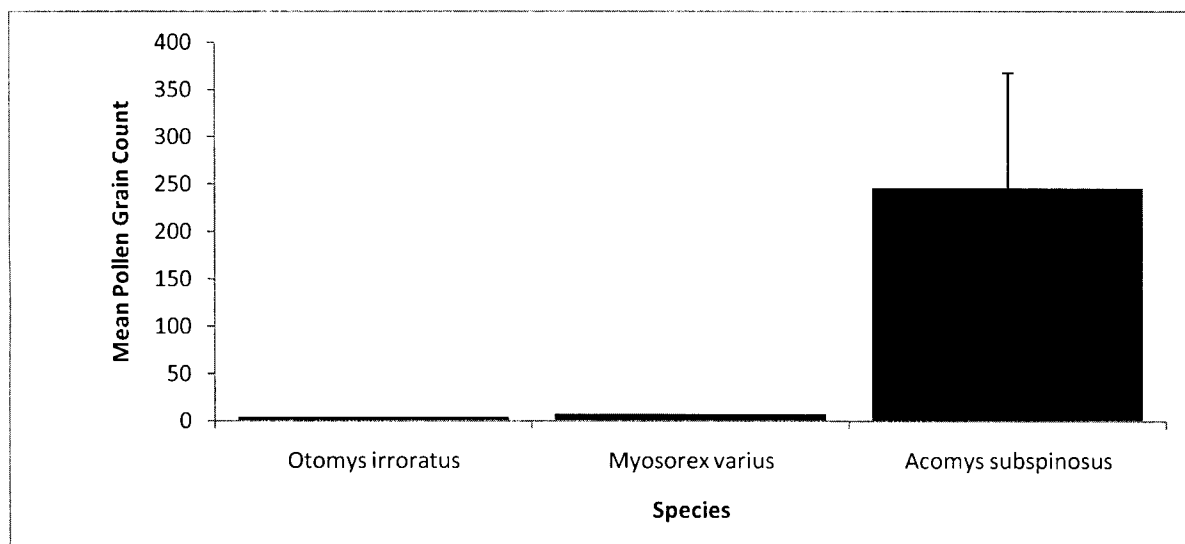


Figure 2: Mean number of *Protea* pollen grains counted in the scat samples for each of the species caught at Site A during August 6th & 7th 2010. A standard error bar has been added where applicable. Values represent \pm 3% of the coverslip surface area.

Data from Site A (Figure 3) and from Site B (Figure 4) during the second trapping period are shown below. The *O. irroratus* and the *A. namaquensis* individuals from Site A both displayed minimal *Protea* pollen counts in their scat samples (2 and 1, respectively) while *A. subspinosus* was found to have a mean *Protea* pollen count of 160 with a large standard deviation (SD=151) (Figure 3). The mean *Protea* pollen count from the scat samples collected from Site B was significantly reduced (46, SD=35) in comparison to the data from Site A (Figure 4).

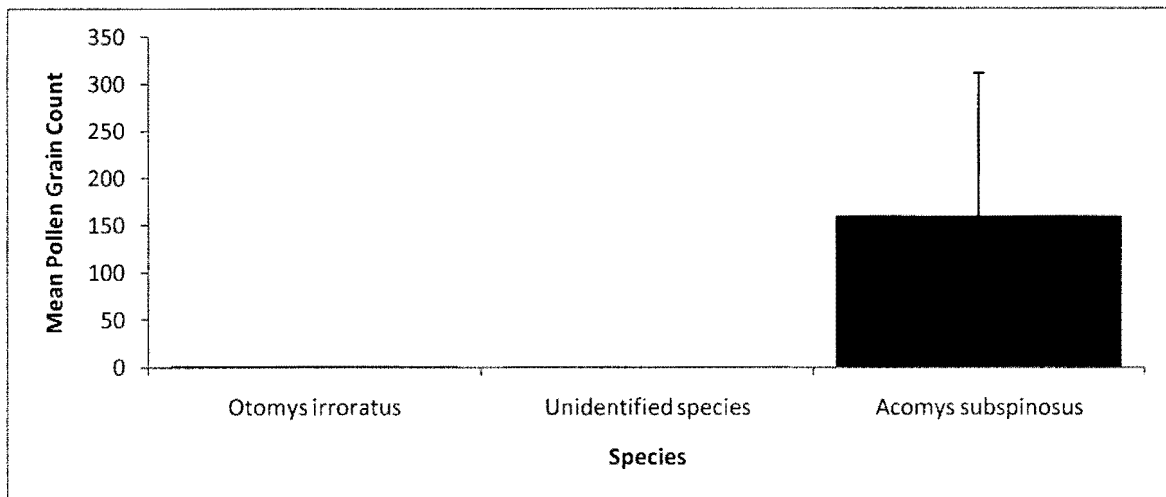


Figure 3: Mean number of *Protea* pollen grains counted in the scat samples for each of the species caught at Site A during September 8th-10th 2010. A standard error bar has been added where applicable. Values represent \pm 3% of the coverslip surface area.

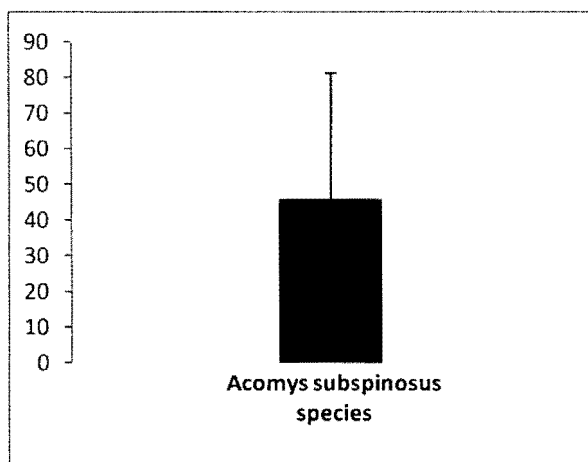


Figure 4 (left): Mean number of *Protea* pollen grains counted in the scat samples for the species caught at Site B during September 8th-10th 2010. A standard error bar has been added. The value represents \pm 3% of the coverslip surface area.

4.3. Phenological Data:

Phenological data for *P. scabra* and *P. cordata* at Site A on August 7th 2010 is illustrated below (Figure 5). The mean near-neighbour distance of the fifty plant pairs was 128cm (± 85 cm SD). No *P. scabra* were found to be in flower nor were there any dead flowers observed on individuals. *P. cordata* individuals were either in flower or they had dead inflorescences, although two individuals still had immature buds present.

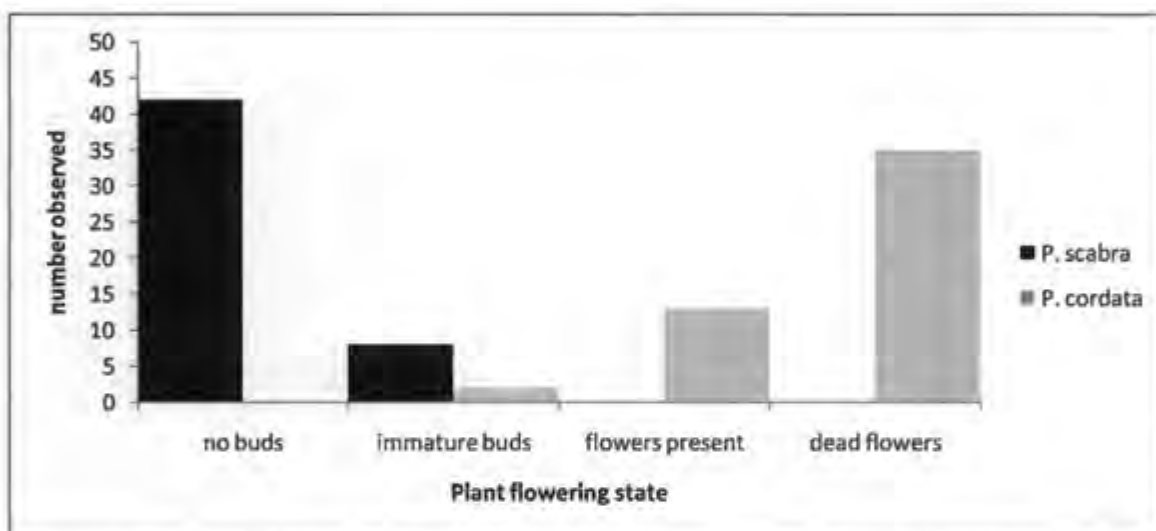


Figure 5: The flowering phenologies of 50 pairs of *Protea scabra* and *Protea cordata* on August 7th 2010 at Site A

Phenological data from Site A was collected a month later on September 9th 2010 (Figure 6). Although the likelihood that many of the plant pairs were the same as those studied a month earlier is high, this was not regulated for. The mean near-neighbour distance was calculated at 87cm (± 61 cm SD). All of the 50 *P. cordata* plants had dead inflorescences whereas *P. scabra* was not as uniform. *P. scabra* plants still had a large percent with no buds present (36%) but 26% of plants were in flower and 26% of plants had dead inflorescences. 12% of *P. scabra* plants had immature buds present.

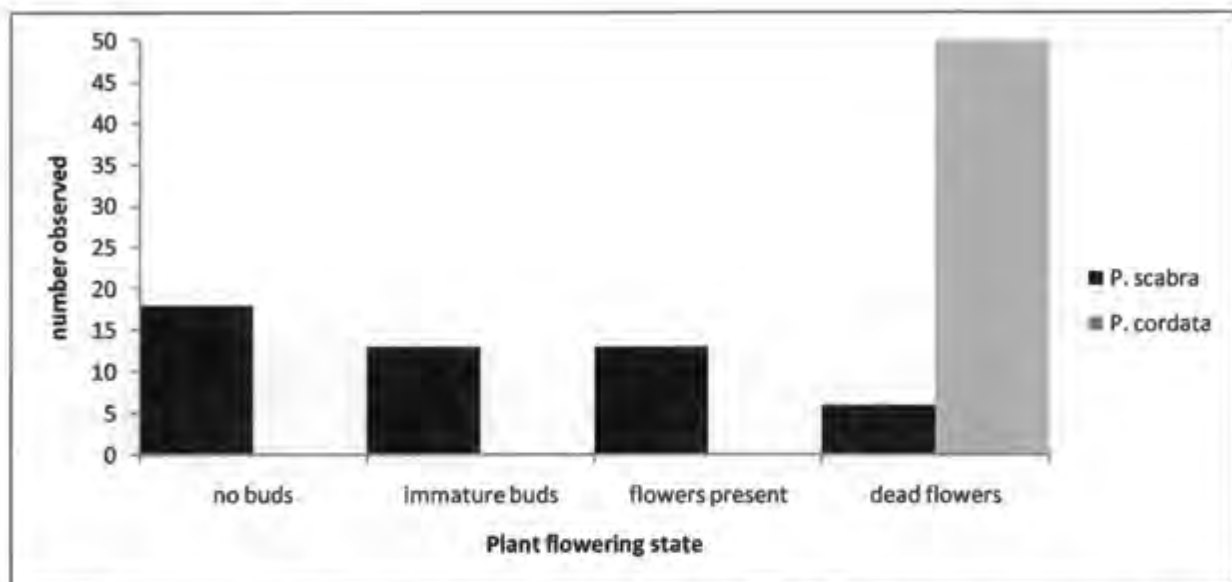


Figure 6: The flowering phenologies of 50 pairs of *Protea scabra* and *Protea cordata* on September 9th 2010 at Site A

5. Discussion

5.1. *Protea cordata*:

This study has provided the first evidence that *Acomys subspinosus* collects *P. cordata* pollen grains in its digestive tract as a result of its feeding habits. If the pollen made its way into the rodents' digestive tract by chance then we would expect to see similar numbers of grains in the scat samples of the other two species of small mammal trapped in the area. This is clearly not the case and the high mean pollen count and the lack of co-occurring flowering *Protea* species at the time of trapping is highly suggestive of the important role that *P. cordata* nectar and possibly pollen plays in *A. subspinosus*'s diet during the winter months.

The lack of pollen in *O. irroratus* is because the species is a destructive vegetarian with a preference for stems and leaves of grasses and reeds (Smithers, 1986). Biccard and Midgley (2009) found minimal *Protea* pollen in *O. irroratus* scats in their study on *Protea nana*. Although the *M. varius* individual caught in this study did not have substantial pollen loads in its scats, shrews have been proposed as a potential pollinators as they pick up pollen on their rostrums

during their hunt for insects within inflorescences (Fleming and Nicolson, 2002). Unfortunately, due to only one individual being captured in this study, I am unable to add to, or refute, this hypothesis.

Although the substantial pollen found within *A. subspinosus* scats, added to the therophilous traits observed in the species, have allowed me to accept my hypothesis that *Protea cordata* is rodent pollinated, the evidence could be further strengthened. Observations of pollination-conducive behavior (either videoed in the wild or witnessed in captured animals), along with a significant reduction in seed-set in rodent-excluded inflorescences, would provide conclusive 'proof' of therophily (Carthew and Goldingay, 1997). Unfortunately, due to time constraints and logistical problems this study did not incorporate the aforementioned tests. Additionally, data on nectar volume and composition would be useful to strengthen the hypothesis that NMP plants are nectar-rich.

5.2. *Protea scabra*:

Like *P. cordata*, *P. scabra* has also been described as a NMP species due to its floral traits. Where *P. scabra* differs from *P. cordata* is its apparent lack of odour and its yellow-coloured inflorescence. Orange-breasted sunbirds (*Anthobaphes violacea*) have been observed feeding on the plant (Rebelo and Breytenbach, 1987), however the plants cryptic nature means that this is probably a rare occurrence as birds are generally attracted to plants visually (Raven, 1973). In addition, rodent activity has been previously observed at *P. scabra* plants (Rourke and Wiens, 1977) and a captive Namaqua Rock Mouse (*Aethomys namaquensis*) was observed to feed on a *P. scabra* inflorescence in a manner likely to be conducive to pollination (Wiens et al., 1983). The lack of pollen in the scats of the *A. namaquensis* in this study suggests that the species may not feed on *P. scabra* in the wild, however as only one individual was caught it is dangerous to infer too much from the data.

Although significant *P. scabra* pollen loads were found in the scats of *A. subspinosus*, the mean count was less than that found when *P. cordata* was in flower. This could be a result of fewer

flowering *P. scabra* (I counted 15 at the site) in comparison to *P. cordata* whose inflorescences were more abundant during the first trapping period. Additionally, *P. scabra* plants have a single inflorescence while several inflorescences are found at the base of a *P. cordata* plant, thus rodents foraging for *Proteas* would possibly ingest more *P. cordata* pollen during a nights feeding. Once again it is unsurprising to find minimal amounts of *Protea* pollen in *O. irroratus* scats for reasons previously outlined.

It could be argued that the presence of other flowering *Protea* species during the second trapping period, added to the inability to distinguish between pollen of different *Protea* species, equates to uncertainty in knowing if the pollen found in the scats is actually that of *P. scabra*. However, the other *Protea* species flowering at the time were large, erect shrubs more than 100m from the study slope are known to be bird-pollinated. In addition, I found no evidence in the literature of arboreal activity in *A. subspinosus*, subsequently I do not see any other possible source of the *Protea* pollen found in scats other than from *P. scabra*. As with *P. cordata*, greater confidence would be had if additional tests had been carried out (as previously outlined), however the presence of substantial amounts of *Protea* pollen in *A. subspinosus* scats gives me reason to accept the hypothesis that *P. scabra* is therophilous.

5.3. *Protea angustata*:

As mentioned in the study's introduction, *P. angustata* has been thought of as a therophilous species, however not all evidence points towards this pollination syndrome, for example the flower-head is borne slightly above the ground on a somewhat delicate peduncle (pers. obs.). Additionally, Wiens et al. (1983) found the nectar of *P. angustata* to have a lower sucrose to glucose and fructose ratio than that found in other therophilous plants, while Orange-breasted Sunbirds have been witnessed defending their territories while feeding exclusively on *P. angustata* (Rebelo, unpublished). Despite these observations, *P. angustata* emits a strong odour and is geoflorous, cryptic, obeys the "10mm rule" and has strongly curved styles.

My results are somewhat inconclusive. While *P. angustata* pollen is present in the scats of each of the four individual *A. subspinosus* caught, the mean (45) is substantially lower than that recorded at Site A. Interestingly, two of the four individuals caught were less than 15g in weight and can be thus considered juveniles (Fleming and Nicolson, 2002). These two individuals had pollen counts of 29 and 24 compared to the heaviest individual whose weight was 22g (still relatively small in comparison to the other sites) and had a pollen count of 98. It is possible that reduced dietary intake and/or inexpert feeding techniques would result in less pollen accumulating in the digestive tracts of juveniles. The floral traits exhibited by *P. angustata* may also have a role in the reduced pollen count as the flower-head, held slightly above ground level, may be less accessible to rodents than true geoflorous species such *P. cordata* and *P. sabra*.

The presence of *A. subspinosus* in a sandy habitat is unusual as it prefers a rocky terrain (Smithers, 1983), however the adjacent slope was fairly rocky and the individuals may nest in their preferred habitat but forage elsewhere provided the distance is not too great. The presence of *P. angustata* pollen in the scats of *A. subspinosus*, albeit in lower quantities than the other two species of study, allows me to accept my hypothesis that the species is therophilous. More work is needed in clarifying this as my results are somewhat inconclusive and birds have been observed feasting on *P. angustata* stands. Indeed, the species is listed as bird pollinated by the *Protea* Atlas Project (www.Protea.worldonline.co.za). Irrefutable proof of a pollination syndrome for *P. angustata* could be vital in its conservation as it is listed as vulnerable (Rebelo, 1995).

5.4. Phenology and niche differentiation between *Protea cordata* and *Protea scabra*:

This study has provided the first empirical evidence of staggered flowering phenologies between two co-occurring therophilous *Protea* species. I found no occurrences of overlapping flowering but a few noteworthy observations warrant further mention. Firstly, a large proportion of the study's *P. cordata* plants had dead inflorescences on August 9th suggesting that their peak flowering season is earlier than this, probably June and July (Rebelo, 1995).

Secondly, the ecology of the two species differs substantially: *P. cordata* regenerates exclusively from seed and take at least four years to produce their first flowers, while *P. scabra* is rhizomatous and reportedly flowers almost exclusively in the first few years following a fire (Rebello and Breytenbach, 1987). As the previous fire in Site A was in 2001 and possibly even further back in time, this could explain why so many *P. scabra* plants did not have flowers or buds present. Valente et al. (2010) showed that the two species are not closely related phylogenically, thus the possibility of hybridization is unlikely to be the reason for non-overlapping phenologies.

There is uncertainty in the literature as to the reasons for co-occurrence within localized therophilous species (Wiens et al., 1983; Rebello and Breytenbach, 1987). Wiens et al. (1983) suggest that it may be a result of therophily reducing gene flow due to the limited movement of rodents in comparison to birds. Limited gene flow may cause therophilous *Proteas* to be more prone to speciation, genetic drift and extinction, thus favouring the co-occurrence of several therophilous species in small localized populations. I propose that they may be forced to co-occur in a region where the habitat is suitable for the existence of their pollinator. Competition for pollination among sympatric flowering plant species has been shown to be an important force structuring plant communities and studies have shown sympatric plants with similar floral structures to have flowering periods separated in time (Stone et al., 1996). Competition between the two species for a shared pollinator due to 1) a reduction in number of visits due to an increased total number of flowers and 2) reduction of intra-specific pollination would favour the divergence of flowering phenologies and increase the likelihood of reproductive success.

5.5. Relationship between *Acomys subpinosus* and the study species:

The identification of *Acomys subpinosus* as a pollinator was expected because the rocky habitat and foliage cover is ideal habitat for the species (Rickart, 1981) and it has been recorded as a pollinator before (Johnson et al., 2001; Fleming and Nicolson, 2002; Letten and Midgley, 2009). Much is still unknown about the ecology of the species (Fleming and Nicolson, 2002) with exception that *A. subpinosus* is a Fynbos endemic (Breytenbach, 1982). The importance of

Protea resources should not be underestimated as a determinant of the species' distribution, while the importance of *A. subspinosus* to the distribution of certain therophilous *Proteas* could also be significant.

Unlike Australia where evidence of co-evolution exists (Wiens et al., 1979), therophily in southern Africa is considered a non-coevolved system with rodents treating the *Protea* nectar as a "dessert" during the harsh winter months (Wiens et al., 1983). More recent work highlights the importance of therophilous *Protea* resources in certain rodents' diets: Fleming and Nicolson (2002b) conducted a detailed study on the relationship between *A. subspinosus* and *Protea humiflora*, a therophilous species. Their results showed that *A. subspinosus* density was strongly correlated to *P. humiflora* fecundity and that breeding was opportunistic and coincides with greatest resource availability.

The evidence shown in this study of the co-occurrence of therophilous *Proteas* with staggered flowering phenologies provides further support to the idea that *Protea* resources are an essential component *A. subspinosus*' diet during the harsh winter months and breeding period. This is further strengthened by work by van Tets (1997) which showed that many rodents are capable of extracting protein from pollen grains, thus I propose that the *Protea* nectar and pollen are more than a "dessert" as described by Wiens et al. (1983). More investigation is needed to unravel the mutualism displayed between *A. subspinosus* and the geoflorous *Proteas* within its habitat.

6. Conclusion

This study has provided the first evidence that *Acomys subspinosus* ingests pollen from the three species during foraging and consequently acts as a potential pollinator. In addition, the phenological data collected on *P. cordata* and *P. scabra* provide the first empirical evidence that sympatric therophilous species stagger their flowering phenologies. To obtain irrefutable proof of rodent pollination in all three species additional tests need to be performed. These include observation of pollination-friendly behavior, enclosure experiments to assess seed set, and nectar analysis. Additionally, further investigation into the mutualism between the endemic *A. subspinosus* and therophilous *Proteas* would enhance our knowledge on possible dependence between the species. Investigating the phenologies of other co-occurring therophilous *Proteas*, such as *P. nana* and *P. amplexicaulis* (Midgley, pers. comm.) would broaden our understanding on the topic and add insight into its evolution. Understanding the pollination ecology of plant species is a crucial tool in conservation management and it would be prudent to obtain this knowledge before endemic species come under even greater threat from habitat loss, alien invasives and a changing climate.

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