A Squeaky Suspicion: Rodent pollination in *Protea nana*

Aiden Biccard

Supervisor: Prof. Jeremy J. Midgley

A Squeaky Suspicion: Rodent pollination in *Protea nana*

Aiden Biccard
Supervisor: Prof. Jeremy J. Midgley
Botany Department, University of Cape Town

ABSTRACT

The pollination syndrome of *Protea nana* has been described as uncertain throughout the literature. Floral morphology, presence of pendulous inflorescences, winter flowering time and a distinct yeasty odour make it the perfect candidate for rodent pollination (therophily). To test this suspicion, rodents were trapped in a stand of *P. nana*, examined for presence of pollen (on their noses and in their scats) and then observed in the laboratory for pollination behaviour. Inflorescences were placed in wire exclosures and seed set compared to controls in the field. Nectar samples were taken and analysed for percentage sucrose content. *Otomys irroratus*, *Aethomys namaquensis*, *Rhabdomys pumilio* and *Praomys verreauxi* were the rodent species captured. All species tested positive for presence of *Protea* pollen. Each rodent species, except *O. irroratus*, displayed legitimate pollination of *P. nana*. *P. verreauxi* was regarded as the most competent pollinator as it displayed superior climbing ability in comparison to the other rodent species. *R. pumilio* displayed highly destructive behaviour towards *P. nana* inflorescences and is the likely explanation for the observed 20% reduction in average number of inflorescences per plant over a two month period based on its predatory behaviour. Seed set was lowest in shade-cloth covered inflorescences and highest in the cones from the previous year's cohort. A large percentage of sucrose (29.4%) in the nectar of *P. nana* was found to be similar in comparison to known therophilous species. Insurmountable evidence from the results of this study concludes that *P. nana* is indeed therophilous.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Prof. Jeremy Midgley, for funding this project and for his valued input. Thanks to Irene Van Den Heuvel for her advice on trapping rodents and assisting in field-work. Finally, I would like to acknowledge Dr. Gary Bronner for assisting in the identification of rodent species.

INTRODUCTION

Proteaceae are one of the oldest known groups of flowering plants (Rourke 1980). Of the 360 South African species, more than 330 are restricted to the Mediterranean climate of the Cape folded mountain region (Rebelo 1995). This phenomenon has primarily been attributed to the highly dissected topography of the land, which is comprised of many small mountain ranges with diverse elevations, precipitation and soils (Rourke 1980, Wiens et al. 1983). Very few studies on pollination biology in Proteaceae have been undertaken, despite the suggestion that this may be a significant contributor to the observed high species richness in this family (Rebelo 1987). In comparison, virtually none of the existing pollination studies have regarded small nonflying mammals as pollinators in Proteaceae.

Small mammals, particularly rodents were first proposed as pollinators of South African Proteaceae by Rourke and Wiens (1977). This speculation was largely based on a series of fundamental characteristics present in certain Cape *Protea* species, which favoured such a pollination syndrome. Subsequently, the first evidence of therophily in two Cape *Protea* species, *Protea amplexicaulis* and *Protea humiflora*, was published a year later (Wiens and Rourke 1978). Apart from observed rodent pollination activity, it was also shown that the flowers of these proteas were specifically adapted for rodent pollination behaviour (Wiens and Rourke 1978). Furthermore, Rourke (1982), states that a probable 35 additional species may be pollinated in this way. However one such species, *Protea nana*, has been described as entomophilous (Bond and Goldblatt 1984) yet displays some of the traits similar to that of therophilous species (Rourke and Wiens 1977). Observational studies reveal that ornithophily is unlikely (Rourke and Wiens 1977) and that *P. nana* may indeed be pollinated by nonflying mammals but this proposition lacks verification (Wiens et al. 1983).

In contrast to Australian Proteaceae, where flower products are an important dietary supplement for mammals, South African therophilous proteas have been described as a non-coevolved system (Rourke and Wiens 1977). Several explanations for this proposition have been made, one of the most important being the brief flowering season and limited plant distribution of therophilous proteas (Rourke and Wiens 1977,

Wiens et al. 1983, Rebelo and Breytenbach 1987) not to mention the contrast between morphologically specialised plants and generalist rodents (Fleming and Nicolson 2002). Hence therophilous proteas have been thought of as unilaterally evolved species (Wiens et al. 1983).

Basic floral structures of proteas used to infer pollination by nonflying mammals have been summarised by Wiens and Rourke (1978) and compared to Australian Proteaceae by Rourke and Wiens (1977). These structural characteristics include: "(1) bowl shaped heads borne on short (3-4 mm) stout peduncles, often with the outsides of bracts darkly coloured, (2) copious, sucrose-rich nectar production with a high (36%) total carbohydrate composition, (3) often inflexed, wiry styles ca. 30-40 mm long , (4) cryptic, geoflorous, axillary positioning of the heads, and (5) a distinctive "yeasty" odour" (Wiens et al. 1983). In addition a critical stigma-nectar distance of approximately 10 mm is one of the most important for a functional "fit" between the rostrum of a pollinating rodent and the stigma (Wiens et al. 1983).

P. nana partly meets these requirements, and it is this partial conformity which raises considerable doubt as to whether the species is therophilous or not. Rourke (1980) describes *P. nana* as an erect, rounded shrub seldom exceeding a metre in height. The inflorescences are pendulous, 30 – 45 mm in diameter (Plate 2 A) and cup-shaped with involucral bracts scarlet to green, hairless, 10 – 15 mm long and 6 – 8 mm wide (Rebelo 1995). The styles are inflexed, 20 – 25 mm long and hairless and the pollen presenter is 2 – 3mm long, linear, tip pointed and knee-bent at the base (Rebelo 1995). Brief visual examination of this shrubby species reveals that it is not geoflorous as inflorescences are held well above the ground. Apart from this characteristic however, the remainder of the morphological traits lead one to suspect that *P. nana* could be rodent pollinated. One such factor is the presence of inflexed styles, a consistent feature amongst known therophilous *Protea* species. Wiens and Rourke (1978) demonstrate that the bases of such styles effectively block access to the nectar if approached from the perimeter of the inflorescence. Pollinators are therefore forced to forage outward along a radius from the centre of the inflorescence in an attempt to access nectar which collects in a bowl-like structure formed from the petals (Wiens and Rourke 1978). There are no records of nectar sucrose content, yet the inflorescences possess the characteristic yeasty smell of

well known therophilous, species such as *P. amplexicaulis*. The inflorescences are also pendulous, concealed from bird pollinators (Rourke and Wiens 1977), yet visible to ground-dwelling rodents. Furthermore the flowering season of *P. nana* (July – October) coincides with that of known therophilous *Proteas* which is not conducive to entomophily. The combination of these factors provides sufficient evidence to suggest that *P. nana* could be pollinated by small nonflying mammal species.

Quite clearly the pollination type for *P. nana* has not yet been established and the uncertainty regarding this issue is echoed throughout the literature (Rourke and Wiens 1977). The main objective of this study is to resolve the pollination syndrome in *P. nana* and, more specifically, to test for evidence of therophily within this Cape *Protea* species. Given the morphology of the plant, one would expect potential rodent pollinators to be competent climbers, perhaps making use of a semi-prehensile tail in order to hold on whilst reaching underneath pendulous inflorescences in search of nectar. This led us to suspect that *Dendromys melanotis* (grey climbing mouse) or *Dendromys mesomelas* (Brants' climbing mouse) could fill the role of a climbing pollinator in *P. nana* as both are extremely light weight and make use of their tails when climbing (Smithers 1983). Rourke and Wiens (1977) suggest the Cape striped field mouse, *Rhabdomys pumilio*, is perhaps the most plausible mammal pollinator of cryptic geoflorous species of *Protea*. Judging by observations in the field and having trapped a *R. pumilio* individual at this plant, they suggest that this may also be the case in *P. nana*. In late winter the soft, fleshy floral parts of *P. nana* are one of the best sources of vegetable matter (Smithers 1983). *Praomys verreauxi* (Verreaux's mouse) and *R. pumilio* have been known to exploit this resource and in doing so may play an important part in fertilisation of the flowers by carrying pollen on their heads (Smithers 1983). Dr. J. Jarvis, of The University of Cape Town, commented in Rourke and Wiens' (1977) paper that *D. melanotis*, *Mus minutoides* (dwarf mouse), *Otomys irroratus* (vlei rat) and *Acomys subspinosis* (Cape spiny mouse) may be responsible for pollination behaviour in Proteaceae. Based on previous research, it is clear that many candidates exist and the objective behind this paper is to identify those which are legitimate pollinators of *P. nana*. The efficiency of different "potentially legitimate" pollinators will be determined and a conclusion reached highlighting those deemed to be most suited.

MATERIALS AND METHODS

Exclosure experiments:

This study was carried out within a small (< 1 km^2), dense population of *P. nana* located along Bain's Kloof Pass outside Wellington in the Western Cape (33°37'44.5"S, 19°05'58.3"E, 514 m altitude). All experiments were setup at this site in mid-August (late winter) during the start of the flowering season. In an attempt to exclude small mammals, wire cages constructed out of 13mm "chicken mesh" were placed around 40 *P. nana* inflorescence buds on different bushes (Plate 1A). In order to avoid weighing the plants down, or damaging inflorescences, the wire cages were secured with a cable-tie to a dowel stick which was anchored into the ground. An additional 20 wire cages were covered in shade-cloth and placed around *P. nana* inflorescences in a similar fashion (Plate 1B). These served to exclude all potential pollinators (including insects) and tested for self-pollination. Throughout the experiment inflorescences were randomly selected at various heights and distances from the centre of each bush. Great care was taken when selecting inflorescence buds for this experiment: each inflorescence was inspected for fungal contamination and insect predation prior to being enclosed within a cage. Only immature (closed) inflorescences were selected. Ten *P. nana* bushes were then randomly chosen as controls and the total number of fresh inflorescences recorded for each bush.

Two months later, all inflorescences within the exclosures were removed from the bushes and retained for seed set analysis. The inflorescences on the control bushes were then recounted and five randomly sampled from each bush for additional seed set analysis. Determination of seed set in *Protea spp.* is somewhat complicated given that the individual fruits remain attached to the head whether or not they develop into viable seed (Wiens et al. 1983). Furthermore, viable and sterile achenes do not differ morphologically, however viable, endosperm-containing seeds can be readily identified by their soft, milky-white texture; whereas seeds interpreted as sterile have a dull-white, dry, fibrous content (Wiens et al. 1983) – Plate 1C and D. Hence ovules with swollen endoderm were used as a proxy for seed set. Such "viable seeds" were identified in the laboratory by sequentially cutting a transverse section through all florets at the level of the ovules under a dissecting microscope. Ten cones from the previous flowering season

were randomly harvested from the control bushes and used as a reference (Plate 1C) for identifying swollen ovules in experimental samples (Plate 1D).

Percentage sucrose content in nectar:

Nectar was randomly sampled from ten newly opened *P. nana* inflorescences (Plate 2A) using capillary tubes. Each sample was optically analysed for percentage sucrose content using an Eclipse handheld refractometer (Bellingham & Stanley Ltd.) which had been calibrated with distilled water at 20° C. Readings were corrected according to the International Temperature Correction Table for °Brix (% Sucrose) scale.

Mammal trapping:

Trapping was conducted on 15, 16, 22 and 23 August 2007. Sherman live traps were baited with a mixture of peanut butter and rolled oats and spaced at approximately 10 m intervals within the *P. nana* stand. Traps were set in the early evening before sunset and then inspected the following morning. A few were left open during the day for the capture of diurnal species. Captured rodents were identified, sexed, weighed and ventrally marked using black hair dye. Breeding status for each individual was recorded.

Each trapped animal was tested for the presence of pollen grains on their nose to assess which species had visited *P. nana*. Pollen was collected from the rostra of captured rodents by rubbing a cube of gelatin (impregnated with basic Fuchsin dye – Beattie 1971) mounted on a dissecting needle against the fur from the eye-level down to the nose and around the mouth. The size of the gelatin cubes and the amount of effort applied when collecting pollen from the rostra of rodents was not standardised. Gelatin cubes were immediately placed into plastic vials in order to avoid contamination. In the laboratory the cubes were melted inside the plastic vials by placing them in a bath of boiling water. Two drops of the molten Fuchsin Gelatin solution were taken from each sample and mounted on separate microscope slides. Pollen grains were counted for the full field of view at 40× magnification (0.45 mm diameter) over three different scans of the entire coverslip length (22 mm). This count yielded an approximate 3% sample of the area of the coverslip. Total pollen counts were recorded for each sample.

Scats were either obtained directly from the colon of the captured specimen or from obtained from the traps in which they were caught. Scats were placed in plastic vials to avoid contamination and stored in a household freezer for preservation until processed in the laboratory. The entire faeces sample was pulverised in a mixture of basic fuchsin gelatine (Beattie 1971) and 96% alcohol with the end of a sterile stainless steel spatula. Again, two drops of the suspension from each sample were placed onto separate microscope slides and scanned in the same way as previously mentioned. Where the number of pollen grains in a single scan (approximately 1% of the area of the coverslip) exceeded 200, the remainder of that scan was completed and no additional scans were done.

Random individuals of each species sampled were taken back to the laboratory for behavioural observation. Individuals were observed in a glass tank which contained two *P. nana* bushes with a total of 15 undamaged newly opened inflorescences. Conditions in the tank were setup in an effort to simulate a scenario in the field. Sunflower seeds were provided as a backup food source to maintain energy levels in captured rodents. Individuals were observed immediately after being placed in the tank and their behaviour within the first ten minutes was documented. Each individual was observed for a minimum of 30 minutes in total. Nocturnal species were observed at night with a red light and diurnal species were observed during the day. Digital video footage of each individual was taken.

Species which displayed viable pollination behaviour were dusted on the rostrum with ultraviolet (UV) fluorescent powder and left in the tank for an extended period of time (>2 hours). Flowers were then inspected with a UV light for presence of UV powder, with particular attention to the surface of stigmas.

Statistical analyses:

Given the nature of the data and inconsistent *n* for each species caught, pollen-count data were represented graphically as a bar graph with one standard deviation added. It was noticed that many of the shade-cloth exclosures (8 out of 20) had been removed from their respective *P. nana* bushes. This could be attributed to wind or curiosity of *Papio ursinus* (Chacma baboons) observed in the area. Additional exclosures had also pulled

inflorescences off from their peduncles as a result of strong winds in the area. Again, no statistical test could be performed and seed set data were represented as a bar graph with one standard deviation added. A paired t-test was performed for comparing the number of control flowers at the beginning of the experiment and those still present two months later. Statistical analyses were performed using STATISTICA® 7.0 (Statsoft, Inc. 2004).

Plate 1: A – Wire cage experiments designed to exclude small mammals, yet allowing for the passage of potential insect pollinators. B – Shade-cloth covered cages designed to exclude all pollinators. C – Transverse section through ovules from a year-old (2006) *P. nana* cone. D – Transverse section through a fresh cone (2006). Arrows indicate swollen endoderm, which was used as a proxy for seed set. Photographs – A. Biccard (2007).

RESULTS

Rodent captures

In total 45 rodents were caught with 11 recaptures, totalling 56 captures for the study period (Table 1). Four rodent species were captured, each of which displayed presence of *Protea* pollen on their noses and in their scats (Figure1A and B). Captured species included *Rhabdomys pumilio*, *Otomys irroratus*, *Praomys verreauxi* and *Aethomys namaquensis*. *R. pumilio* was by far the most common of the rodents captured at the site followed by *A. namaquensis*, *P. verreauxi* and lastly *O. irroratus* (Table 1).

Table 1: Composition of mammal captures in the present study.

Species	No. Individuals	No. Captures	Mean Body Mass (g)
Rhabdomys pumilio	25	32	41
Otomys irroratus	1	1	73
Praomys verreauxi	5	5	21.4
Aethomys namaquensis	5	7	59
Total	36	45	

Pollen counts

The greatest number of pollen grains present on the nose of a rodent was that of *O. irroratus* (Figure 1A). However, only one individual was captured throughout the study period, hence this value merely serves a qualitative purpose in that it shows presence of *Protea* pollen on the rostrum of this species. In contrast, only 25 pollen grains were present in the scat of *O. irroratus* – the lowest in comparison to the other species (Figure 1B). Again this result should be treated with caution for the same aforementioned reason. *A. namaquensis* had the second highest average of *Protea* pollen grains on its nose (361), and the highest average of pollen grains (8) in its scat (Figure 1 B and A). *P. verreauxi*

was observed to have the third highest average of pollen on its rostrum (7 grains). However, this average was only taken over two individuals, one of which had 11 pollen grains removed off its nose. Furthermore *P. verreauxi* had the second highest average of pollen grains in its scat (196). The most common species, *R. pumilio* (n = 32), had the lowest average of pollen grains on its nose (5) and the second lowest average of pollen grains in its scat (153).

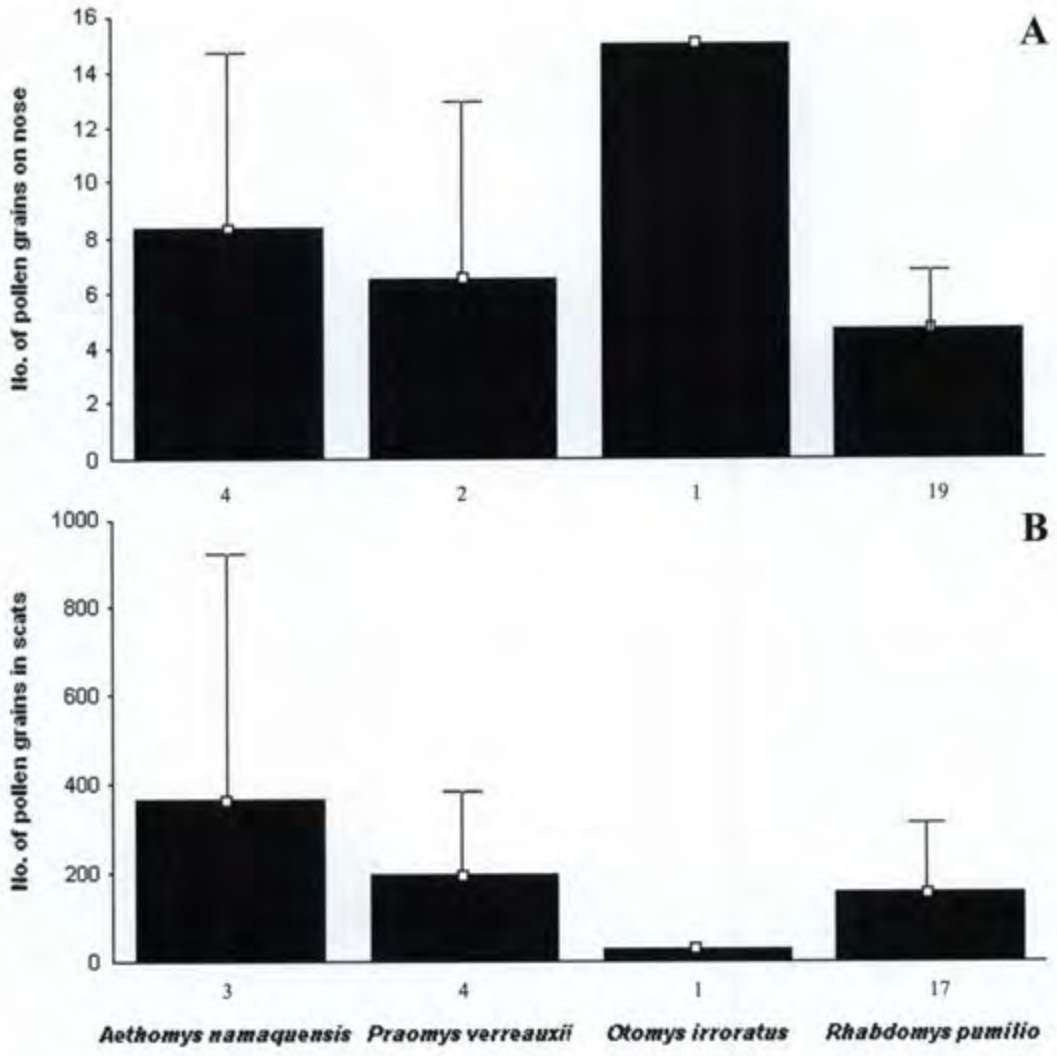

Fig. 1: Relative number of pollen grains counted for nose (A) and scat (B) samples from rodent species which were trapped. The numbers of individuals upon which these results are based are represented below each bar. The number of pollen grains per sample (n) represent 3% surface area of the coverslip. Values are the means between the number of individuals for each species + 1 SD.

Rodent behavioural observations

Rhabdomys pumilio:

Upon being released into the tank, *R. pumilio* was observed lapping nectar from *P. nana* inflorescences whilst standing upright on its hind feet. Having visited all the inflorescences within reach from the ground, *R. pumilio* then proceeded to climb into *P. nana* in search of more nectar-laden inflorescences. It was quite obvious that *R. pumilio* was in an unfamiliar habitat as it often lost its balance and fell, confirming that it is indeed a poor climber. However the most surprising observation was the ability of this species (average mass of 41 g) to climb throughout *P. nana* and manage to lap nectar from even the highest inflorescences. More importantly this was performed without inflicting any significant damage to florets. However, a female individual displayed highly destructive behaviour, chewing away at the bases of florets in order to access nectar. This same individual was later observed chewing off entire inflorescences (one at a time) whilst climbing in *P. nana.* Chewed-off flower heads were carried down to ground-level where florets were chewed away at the base to allow for easy access to nectar. During an extended observation of more than three hours, it was observed that every inflorescence within the tank had been removed from *P. nana* and some of the florets had been chewed or torn away. This was by far the most destructive behaviour observed throughout the study. Most importantly it was obvious that this destructive behaviour was performed deliberately and almost immediately after *R. pumilio* had been released into the tank. It clearly recognised the nectar of *P. nana* as a food source and knew exactly how to obtain it in the most efficient manner without any uncertainty. Apart from this, legitimate pollination behaviour of *P. nana* by *R. pumilio* was observed.

Praomys verreauxi:

This species displayed remarkable climbing ability and made use of its disproportionately long tail and low average mass of 16.5 g (average mass excludes a mature female of 41g which was most likely pregnant at the time of capture) to maintain balance whilst lapping nectar from the topmost *P. nana* inflorescences. Large quantities of pollen covered the entire face of *P. verreauxi* after visiting flowers, and regular wet-preening of its snout

was observed. Despite this, fluorescent powder, which had been applied to the rostrum of a single individual, was found on the stigmas of every inflorescence the following morning when inspected under UV light in the dark. Virtually no damage was sustained by those flowers which had been visited. Legitimate pollination behaviour was observed between this rodent species and *P. nana*.

Aethomys namaquensis:

Of all the species observed, *A. namaquensis* was clearly the least efficient at climbing. In comparison to the other rodent species (barring the single *O. irroratus* specimen) *A. namaquensis* showed the greatest average mass of 59 g and, despite poor climbing capabilities, maintained a keen interest in *P. nana* inflorescences. Most of the inflorescences which were visited by this species were in close proximity to the ground, allowing relatively easy access for this comparatively large and rather clumsy rodent. However *A. namaquensis* was still able to climb and access inflorescences at about mid-height in *P. nana*, above which the branches could not support the weight of this rodent. Overall, legitimate pollination of *P. nana* was displayed.

Otomys irroratus:

Only a single specimen was caught and observed, yet it was apparent that this species showed no interest in *P. nana* inflorescences whatsoever (even when nectar-laden inflorescences were placed on the ground). *O. irroratus* was observed eating leaves and other plant material around the base of *P. nana*. No climbing or pollination behaviour was observed.

Seed set results:

Lowest average seed set (<1) was observed in those inflorescences which were enclosed with wire and shade-cloth (Figure 2). Surprisingly, controls for the current year of study displayed lower average seed set (approximately 2) than those inflorescences which were enclosed only with wire (3.7). Highest average seed set of 5 per inflorescence was found in the controls from the previous flowering season.

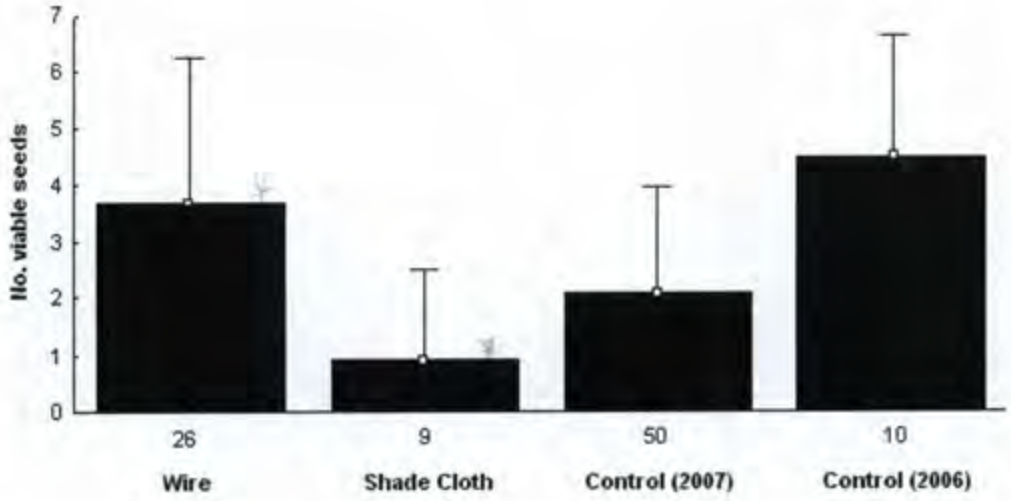

Fig. 2: Seed set in wire cage, shade cloth covered and control (from the current and previous flowering season) *P. nana* inflorescences. The number of inflorescences for each result is shown below the bars. Values are means + 1 SD.

Inflorescence counts and nectar sucrose content

The mean number of inflorescences per *P. nana* individual counted at the beginning of the experiment was 40. Two months later, after recounting the number of inflorescences on the same individuals, the mean number of inflorescences had decreased to 32 per *P. nana* individual. This difference was found to be statistically significant ($n = 10$, $t = 2.53$, $df = 9$, $P < 0.05$) with 20% of inflorescences lost to predation.

The mean percentage sucrose content recorded for the nectar of ten inflorescences was 29.4% ($S^2 = 38.3$, $SD = 6.2$). It was also observed that *P. nana* produced noticeably high amounts of nectar (>1 ml) which had a distinct yeasty smell and sweet taste.

Additional observations

Throughout the study many *P. nana* inflorescences were found lying on the ground, some in distinct piles with all the florets torn out. However the most common observation was the systematic removal of florets on only one side of the inflorescence whilst still attached to the bush (Plate 2B). Some of these inflorescences were also observed along the "runways" and burrow entrances of rodents. On warm, sunny days domestic bees (*Apidae*) were observed visiting *P. nana* flowers.

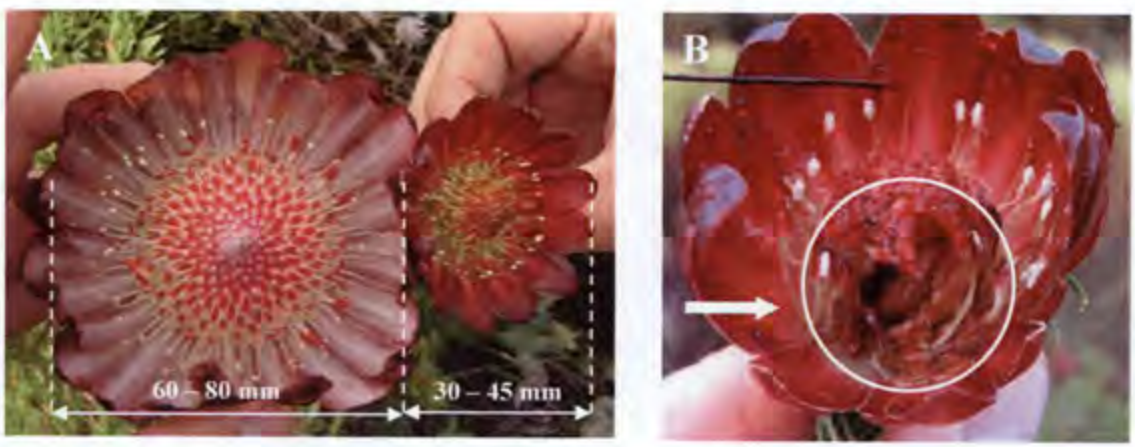

Plate 2: A – Comparison between morphology of known therophilous *Protea amplexicaulis* (left) and the subject of this study, *Protea nana* (right). Note the different diameters of the flower heads and the contrasting colours of the involucral bracts. B – Evidence of small mammal visitation of *Protea nana* (encircled). Note the removal of florets at the base of the inflorescence. Photographs – A. Biccard (2007).

DISCUSSION

Prior to this study, *P. nana* was not regarded as a rodent pollinated species. Its shrubby growth form and red inflorescences borne well above the ground have lead many to accept various pollination syndromes other than therophily. Comments by Rebelo (unpublished) and a publication by Bond and Goldblatt (1984) consider *P. nana* as an entomophilous *Protea*. Other publications simply describe *P. nana* as a species of

uncertain pollination type (Rourke and Wiens 1977). However in accordance with our suspicions, results from this study show incriminating evidence that *P. nana* is in actual fact a therophilous species. The presence of pollen grains in scats and on the noses of captured rodents (Figure 1A and B), in addition to observational results, provides clear evidence of this. These results agree with previous publications which state that *R. pumilio*, *A. namaquensis* and *P. verreauxi* are pollinators of various Cape *Protea* species (Wiens and Rourke 1978, Rourke 1980).

The suggestion of *O. irroratus* as a pollinator of Proteas (J. Jarvis in Rourke and Wiens 1977) is not well supported by the findings of this study. Behavioural observations and the average mass alone (122 g in males and 114 g in females – Smithers 1983) provide sufficient evidence that this rodent would not be able to climb in *P. nana* as it is simply too heavy. Its complete ignorance of nectar-laden inflorescences lying on the ground during observational trials in the laboratory confirms that *O. irroratus* is not a likely pollinator of *Protea* species. Low amounts of *Protea* pollen in the scat of *O. irroratus* reiterates this point. Given that only a single *O. irroratus* individual was caught, the high amount of *Protea* pollen present on its rostrum (Figure 1A) could be explained by potential feeding on the numerous chewed off inflorescences in the field. This is to be expected as the basal parts of the bracts and styles constitute some of the most succulent and fleshy vegetable matter available in late winter (Smithers 1983). With such an attractive food source, and as a well known vegetarian (Smithers 1983), *O. irroratus* is more likely to eat flowers than pollinate them (Perrin 1980, Vlok 1995).

Speculation by Rourke and Wiens (1977) that *R. pumilio* is a potential pollinator of *P. nana* can be verified by the results of this study. The presence of pollen on its rostrum and an average of 153 grains in its scat (Figure 1A and B), together with observational results, confirm this rodent is a legitimate pollinator of *P. nana*. Its destructive behaviour observed in the laboratory has also been recorded in previous studies where *R. pumilio* has chewed away the soft floral parts of *P. nana* and *Protea subulifolia* (Rourke an Wiens 1977, Wiens and Rourke 1978). Judging from laboratory observations and the high number of inflorescences observed along rodent runways in the field, it can be assumed that the decrease in mean number of inflorescences for each *P. nana* individual is the result of predation by *R. pumilio*. Results showing a significant

decrease of 20% in inflorescences per *P. nana* individual over a period of two months support this notion.

There is no mention of complete removal of inflorescences from Proteaceae by rodents in the literature, but judging from the behaviour of *R. pumilio* and its relatively high frequency in the area (n = 25 – Table 1), it is likely that most of the inflorescences observed on the ground during fieldwork have been chewed off by *R. pumilio*. An alternative explanation could be the foraging behaviour of *P. ursinus* (Chacma baboons) which were observed near the study site. These primates have been known to remove large amounts of inflorescences from Proteaceae for their nectar (Wiens et al. 1983, Rebelo and Breytenbach 1987). However, most of the inflorescences on the ground had clearly been bitten off and the florets chewed away at the base (Plate 2B), in an analogous manner to that observed in the laboratory by *R. pumilio*. Those inflorescences on the ground which had all their florets torn out are most likely those which have been removed by *P. ursinus* but these constituted the minority.

The high number of florets within a *P. nana* inflorescence is commonly observed throughout the Proteaceae. Such an increase in reproductive units for a single inflorescence is far beyond that required to maintain successful reproductive levels (Rourke and Wiens 1977). This has been regarded as an adaptation to cope with the destructive activities of potential pollinators like that of *R. pumilio* (Plate 2B) (Rourke and Wiens 1977). In spite of this, no actual benefit can be realised by *P. nana* if entire inflorescences are removed from the plant. Therefore *R. pumilio* can only be regarded as a legitimate pollinator for those inflorescences which it does not chew off. Hence the efficiency of *R. pumilio* as a pollinator of *P. nana* is suboptimal.

Pollen grain exines are indigestible and pass directly through the gut, yet a number of mammal species are capable of extracting nutrients from *P. humiflora* and *P. subulifolia* pollen (Van Tets 1997) and *Aethomys* has been noted to satisfy its nitrogen requirements based on a diet of pollen (Van Tets et al. 2000). This could explain the highest number pollen grains counted in the scat of *A. namaquensis* (Figure 1B). Together with the observational results and number of pollen grains on its nose (Figure 1A) *A. namaquensis* can be regarded as a legitimate pollinator of *P. nana*. Despite supportive results from previous studies (Wiens and Rourke 1978, Wiens and Rourke

1983, Fleming and Nicolson 2002) this species was not included in the list of potential small mammal pollinators of proteas as suggested by J. Jarvis in Rourke and Wiens (1977).

Judging from its relatively high average body mass (Table 1) and subsequent poor climbing ability, *A. namaquensis* is one of the least efficient pollinators of *P. nana* in comparison to the other rodents captured in this study. The few inflorescences within reach from the ground are the most likely to be visited by this rodent as it would require too much effort to climb for nectar when an alternative source is available at ground level from *P. amplexicaulis*. Furthermore it is likely that *P. amplexicaulis* is the preferred source of nectar for *A. namaquensis* as the inflorescences are markedly larger and should theoretically yield a greater volume of nectar in comparison to *P. nana* (Plate 2A).

P. verreauxi was undoubtedly the most efficient pollinator of *P. nana* observed during this study. In comparison to the other rodent species *P. verreauxi* individuals displayed impressive climbing ability, visiting numerous flowers without inflicting noticeable damage and spending the greatest amount of time climbing in *P. nana*. It was also observed that *P. verreauxi* spent a great deal of time wet-preening its snout and it presumably ingested much of the pollen which had been deposited there. This could account for the comparatively lower number of pollen grains on its nose and higher number in its scat (Figure 1A and B). The relatively low mass (Table 1) of *P. verreauxi* allowed it to climb with ease, as the branches of *P. nana* were able to withstand its weight and its disproportionately long tail was used as a counter-balance to access inflorescences at the outer most extremities of the bush. Out of all the rodents captured in this study, *P. verreauxi* is most suited for climbing and pollinating *P. nana*.

The nectar samples of *P. nana* inflorescences in this study were found to have an average sucrose content of 29.4%. Known therophilous species such as *Protea cryophila*, have nectar recorded with percentage sucrose levels as low as 27.6% and ornithophilous species (e.g. *Protea repens*) at around 12% (Wiens et al. 1983). Clearly the mean sucrose content in the nectar of *P. nana* is high enough to make it worthy of rodent pollination. This result, in combination with floral morphology, flowering time, and a yeasty scent confirm therophily in *P. nana*. However, the distinct red colour of the involucral bracts introduces some confusion as this is a common feature of ornithophilous plants (Raven

1973). Rebelo and Breytenbach (1987) argue that appearance is overruled by odour which seems to play the major role in attracting rodents to therophilous Proteaceae. Hence one would expect therophilous species to be selected on account of the odour they emit and not their appearance. Another possible explanation could be attributed to the suggestion that therophily is a derived system from an ancestral ornithophilous prototype (Wiens et al. 1983). Such prototypes would have been somewhat "pre-adapted" for non-flying mammal pollinators, producing a volatile olfactory attractant, copious amounts of sucrose-rich nectar and possessing the necessary structural features such as mechanically strengthened inflorescences able to accommodate a relatively large animal without undue destruction of floral parts (Wiens et al. 1983). It is plausible that this could have been the case in *P. nana* and the red bracts observed today are merely remnants of a past ornithophilous syndrome.

Morphological features present in the inflorescence of *P. nana* are analogous to those of well known therophilous species such as *P. amplexicaulis* (Plate 2A). The major difference however is the elevation of inflorescences well above the ground in *P. nana*. Based on this observation, it is clear that *P. nana* selects for an efficient climbing rodent pollinator like that of *P. verreauxi* found in this study. It was however predicted that *D. melanotis* or *D. mesomelas* would fill this capacity but none of these species were captured during this study. Nevertheless *P. verreauxi* can be regarded as a competent pollinator of *P. nana* and it remains to be found whether other rodents with similar climbing abilities (i.e. *Dendromys*) are as efficient.

The results obtained from the seed set experiments (Figure 2) are somewhat dubious as it was difficult to tell which ovules from inflorescences of the current flowering season were fertilised and which were not. Inflorescences were harvested after only two months given the time constraint of this study, and as a result, not enough time was allowed for the endoderm of potentially pollinated ovules to swell. Plate 1C shows that fertilised ovules from *P. nana* cones of the previous flowering season (2006) can be clearly distinguished from unfertilised ones. This is because they have had enough time to develop. However many *P. nana* inflorescences from the current flowering season (2007) were simply too immature to allow for discrimination between fertilised and unfertilised ovules – Plate 1D represents one of the best examples obtained during this

study. Therefore it is likely that the lower average seed set of controls observed this year in comparison to that of last year (Figure 2) can be explained by the inadvertent overlooking of fertilised ovules in immature control inflorescences. Ultimately this would imply that the observed average seed set for controls of the current flowering season are underestimates and the results obtained for the controls of last year's cohort a more accurate approximation of natural seed set in *P. nana*.

Lowest average seed set was observed in those inflorescences which were covered with shade-cloth exclosures (Figure 2). This was to be expected as all potential pollinators were excluded, implying that fertilised ovules were most likely self-pollinated. An average seed set of <1 per inflorescence for this experiment, is supported by the findings of Rourke and Breytenbach (1987) who mention that few seeds are normally set by self pollination in Proteaceae. Inflorescences which were covered in wire exclosures were observed to have higher average seed set in comparison to shade-cloth experiments and controls from the present cohort (Figure 2). This could be explained by the observed pollination behaviour of honeybees, which were otherwise excluded from shade-cloth controls. This observation is common in therophilous proteas and is not surprising since bees are regarded as generalist pollinators (Rourke and Wiens 1987). Geoflory of inflorescences in known therophilous proteas have been recognised as a cryptic adaptation to reduce insect visitation (Rourke and Breytenbach 1987). Although the inflorescences of *P. nana* are not geoflorous nor very cryptic, it is suspected that their red appearance makes them less conspicuous to insect pollinators, particularly bees – a trend observed throughout the non-entomophilous inflorescences of Proteaceae (Raven 1973, Rourke and Breytenbach 1987). The impact of rodents on seed set in this study was difficult to detect given the lack of time for fertilised ovules to develop in experimental inflorescences. However, higher average seed set in the control inflorescences from last year's flowering season suggest that rodents were in fact excluded from wire and shade-cloth cages. This would imply that they do have an effect on seed set and are therefore important pollinators in *P. nana*.

Conclusion:

From observational results, it is evident that the different rodent species captured during this study can be ranked according to their relative pollination efficiency of *P. nana*. In order from least to most efficient, rodents are ranked as follows: *O. irroratus*, *A. namaquensis*, *R. pumilio* and *P. verreauxi*. Ability to climb and frequency at which inflorescences were visited were the major factors which determined this ranking of pollination efficiency.

Floral structure, pendulous inflorescences, highly branched growth form and sucrose-rich nectar with a distinct yeasty odour in *P. nana* clearly select for a climbing rodent pollinator. *P. verreauxi* displayed legitimate pollination behaviour and was by far the most efficient of all the rodents captured in this study. It is likely that the other rodent species, apart from *O. irroratus*, do pollinate *P. nana* but are nowhere near as competent as *P. verreauxi*. With alternative sources of nectar, provided by *P. amplexicaulis* at ground level it is likely that *A. namaquensis* (nocturnal) and *R. pumilio* (diurnal) would exploit this resource before turning to *P. nana* for nectar. Being the smallest nocturnal rodent captured in this study, *P. verreauxi* is probably out-competed by the larger *A. namaquensis* at ground level for nectar. With superior climbing abilities, it seems logical that *P. verreauxi* would opt to exploit the relatively "untapped" resource of *P. nana*.

In conclusion, there exists insurmountable evidence to regard the pollination syndrome of *P. nana* as therophilous. Additional research, allowing more time for excluded inflorescences to set seed is needed to gain a perception of how dependant *P. nana* is on rodent foraging activity as a means for pollination. Trapping at other populations of *P. nana* may provide further insight and perhaps explore the possibility of additional rodent species as pollinators.

REFERENCES

Beattie, A. J. (1971). A technique for the study of insect-borne pollen. *Pan-Pacific Entomology.* **47**: 82.

Bond, P. and Goldblatt, P. (1984). Plants of the Cape flora: a descriptive catalogue. *Journal of South African Botany* Supplementary Volume: 13.

Fleming, P. A. and Nicolson, S. W. (2002). How important is the relationship between *Protea humiflora* (Proteaceae) and its non-flying mammal pollinators? *Oecologia* **132**: 361 – 368.

Perrin, M. (1980). The feeding habits of two co-existing rodents, *Rhabdomys pumilio* (Sparrman, 1784) and *Otomys irroratus* (Brant's, 1827) in relation to rainfall and reproduction. *Acta. Ecol.* **1**: 71 – 89.

Raven, P. H. (1973). Why are bird flowers predominantly red? *Evolution* **26**: 674.

Rebelo, A. G. and G. J. Breytenbach. (1987). Mammal pollination in the Cape Flora. In: Rebelo, A. G. (ed.), A preliminary synthesis of pollination biology in the Cape Flora **141**. South African National Scientific Programmes Report, CSIR, Pretoria.

Rebelo, A. G. (1995). Sasol Proteas. A field guide to the Proteas of Southern Africa. Fernwood Press and National Botanical Institute, Vlaeberg, South Africa. 224 pp.

Rourke, J. P. and Wiens, D. (1977). Convergent floral evolution in South African and Australian Proteaceae and its possible bearing on pollination by non-flying mammals. *Annals of the Missouri Botanic Gardens* **64**: 1 – 17.

Rourke, J. P. (1980). The proteas of southern Africa. Purnell, Cape Town. 240 pp.

Smithers, R. H. N. (1983). The mammals of the southern African subregion. University of Pretoria. 736 pp.

Tets, I. G. van (1997). Extraction of nutrients from *Protea* pollen by African rodents. *Belg. J. of Zool.* **127**: 59 – 65.

Tets, I. G. van, Hutchings, K. and Nicolson, S. W. (2000). Relative merits of using purified proteins and natural foods in studies of wildlife nutrition: the nitrogen requirements of a rodent pollinator. *African Journal of Zoology* **35**: 207 – 215.

Vlok, J. (1995). Fynbos and rodents. *Veld flora* **81**: 105 – 107.

Wiens, D. and Rourke, J. P. (1978). Rodent pollination in southern African *Protea* species. *Nature* **276**: 71 – 73.

Wiens, D., Rourke, J. P., Casper, B. B., Rickart, E. A., Lapine, T. R., Petersen, C. J. and Channing, A. (1983). Non-flying mammal pollination of southern African *Proteas*: a non-coevolved system. *Annals of the Missouri Botanic Gardens* **70**: 1 – 31.

Plagiarism Declaration:

1. I know that plagiarism is wrong. Plagiarism is to use another's work and to pretend that it is one's own.
2. Each significant contribution to, and quotation in, this project from another source has been attributed, cited and referenced.
3. This project is my own work
4. I have not allowed and will not allow anyone to copy my work with the intension of passing it off as his/her own.

Signature: