The ecology of rodent pollination in *Liparia parva* (Fabaceae)

Andrew Letten

Supervisor: Jeremy Midgley
University of Cape Town

Cape spiny mouse, *Acomys subspinosis* foraging for nectar at *Liparia parva* flower. Photograph by author.

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Abstract

The evolution of non-flying mammal pollination has given rise to a broad suite of adaptive plant traits including dull coloured and geoflorous flowers, copious sucrose rich nectar, nocturnal anthesis and nectar production and a musky odour. The Fynbos endemic, *Liparia parva* (Fabaceae) has been recognised to exhibit several of these traits. Based on this observation, field studies were carried out on the Cape Peninsula, South Africa, to investigate the prediction that *L. parva* is rodent pollinated. Several lines of evidence indicate that flowers of *L. parva* are visited by rodents. These include; the presence of *Liparia* pollen in the faeces of live trapped rodents; observations of captive rodents selectively foraging at flowers of *L. parva*; floral debris underneath *L. parva* plants; and video footage captured of a rodent visiting *L. parva* flowers in the field. However, a strong plant-pollinator relationship was apparent only in the Cape spiny mouse, *Acomys subspinosis*. Captive *Acomys* foraged non-destructively at flowers of *L. parva,* becoming visibly dusted with pollen. The exclusion of rodents from flowers resulted in a significant reduction in seedpod set, indicating rodents do contribute to pollination success in *L. parva*. Additional evidence that *L. parva* is adapted to pollination by rodents includes nocturnal floral anthesis and large amounts of total nectar in inflorescences. The findings of this study provide substantial evidence for rodent pollination in *L. parva* and thus represent the first report of rodent pollination in a legume.

Introduction

The pollination of flowers by rodents was first documented almost 30 years ago in South African Proteaceae (Wiens and Rourke, 1978; Wiens et al. 1983). Since then the rodent pollination syndrome has been demonstrated in only a handful of novel plant species (Lumer, 1980; Cocucci and Sersic, 1998; Johnson et al. 2001). It is probable however, that the scarcity of documented cases exaggerates the rarity of the syndrome. For instance, in the Cape Floral Kingdom, South Africa, around 35 putatively rodent pollinated *Proteas* (Rebelo and Breytenbach, 1987) remain to be experimentally tested. Furthermore, more species are likely in those areas of the world such as the neotropics and Asia, where the ecology of nocturnal mammals has been poorly studied (Carthew and Goldingay, 1997). Part of the problem in rodent pollination studies stems from the difficulty in observing these small, mostly nocturnal animals under natural conditions. For example, the visitation of *Protea* flowers by their rodent pollinators has yet to be observed in the wild (Fleming and Nicolson, 2002). This paper presents the first rodent pollination study to employ field cameras to observe rodents under natural conditions and the first account of rodent visitation/pollination in a legume.

Non-flying mammal pollination (therophily) is associated with a broad suite of adaptive plant traits. These include; dull coloured flowers, copious sucrose rich nectar, inflorescences borne close to the ground, nocturnal anthesis and nectar production and a musky odour (Turner, 1982; Rebelo and Breytenbach, 1987, Carthew and Goldingay, 1997) In addition, the syndrome in Proteaceae is characterised by a winter flowering period and a distinctive 10mm stigma-nectar distance (Wiens et al, 1983). The relative importance of each of these traits varies depending on the specific type of non-flying mammal pollinator (Johnson et al. 2001). For instance, the constraint on geoflorous flowers is likely to be much more pronounced in rodents (Wiens et al, 1983) than in canopy dwelling marsupials (Turner, 1982).

The papilionoid legume, *Liparia parva*, is a long-lived perennial shrub, endemic to the Cape Peninsula of South Africa (Schutte, 1997). The possibility of rodent pollination in *L. parva* has been suggested, based on its morphological similarities to proven rodent pollinated species (Rebelo and Breytenbach, 1987, Schutte and Van Wyk,

1994). However, until now there has been no investigation into this hypothesis. The most noticeable features of *L. parva* that indicate adaptation to rodent pollinators include pale coloured flowers, inflorescences close to the ground and a winter flowering period.

Demonstrating non-flying mammal pollination of flowers requires the synthesis of several lines of ecological evidence. Firstly, animals must be observed visiting flowers frequently and non-destructively. Secondly, it must be shown that in the process of visiting flowers they acquire pollen in such a way that it could be transferred from one flower to another. Thirdly, evidence should be provided that visits to flowers do result in active pollination and consequential seed production (Carthew and Goldingay, 1997).

The primary aim of this study was to test the hypothesis that *L. parva* is indeed rodent pollinated. For this purpose, data were collected to address the following questions: (1) Do rodents visit flowers of *L. parva*? (2) Do rodents transfer *L. parva* pollen? (3) Does rodent visitation to *L. parva* flowers result in seed set? (4) Does *L. parva* exhibit adaptive traits for rodent pollination?

Study Area and plants

The field component of the study was carried out near Redhill (34°11′ S, 18°24′ E, elevation ≈ 250m) adjacent to the Cape of Good Hope Nature Reserve, Western Cape Province, South Africa. *Liparia parva* is relatively common in the area but tends to be more clustered around rocky outcrops and less abundant in the intervening surrounds.

Materials and methods

The total time spent at the site was approximately 70 daylight hours over a two-month period from the beginning of July to the end of August 2005.

Field photography

The equipment used for the photographic component of the study consisted of one Trailmaster TM700v passive Infrared Video Trail Monitor connected to a Sony Handycam and TM Video Light controller, three Trailmaster TM1500 Active Infrared Trail Monitors used in conjunction with three TM35-1 Camera Kits and one video surveillance system that was custom built for the purposes of this project using a LANC *Pix*Controller board connected to a Sony digital Handycam. This combination made it potentially possible to have two video cameras and three still cameras in operation at any one time. The cameras in these systems are triggered by sensors that detect either motion (TM1500) or a combination of body heat and motion (TM700v and LANC *Pix*Controller board) but need to be calibrated depending on the size of the animal being studied. It was thus necessary to spend a certain amount of time experimenting with the camera set-ups in order to determine the settings that would be optimal for detecting small animals such as those anticipated in this study.

Photographic equipment was deployed in the field on five separate nights between the end of June and the beginning of September 2005. Preliminary experimentation indicated that the level of sensitivity required to detect a small animal was susceptible to

false triggers due to the movement of vegetation in anything more than a light breeze. This finding coupled with the vulnerability of the equipment to water damage made it necessary to select clear and relatively windless nights. On every night of photographic investigation, the equipment was set-up in the field at approximately 5.00 pm and collected the following morning at approximately 8.00 am. On the first night of the photographic study all the camera set-ups were deployed, however on the following nights the still photography set-ups were abandoned, as they were judged unsuitable for the study given the precision required.

Rodent trapping – species composition

A preliminary investigation of the rodent species composition on and off *L. parva* patches was done through live trapping of rodents on the nights of the 2nd and 3rd August, 2005. On both nights, sites selected for trapping included areas where *L. parva* was either relatively abundant (mean = 5 plants/25m^2) or entirely absent. Trap sites consisted of between 6-15 traps spaced at 5m intervals in two rows and baited with peanut butter and oats. Traps were set up at 5.00 pm and rechecked at 7.00 am the following morning. Captured rodents were identified and either released immediately onsite or kept in a tank overnight for observations and returned to the trap site the following morning. On the first night a total of 47 traps were laid out on 5 separate rocky outcrops populated by *L. parva* and 20 traps over 2 control sites where there was no *L. parva*. On the second night a total of 37 traps were laid out on 3 separate rocky outcrops populated by *L. parva* and 47 traps over 4 control sites where there was no *L. parva.* Combining the two sample nights together gave a total of 84 traps (0.21ha) on *L. parva* patches and 67 traps (0.17 ha) off *L. parva* patches.

Rodent trapping – pollen transfer

A second round of rodent rapping for pollen load sampling was carried out over the three nights of the 19th, 20th and 21st of August. A total of 10 (2x5), 64 (8x8) and 56 (8x6)

traps were setup respectively on the three nights at a different rocky outcrop populated by *L. parva* and separated by a minimum distance of 200m. On each trap night, traps were laid in a grid, spaced at 5m intervals and baited with peanut butter and oats. Captured rodents were removed from traps at 7.00 am and identified. In order to check for pollen, rodents were placed in a mesh bag so that their snouts protruded from the mesh. A standard-sized (5 mm^3) block of agarose gel dyed with fuschin was then used to swab the snouts of rodents for approximately 20 seconds. The agarose block was subsequently melted and the total amount of pollen in a 5µl sample was quantified microscopically. In addition to the gel swabs, faeces were collected from the traps and stored in 70% alcohol until they could be processed. To check for pollen in faeces a small amount (approx $2mm^3$) was crushed on a slide and mixed with fuschin. Slides were examined at 40x magnification and the amount of *L. parva* pollen quantified over four scans of the length of the coverslip. All captured rodents were kept for 24h in individual glass tanks for observations and foraging choice experiments, following which they were released at the trap site.

Foraging choice experiments

In addition to observations and photographic recording of the foraging behaviour of captive rodents at flowers of *L. parva*, foraging choice experiments were carried out to test whether rodents visit *L. parva* flowers selectively. These were conducted using a T-maze after the procedure described by Wiens et al. (1983). All the rodents (N = 17) captured during the trap nights of the 19th, 20th and 21st were tested once only. Tests were either run between 8.00pm and 12.00am on the night following the trap night (nocturnal species) or between 10.00am and 12.00pm the same day (diurnal species), to approximate the animals' normal foraging periods. The T-maze was constructed from transparent perspex tubing (5×5cm) that included an initial runway of 22cm in length that split at right angles into two interchangeable arms each of 48cm and each terminating with an accessible chamber. For each trial a freshly picked head of each of *L. parva* and *Mimetes fimbriifolius* (bird pollinated Proteaceae) were assigned randomly to opposite chambers

of the T-maze. Prior to testing, rodents were transferred in a box trap from their tanks to the T-maze entrance. Each trial began when the front door of the box trap was opened allowing the rodent access to the maze. If a rodent did not emerge within 5 minutes a light was shone briefly from the opposite end of the box trap. After a rodent entered the T-maze and for the duration of the experiment (5 min) several records were made, including: initial arm choice, initial foraging choice, secondary foraging choice if relevant and total foraging time. Rodents still feeding at the end of the 5 min test period were timed until they stopped feeding.

Petal loss vs. flower height

It was observed before the start of the study that captive rodents removed the standard petal of *L. parva* flowers while foraging for nectar. It was thus assumed that the loss of the standard petal on the flowers of naturally growing plants was an indication of rodent visitation to a flower/inflorescence. Based on this assumption it was secondarily hypothesised that if a non-flying mammal is the main pollinator of *L. parva*, then standard petal loss (visitation) should decrease with height. Subsequently the intensity of "visitation" and the height above ground was measured for 30 randomly chosen inflorescences on nine plants. The intensity of "visitation" was measured as a percentage of the flowers on an inflorescence that had lost the standard petal of the total number of flowers on that inflorescence.

Pollinator exclusions and seed set

In order to determine whether rodents are important for seed set in *L. parva*, 20 pairs of inflorescences with equal numbers of flowers at the same height were selected from 10 individual plants i.e. four inflorescences per plant. From each pair one inflorescence was enclosed in a wire mesh, with a mesh diameter of 14×19 mm, that allowed access to insects but excluded rodents. The other inflorescence in each pair was marked as a control but not caged. It was necessary to utilise inflorescence buds that were already

partially opened due to the rarity of closed flowers at the time of the start of the experiment. However only inflorescences in which all the flowers possessed intact standard petals were used for rodent exclosures. Inflorescences were harvested a month later and the number of seed pods counted in each inflorescence. Wilcoxin's paired t-test was performed to determine if there was a significant difference in the number of pods in inflorescences protected from rodents compared with controls. In addition to their primary function, the caged exclusions were also used to test whether in the absence of visitation, flowers of *L. parva* retained all their standard petals.

Floral Characteristics

A coarse measure of the pattern of floral anthesis in *L. parva* was obtained by recording the number of open flowers on 24 marked inflorescences (322 flowers) on 6 plants twice a day (8.00am and 5.00pm) over a 72 hour period. A flower was considered open when the standard petal had reflexed at least 5mm from the fused keel petals that enclose the androecium. A Chi-square test was performed to determine whether timing of flower opening differs significantly from random.

Nectar volume, sugar concentration and nectar-stigma distance was measured for 20 flowers. A maximum of 2 flowers per inflorescence and 2 inflorescences per plant were sampled. Nectar volume was measured using 5µl capillaries and the nectar sucrose concentration was measured using a refractometer.

Results

Qualitative field observations

The standard petals of recently mature *L. parva* flowers were frequently observed deposited underneath *L. parva* plants throughout the study area (Fig. 1A). Distinct from the scatterings of standard petals were less frequent piles of shredded floral material under *L. parva* plants (Fig. 1B) and also at the entrance to rodent burrows associated with *Rhabdomys* scats. Sorting of these shredded piles into their separate floral parts indicated that the anthers had mostly been removed.

African honey bees were observed foraging for nectar on *L. parva* flowers and those of co-occurring plants on multiple occasions during the study. Bees accessing the nectar of *L. parva* flowers did not appear to come into contact with the pollen laden anthers. Sunbirds were also sighted frequently visiting the flowers of the protea *Mimetes* amongst *Liparia* patches but were never seen visiting *Liparia* flowers.

In some *Liparia* patches, herbivory on *Liparia* flowers by the Argentine ant, *Linepithema humile,* was substantial, with some plants devoid of any viable flowers. Ants removed pollen and possibly nectar from the flowers as evidenced by excisions at the top and base of flowers. As a result of ants foraging from *Liparia* flowers the anthers would first wilt, following which the whole flower would typically go brown and die.

Field photography

A single clip of footage was captured of a rodent foraging at a flower of *L. parva*, under natural conditions in the field, for 18s at 8.30pm (Clip1.avi in appendix).

Rodent trapping – species composition

The joint trap nights of the 2nd and 3rd of August generated a total capture of 11 animals. The species composition of rodent captures on and off *L. parva* patches is shown in Table 1. The rodent species most commonly trapped on *L. parva* patches was the Cape spiny mouse, *Acomys subspinosis*.

Table 1: Composition of rodent captures on and off sites populated by *L. parva*

Species	*L. parva* sites	Control sites
Acomys subspinosis	5	1
Otomys unisalcatus	0	2
Rhabdomys pumilio	0	2
Shrew sp.	0	1

Following the first trap night, tank observations were made of two captive *Acomys subspinosis* individuals foraging repeatedly at freshly picked *L. parva* inflorescences between 8.00pm and 12.00am. Both individuals exhibited a distinctly similar and specific non-destructive foraging method. This included initially inserting their snouts into the gap between the standard petal and the fused keel petals. The resultant pressure on the base of the flower caused the anthers to protrude from the flower and release pollen in a 'pump action' on to the snouts of the foraging animal. The animals then used their front paws to remove the standard petal from the inflorescence to lap nectar from the nectar cup (Figs. 1 D & E). The animals then discarded the standard petal and lapped up the remaining nectar in the flower, coming into contact with the anthers a second time. This behaviour pattern was repeated at multiple flowers on the same inflorescence and on other inflorescences in a single foraging bout (Clip2.avi in appendix). The removal of the standard petal caused no apparent structural damage to the flowers.

A single *Otomys* kept in an observation tank overnight did not forage from *L. parva* flowers at any time.

Fig. 1 - A. Standard petals of *L. parva* as observed deposited underneath *L. parva* plants. **B.** Shredded floral material found underneath *L. parva,* and at entrance to rodent burrows associated with *Rhabdomys* scats. **C.** *L. parva* individual showing drooping, geoflorous infloresences. **D.** Inflorescence of *L. parva* showing nectar cup (arrow) at the base of the keel petals. **E.** *Acomys* inserting it's snout into *L. parva* flowers to access nectar.

Rodent trapping - pollen transfer

The second round of trapping on the nights of the 2nd, 3rd and 4th of August resulted in a total number of captures on each night of two, thirteen and two animals respectively. Rodent species captured included *Acomys subspinosis*, *Rhabdomys pumilio*, *Mus minutoides* and *Lemniscomys rosalia*. The low trap rate on the third night was attributable to wind blowing over 60% of the traps. Thirteen of these blown over traps had been visited by rodents as indicated by the presence of scats in the traps. *Liparia parva* pollen was obtained from the snout of only one rodent but was found in the faeces of 14 of the 17 animals (Table 2). Pollen found in *Acomys* scats was almost exclusively *Liparia* pollen. In contrast, *Rhabdomys* scats had much larger quantities of pollen from other plants.

Table 2. *Liparia* and non-*Liparia* pollen loads in faeces of trapped rodents

Rodent species	No. of animals caught	Animals with *Liparia* pollen in faeces	Mean *Liparia* pollen count in faeces (SD:range)	Animals with non-*Liparia* pollen in faeces	mean non-*Liparia* pollen count in faeces (SD:range)
Acomys subspinosis	11	11	40.4 (37.5: 6-105)	5	5.6 (6.4: 2-17)
Rhabdomys pumilio	4	2	9 (9.9: 2-16)	4	128.5 (150.7: 35-352)
Mus minutoides	1	0	-	1	4.0
Lemniscomys rosalia	1	1	2	1	58.0

Foraging choice experiments

All rodents that entered the T-maze spent a portion of the trial period selectively foraging on flowers of *L. parva* (Table 3). In only one trial did a rodent (*Rhabdomys pumilio*) forage secondarily for a very short period (8s) on the anthers of *Mimetes*. Rodents did not appear to show a species specific preference in initial lane choice. The foraging behaviour of *Acomys* individuals was characteristic of that observed in earlier observations of captive *Acomys*. All *Acomys* fed non-destructively with most individuals removing at least one standard petal (mean = 5) to obtain nectar (two *Acomys* foraged without removing standard petals), during which pollen was visibly dusted onto the snouts of the foraging animal. The average amount of time spent by *Acomys* individuals

foraging at flowers of *L. parva* was 52s (16-150). All *Rhabdomys* individuals foraged highly destructively on flowers of *L. parva*. This included considerable time (up to 12 minutes) spent ripping the inflorescence apart to feed mainly on the stamens of *L. parva* flowers but also on the petals and bracts.

Table 3. Foraging choice of rodents in t-maze experiment

Rodent species	#. Animals	Initial lane choice		Initial foraging choice	
		L. parva	*Mimetes*	*L. parva*	*Mimetes*
Acomys	8	3	5	8	0
Rhabdomys	3	2	1	3	0

Trials were discarded if animals failed to enter the maze (N=6).

Effect of height on visitation

The relationship between flower height and visitation intensity, as indicted by the absence of standard petals, was not found to be significant ($R^2 = 0.1034$; $p = 0.0831$) (Fig.2).

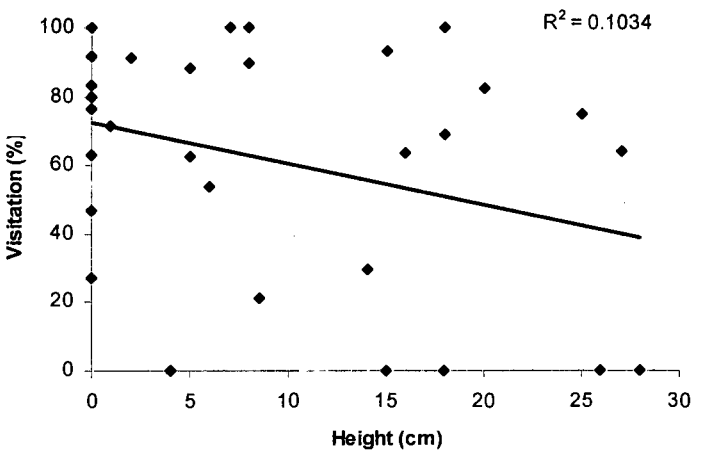

Fig. 2 Relationship between flower height and visitation intensity

Pollinator exclusions and seed set

Only 18 exclusion pairs were available for analysis at the end of the experimental period due to the loss of flowers in two pairs that had been chewed off presumably by a rodent. The average number of pods that developed in control flowers (5.8) was significantly greater than the average in flowers excluded from rodents (2.2) ($T = 10.5$, $p = 0.0049$). Pods were also noticeably larger in the controls than in the exclusions. In none of the exclusions did the standard petals become detached from flowers.

Floral Characteristics

A total of 76 flowers opened during the 72 hours of observation. Of those, 60 (79%) opened between the hours of 4.30pm and 8.00am, which is significantly different from expected values if flower opening is random with respect to time of day ($\chi^2 = 6.86$, $p < 0.009$).

The nectar volume and sucrose concentration in *Liparia* flowers were both found to be highly variable (Table 4). When the nectar volume in a single flower is extrapolated to that within an inflorescence, the total nectar reward increases substantially from a mean of 10.73µl per flower to 140µl per inflorescence (mean flowers/inflorescence = 13 (N=24)). The nectar-anther distance was relatively invariant with a mean of 12.84mm (Table 4).

Table 4. The nectar volume, nectar sugar concentration and nectar-anther distance of *L. parva* flowers.

	Nectar Volume (µl)	Sucrose Content (%)	Nectar-anther distance (mm)
Mean	10.73	19.73	12.84
SD	6.24	5.11	0.71
Range	3.7-31	11.5-28.5	11.4-13.64

N = 20

Discussion

Rodent visitation to *L. parva* flowers

The synthesis of a range of data in this study strongly indicates that rodents frequently and actively visit flowers of *L. parva*. The abundance of deposited standard petals and less frequent piles of floral parts underneath *L. parva* plants provided initial evidence that flowers were being visited by a small vertebrate. This was corroborated by the finding that *L. parva* flowers do not release the standard petal passively as would have been evidenced in caged inflorescences. Observations of captive rodents provided strong support that two species of rodent (*Acomys subspinosis* and *Rhabdomys pumilio*) are distinctly associated with the floral debris seen in the field. Captive individuals of the nocturnal Cape spiny mouse, *Acomys subspinosis*, typically removed and discarded the standard petals of *L. parva* without damaging the reproductive parts of the flower. This produced petal litter identical to that observed in the field (Fig. 1A). The observation that *Acomys* does not consume floral parts in captivity has been made in other rodent pollination studies (Vlok, 1995). Furthermore, *Acomys* has been shown to be a significant pollen vector of several rodent pollinated fynbos species including *Protea humiflora* and *P. amplexicaulis* in the Proteaceae (Wiens and Rourke, 1978; Wiens et al., 1983; Fleming and Nicolson, 2002) and *Massonia depressa* in the Hyacinthaceae (Johnson et al, 2001). In contrast with the non-destructive feeding habit of *Acomys*, captive individuals of the striped field mouse, *Rhabdomys pumilio,* foraged highly destructively on flowers of *L. parva* to produce piles of floral parts again very similar to those observed in the field. This was supported by the observation of these piles near the entrance to rodent holes associated with *Rhabdomys* scats. *Rhabdomys* has also been associated with destructive foraging at flowers of rodent pollinated *Protea* species (Wiens and Rourke, 1978). The foraging choice experiments also indicated that captive individuals of both species were not foraging at *L. parva* flowers solely on the basis of it's availability but were actively selecting to forage on *L. parva* flowers over an alternative nectar-producing species. None of the other species of rodent captured during the study were observed foraging on

flowers of *L. parva* except for the single individual of *Mus minutoides* which was observed briefly nibbling at the anthers.

The removal of the standard petal of *L. parva* by *Acomys* is unique and fortuitous in that it provides a means to assess indirectly the intensity of rodent visitation at *L. parva* plants. Alternative explanations are unlikely, as birds were never observed visiting flowers of *L. parva* and large insects presumably lack the force or manipulative ability to cause the standard petal to detach from the flower. This discovery should facilitate the acquisition of more comprehensive data on the relationship between *Acomys* and *L. parva* in future studies.

While it was initially hypothesised that if the main pollinator is a rodent, the intensity of visitation should decrease with height, in the light of new evidence it was not a surprise to find that the relationship was not significant (Fig. 2). Tank observations of *Acomys* indicated that they are exceptionally good climbers. Furthermore, Fleming and Nicholson (2002) accredited the lack of any relationship between seed set and height in *P. humiflora* to the abundance of *Acomys*, going on to suggest that they may be to some extent arboreal. Thus the implications of figure 2, rather than being contrary to the hypothesis of rodent pollination, provide further confirmation that *Acomys* is the main visitor to *L. parva* flowers. Interestingly, the trapping densities of *Acomys* recorded throughout this study were apparently much greater than those recorded previously at other sites in the Western Cape (Fleming and Nicholson, 2002).

The presence of *L. parva* pollen in the scats of almost all the rodents captured is yet further evidence that rodents visit the flowers of *L. parva*. The larger implications of pollen transfer with respect to pollination are dealt with later in this discussion.

In addition to the accumulative indirect evidence of rodent visitation to flowers of *L. parva*, the most direct support comes from the short clip of video footage of a rodent foraging at a flower of *L. parva* (Clip1.avi in appendix). Alone the footage is not definitive but in combination with several other lines of evidence, it provides compelling proof that rodents do visit flowers of *L. parva*. Unfortunately, it is very difficult to identify the species of rodent in the clip, however, given that the footage was captured during the night there is a strong possibility that it was *Acomys*. Aside from support for rodent pollination of *L. parva*, the footage has additional value in that it has been

considered in the past virtually impossible to observe rodents in the field at night (Wiens et al., 1983, Johnson et al. 2001). The footage thus apparently represents the first time a rodent has been captured on film visiting flowers under natural conditions.

While it has been suggested that many nocturnal non-flying mammals have acute olfactory senses (Carthew and Goldingay, 1997) the lack of any significant difference in initial arm choice in the T-maze experiments obscures whether rodents are attracted to *L. parva* on the basis of odour. In reference to a similar experiment, Wiens et al. (1983) suggested that the absence of any significant difference in initial arm choice may be due to the saturation of the maze by odours from both heads, precluding selective odour cues. A more parsimonious explanation in this study is that rodents were initially more intent on exploring the maze, with the prospect of escape, than they were on finding food. Even so, while it has been said that *L. parva* flowers do have a yeast-like odour (Schutte and Van Wyk, 1994) this characteristic was not obvious at the time of study.

Pollen transfer

The mode by which pollen is transferred on to rodents (*Acomys*) during foraging was demonstrated visibly during captive observations (Clip2.avi in appendix). The pump-like action, which causes pollen to be deposited from the tip of the keel, represents a variation on the tripping mechanisms that are common in papilionoid flowers (Arroyo, 1981; Tucker, 2002). Such elaborate pump mechanisms are apparently particularly common in Lipariae (Arroyo, 1981). The observation of captive *Acomys* visiting different flowers on the same inflorescence, and different inflorescences, repeatedly triggering the pollen pump, strongly indicates that they would be effective pollinators of *L. parva*. Bees have been documented as visitors to *L. parva* (Bos, 1967) and were observed visiting flowers of *L. parva* during this study, specifically by landing on the standard petal to forage for nectar. However, their abdomens never came into contact with the tip of the keel and they appeared to lack the force to trigger the pump mechanism, indicating that they could not be an effective pollen vector of *L. parva*. In several rodent pollinated fynbos species the observation of bees foraging for nectar is not uncommon (Wiens et al. 1983, Johnson et

al. 2002). However, Wiens et al (1983) maintained that while bees probably do add to some pollination success in rodent pollinated *Proteas* it would only occur haphazardly.

While the absence of pollen on the fur of almost all the trapped rodents may be construed as something of an anomaly, it is not without reasonable explanation. Firstly, a methodological error was made in the size of the gel block that was used for swabbing pollen from the snouts of rodents. The melting of such a relatively large block ($5mm^3$) would have resulted in the considerable dilution of any quantity of pollen that had been collected on the surface. Given that it was only practical to pipette small amounts of the melted gel onto a slide, in hindsight, large quantities of pollen on slides were extremely improbable. It would have been more sensible to have used much smaller blocks such as the 2x2x1 mm blocks used by Fleming and Nicolson (2002). Secondly, live trapped rodents are known to groom themselves and quickly remove much of the pollen from their snouts (Wiens and Rourke, 1978). In concurrence with this finding, captive rodents were observed grooming themselves following bouts of foraging (Clip2.avi appendix). Thirdly, despite there being considerably high densities of *L. parva* at trap sites, flowers with substantial quantities of fresh pollen were relatively scarce. This was primarily because many of the flowers had already been open for some time, but also due to the occurrence of ant herbivory on *L. parva* pollen.

Nonetheless, scat samples from rodent traps provided much informative data to compensate for the gel samples. Nearly all the rodents that were sampled had at least some *L. parva* pollen in their faeces. In particular, *Acomys* scats typically contained significantly more *L. parva* pollen than pollen from other plant species (Table 2). Presumably, *Acomys* ingest pollen solely as a consequence of grooming, as captive animals were never observed consuming pollen directly from *L. parva* flowers. Previous studies have found that in some cases, rodent pollinated plant pollen will make up the bulk of the solid matter in faeces (Fleming and Nicolson, 2002; Johnson et al, 2002). In this study such high pollen counts were not apparent, possibly reflecting the low quantity of *L. parva* pollen that was available during trapping. In contrast with *Acomys*, *Rhabdomys* scats contained considerably greater quantities of non-*Liparia* pollen than *Liparia* pollen indicating much more generalist foraging. Furthermore the presence of pollen in *Rhabdomys* scats is much more likely due to the direct consumption of pollen,

as was evident in captive individuals, than to indirect ingestion during grooming. Pollen found in the scats of the other rodent species showed ratios comparable to those identified in *Rhabdomys* scats suggesting they are not major pollen vectors of *L. parva*. However, such a conclusion would be premature given that only one of each species was trapped.

It was unfortunate that the final trap night was hampered by weather as a larger sample size would have provided much more confidence in the evident relationships between *L. parva* and its rodent visitors. However, by this stage of the investigation, the flowering period of *L. parva* was almost over, making additional trap nights unlikely to add more data.

Nevertheless, it is apparent from the data, that of the rodent species trapped, *Acomys* is the only regular and efficient vector of *L. parva* pollen. This finding contrasts slightly with other studies which have generally found a suite of rodent species to be involved in pollen transfer (Wiens and Rourke, 1978; Lumer, 1980; Wiens et al 1983; Johnson et al, 2001; Fleming and Nicolson, 2002).

Pollinator effect on seed-pod set

The exclusion of rodents from flowers resulted in a reduction in pod development compared with controls, suggesting that rodents do participate in legitimate pollination of *L. parva*. However, while the result is significant, a certain amount of ambiguity was inherited from the method. The rarity of closed buds when the exclusions were setup, made it necessary to select already opened inflorescences for exclusions. In turn, the rarity of opened inflorescences possessing flowers with all their standard petals still in tact, made it necessary to impose an unwanted temporal bias between exclusions and controls. This was because while it did not matter whether controls were already missing standard petals, inflorescences used for exclusions were required to have all their standard petals. Thus, the tendency was for controls to be characterised by slightly older flowers than the exclusions. It was this flaw in the method that possibly explains the noticeable difference in pod size between the exclusions and controls. Assuming only

rodents can pollinate *L. parva*, the implications would be that rodents visited inflorescences, prior to them being caged, without the standard petal becoming detached. Alternatively, it is possible that pod development in caged inflorescences was due to insect pollination, although field observations suggest otherwise. Another strong explanation might be that *L. parva* is self compatible, a trait that is common in papilionoids (Arroyo, 1981). Finding this to be accurate would have considerable fitness implications for out-crossing in *L. parva*, as out-crossed progeny are generally more vigorous than those produced by self ferilization (Wyatt, 1983). However, such a discussion would be premature until the breeding system of *L. parva* has been studied in more detail. Suffice it is to say, that rodents do effect the probability of seed set in *L. parva,* but may not be the only means by which pollination can occur. It would be advantageous in future studies to design an experiment utilising different size meshes that excluded rodents and insects exclusively. In this way, caging inflorescences prior to flower opening would provide significant data on the relative importance of different breeding systems and pollinators in generating seed set in *L. parva*.

Floral characteristics

Nocturnal floral anthesis has been suggested to be an adaptation to pollination by nocturnal animals (Carthew and Goldingay, 1997). The disproportionate increase in *L. parva* flower opening during the night thus supports the notion that *L. parva* is adapted to a nocturnal pollinator.

While nectar volumes were relatively small per flower, when considered at the level of the whole inflorescence, the relative volumes of nectar, fall within measured ranges for other rodent pollinated plants (Lumer, 1980; Johnson et al. 2001). In contrast however nectar sucrose concentrations (mean = 19.73) were well below the ca. 36% nectar sucrose concentration characteristic of non-flying mammal pollinated plants (Rebelo and Breytenbach, 1987). Van Wyk (1983) suggests that phylogenetic constraints may explain the lack of a correlation between nectar sugar concentration and pollination syndrome in some species. Alternatively had nectar been sampled during the night nectar sugar

concentrations may have been different to the mid-morning measurement. Johnson et al. (2001) showed that nectar sugar concentrations in the African lily, *Massonia depressa*, were capable of doubling from midday to early evening.

Other traits exhibited in *L. parva* that are not shared with its therophilous *Protea* counterparts include the possession of relatively fragile anthers and a stigma-nectar distance of approximately 13mm, slightly longer than the proposed 10mm rule. However, the floral structure of *L. parva* is altogether very different from therophilous *Proteas* which possess naked anthers, requiring them to have a certain robustness to prevent damage. In contrast, the androecia of *L. parva* flowers are protected by the keel petals and only emerge in a pump-action when under pressure, negating the need for robust anthers or such a short nectar-anther distance. The rodent pollination syndrome in *Liparia*, therefore departs to some extent from the classic characteristics used to define the syndrome. Nonetheless, the combination of pale flower colour, pronounced geoflory (Fig. 1C), copious nectar, nocturnal anthesis and a winter flowering period in *L. parva,* adequately demonstrate adaptations to rodent pollination that parallel those documented in other species.

The evolution of rodent pollination in *Liparia*

The evolution of rodent pollination in *Liparia* is particularly curious given the strong historical association of the legume family with melittophily (bee pollination) (Arroyo, 1981). It has been suggested that the absence of a rich-bee fauna may have given rise to the evolution of the diverse suite of 'alternative' pollination systems in the flora of southern Africa (Johnson, 2004). This is particularly true for the Cape flora, where temperate conditions are likely to favour pollinators that remain active in cold weather (le Maitre and Midgley, 1992; Johnson, 1992). Preliminary investigations of scaling relationships within *Liparia,* that were conducted during this study, suggests that morphologically, *L. parva,* is a 'dwarf' form of it's putatively bird pollinated sister species, *L. splendens*. Corner's rules (1949) and its derivatives (Midgley and Bond, 1987) suggest a number of plant morphological attributes such as leaf size, stem diameter, plant

height, degree of branching and inflorescence size are under allometric constraint. Thus, selection for one of these traits has a scaling effect on the other traits. Thus given the possibility of a scaling relationship between *L. parva* and *L. splendens,* it is plausible that selection on a single allometrically controlled trait in one of these species could readily bring about a morphological shift towards that of the other. Thus assuming that the rodent pollination syndrome is highly derived in *Liparia*, as is thought likely in Proteaceae (Rebelo and Breytenbach, 1987), adaptation from a bird pollination syndrome to a rodent pollination syndrome may have been a relatively short evolutionary step. Furthermore, there is no doubt that within Papilionoideae, ornithophily has evolved directly from melittophily (Arroyo, 1981). Thus given strong selection for 'alternative' pollination systems in the Cape Flora, the rodent pollination syndrome may have been brought about by relatively simple morphological changes. This is naturally a highly speculative line of reasoning, but nonetheless certainly worth testing by examining scaling relationships in *Liparia* between the full range of pollinator types.

Conclusion

The synthesis of a range of data strongly indicates that rodents are regular visitors to flowers of *L. parva* and in the process participate in pollen transfer. This relationship is most apparent in the Cape spiny mouse, *Acomys subspinosis*, which exhibits a unique non-destructive foraging technique and may be the primary pollinator of *L. parva*. That *L. parva* possesses many of the plant traits characteristic of the rodent pollination syndrome provides further evidence that this is an example of an adaptive plant-animal interaction. Unfortunately, it was not possible to determine the ultimate importance of this interaction for seed production in *L. parva* and is clearly an area requiring further investigation. Nonetheless, as the first description of rodent pollination in the legume family (Fabaceae), this study demonstrates that the rodent pollination syndrome may be less taxonomically restricted then the current body of research suggests.

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Appendix

On disc: download to desktop to play smoothly.

Clip 1 - Footage captured using custom built *Pix*Controller of a rodent visiting flowers of *L. parva* in the field.

Clip 2 – Tank observations of *Acoyms* foraging at *L. parva* flowers.