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EXTRACTION OF NUTRIENTS FROM *PROTEA* POLLEN BY AFRICAN RODENTS

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Abstract. Many of the species of *Protea* that are found in the south-western Cape of South Africa are pollinated by rodents. In Australia, where flowers of the same family are also mammal-pollinated, some of the mammal species that feed on Proteaceae flowers not only gain energy from nectar but also extract protein from pollen. This contrasts with the widely held belief that most mammals are unable to extract nutrients from pollen. To determine whether African rodents are also capable of using pollen as a source of protein, faecal samples were collected from mammals trapped at two sites in the Western Cape where *Protea humiflora* and *P. subulifolia* were common. The mammals included three rodent species, *Rhabdomys pumilio*, *Aethomys namaquensis* and *Mus minutoides*, and an elephant shrew, *Elephantulus edwardsii*. The mean percentage of empty or partially digested pollen grains was 50.3% for *E. edwardsii*, 56.8% for *R. pumilio*, 60.4% for *A. namaquensis* and 83.0% for *M. minutoides*. These four species are clearly capable of penetrating the pollen grains of *Protea* during digestion. Pollen is therefore a potential protein source for these species.

Key words: pollen, rodent, diet, protein, nitrogen.

INTRODUCTION

Many mammalian species, including rodents, feed on flowers or flower products. For example, in Britain, the dormouse, *Muscardinus avellanarius* (L.), feeds on the anthers of hawthorn, *Crataegus monogyna*, in early spring (RICHARDS *et al.*, 1984) and, in the Kalahari desert, *Acacia* flowers are eaten in large quantities by larger mammals such as springbok, *Antidorcas marsupialis* (Zimmerman, 1780), and giraffe, *Giraffa camelopardalis* (L.) (SAUER, 1983; NAGY & KNIGHT, 1994). Although in many cases the plants receive no corresponding benefit, a range of mammal species including bats, rodents, marsupials, primates and insectivores are involved in the pollination of various plant species (REBELO & BREYTENBACH, 1987; GOLDINGAY *et al.*, 1991; FERRARI & STRIER, 1992; FLEMING, 1993).

What do flowers have to offer rodents? One flower product that rodents are likely to feed on is nectar. Nectar is a sugar solution produced by many flowers to attract pollinators and it should provide foraging rodents with an easily obtainable source of energy. A second flower product that rodents may feed on is pollen. Pollen can have a very high protein content. The pollen of some mammal-pollinated *Banksia* species contains over 30% crude protein (TURNER, 1984). The bulk of the protein in a pollen grain is found in the cell contents, known as the protoplast, and this protoplast is encased within a hard cell wall that is extremely resistant to chemical breakdown (RAVEN *et al.*, 1992). The strength of this cell wall has led to a belief that it is difficult for small mammals to extract nutrients from pollen (HUME, 1982). Some dietary studies have identified pollen as a major component of faecal samples but discounted it as a possible source of nutrition because of this perceived difficulty (*e.g.* SMITH, 1982).

Evidence that pollen may not be such an inaccessible food source has been found in Australia in recent years. Laboratory studies on two flower-feeding marsupials, *Petaurus breviceps* (Waterhouse, 1838) and *Cercartetus nanus* (Desmarest, 1818), showed that *Eucalyptus* pollen had a high biological value for both species. Furthermore, the maintenance nitrogen requirements for both species were exceptionally low when they were fed diets in which pollen was the only source of nitrogen (SMITH & GREEN, 1987; VAN TETS, 1996). A flower-feeding bat, *Syconycteris australis* (Peters, 1867), has also been found to have a low maintenance nitrogen requirement on pollen, although not as low as for the marsupials (LAW, 1992a).

A large proportion of the *Banksia* pollen grains found in faecal samples taken from small Australian mammal species have been empty, indicating that these species are capable of extracting the protoplast from the *Banksia* pollen grains they ingest. In the faeces of the obligate flower-feeding marsupial *Tarsipes rostratus,* 95-100% of the *Banksia* pollen grains were empty (RICHARDSON *et al.*, 1986). Other marsupials that frequently fed on flowers, such as *P. breviceps* and *C. nanus,* removed the protoplast from approximately 65% of the pollen grains (VAN TETS & WHELAN, 1997). The flower feeding bat, *S. australis*, was able to extract the protoplast from 53% of the *Banksia* pollen grains it ingested (LAW, 1992b). Even mammals for whom flower products were unlikely to form an important part of their diet were able to digest *Banksia* pollen. For example, the rodent *Rattus fuscipes* (Waterhouse, 1839) and the insectivorous marsupial *Antechinus stuartii* (Macleay, 1841) removed the protoplast from 55% and 37% respectively of the pollen they ingested (VAN TETS & WHELAN, 1997).

In the Cape Floral Kingdom of south-western South Africa, rodents regularly visit the inflorescences of *Protea* in search of food, and rodents are the primary pollinators of a number of *Protea* species (WIENS *et al.*, 1983; REBELO & BREYTENBACH, 1987). These species produce large and often cryptic inflorescences close to ground level (REBELO & BREYTENBACH, 1987). The inflorescences have a strong musky odour and they release nectar at night with maximum flower opening corresponding to maximum small mammal activity (WIENS *et al.*, 1983). When small mammals were excluded from the inflorescences of two species, *P. humiflora* and *P. amplexicaulis*, seed set was reduced by 50 and 95% respectively (WIENS *et al.*, 1983).

Protea and *Banksia* both belong to the same family, Proteaceae. Many relevant species of *Protea* flower between mid-winter and mid-spring (REBELO, 1995). As this is a period when other food resources are in short supply, it is possible that the flower products of *Protea*, including pollen, are an important element in the diet of the rodents during those periods. Although, there is no evidence that the rodents actively select pollen while foraging, they do ingest large quantities of pollen while grooming (WIENS *et al.*, 1983). As Australian mammals, including the rodent *Rattus fuscipes*, could extract the protoplasts from the pollen grains of plants from the same family, it was likely that at least some of the South African rodents could do the same with *Protea*. My aim, therefore, was to determine whether the rodents involved in the pollination of *Protea* were removing the protoplasts from the pollen they ingested.

MATERIAL AND METHODS

Between 31 July and 2 August 1996 and between 17 and 20 September 1996, small mammals were captured at two sites in which the inflorescences of rodent-pollinated species of *Protea* were abundant. The first site was on the foothills of Jonaskop on the western edge of the Riviersonderendberge (33° 56'S 19° 31'E) in which *Protea humiflora* was the only species of *Protea* in flower at the time of sampling. The other site was near Kaaimansgat in the Stettynsberge (33° 56'S 19° 17'E). At Kaaimansgat, *P. subulifolia* was the most common species of *Protea* within the site but there were a few *P. laurifolia* plants in flower near its edge. The mammals were captured in live traps baited with peanut butter, oats and golden syrup and were toe-clipped so that samples were taken from each individual only once. Faeces were taken from the traps in which the small mammals were caught and were stored in 70% ethanol.

Faecal samples were taken from two species of mammal at Jonaskop: the Namaqua rock mouse, *Aethomys namaquensis* (A. Smith, 1834), and Edward's elephant-shrew, *Elephantulus edwardsii* (A. Smith, 1839). At Kaaimansgat, samples were taken from two different species: the striped field mouse, *Rhabdomys pumilio* (Sparrmann, 1784), and the pygmy mouse, *Mus minutoides* (A. Smith, 1834). A number of other species, including

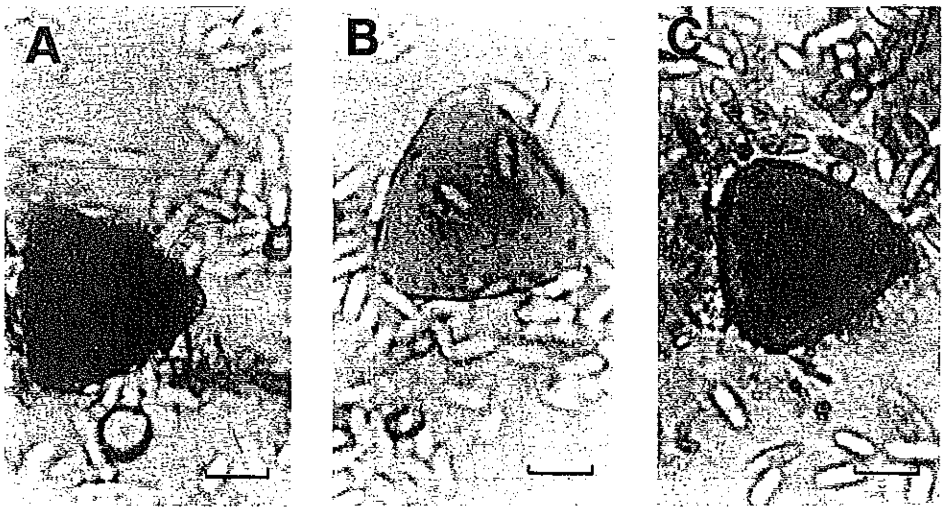

Fig. 1. – *Protea humiflora* pollen grains in the faeces of *Aethomys namaquensis*. A is an intact pollen grain. The darkly stained protoplast fills the entire cell. B is an empty pollen grain. Only the cell wall is visible. C is a partially digested pollen grain, the stained trianglular shaped structure in the centre of the cell is the remnant of the protoplast. The scale bars represent 10 mm.

Acomys subspinosus (Waterhouse, 1838), *Otomys irroratus* (Brants, 1827) and *Myosorex varius* (Smuts, 1832), were also captured at the two sites. These were not included in this study as fewer than five individuals were captured from each of these species.

Approximately 10 mg of faeces from each animal was spread on a microscope slide and stained with a drop of cotton-blue lactophenol. This stained the protoplast dark blue but left the cell wall unstained. On each slide, 100 *Protea* pollen grains were counted and the percentage of grains from which the protoplast had been removed, even if only partially, was recorded (Fig. 1). Grains with partially digested protoplasts were included with the completely empty grains, as in both cases the pollen grain had been penetrated during its passage through the digestive tract. Samples of pollen were also taken directly from the pollen presenters of *P. humiflora* and *P. subulifolia* and assessed in a similar manner. The percentage of empty and partially digested pollen grains in the faeces of the four species was compared using a one way analysis of variance. The values were transformed using an arcsine transformation prior to the analysis.

RESULTS

Over 99% of the pollen grains taken directly from the flowers were intact. However, on average, over half the *Protea* pollen in the faeces of all four species were either empty or partially digested (Fig. 2). The mean percentages were 49.0% for *E. edwardsii*, 58.4% for *R. pumilio*, 60.4% for *A. namaquensis* and 83.0% for *Mus minutoides.* There was no significant difference between the values for *E. edwardsii*, *R. pumilio* and *A. namaquensis*. However, the mean percentage of empty or partially digested grains for *Mus minutoides* was significantly higher than for the other three species ($P < 0.05$).

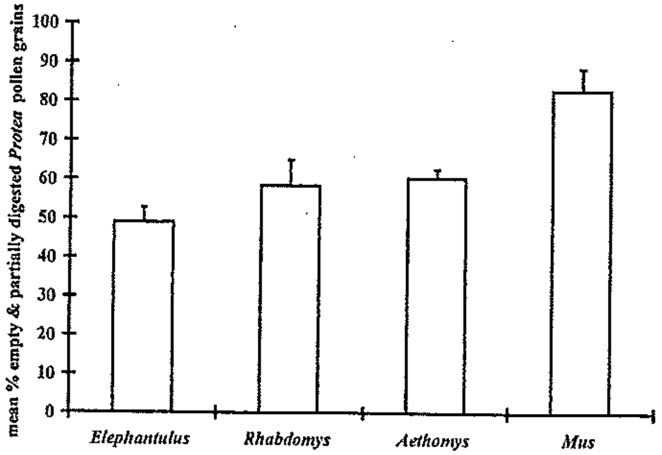

Fig. 2. – Mean percentage of penetrated *Protea* pollen grains in the faeces of four small African mammal species. Error bars represent standard errors. N = 10 for *Elephantulus edwardsii*, 8 for *Rhabdomys pumilio*, 8 for *Aethomys namaquensis* and 5 for *Mus minutoides*.

DISCUSSION

As all four species are capable of extracting the protoplast from at least half the *Protea* pollen grains that they ingest, the pollen cell wall does not prevent them from gaining access to the protein of most of the *Protea* pollen that has been ingested. None of these species is a specialist flower feeder. Their ranges all include areas where the genus *Protea* does not occur (SKINNER & SMITHERS, 1990). Therefore, it is likely that most small African rodent species will also be able to digest *Protea* pollen.

The percentages of empty *Protea* grains were very similar to the percentages of empty grains found in the faeces of eutherian mammals feeding on *Banksia* pollen in Australia: 55% for *R. fuscipes* and 53% for *S. australis* (LAW, 1992b; VAN TETS & WHELAN, 1997). This similarity between the digestibility of *Protea* and *Banksia* suggests that similar values could be expected for other Proteaceae pollens ingested by small mammals.

The mechanism used to extract the protoplast is unclear at this stage and a number of mechanisms have been proposed by various researchers. These include the induction of germination, osmotically or chemically induced bursting and direct enzymatic digestion (TURNER, 1984; RICHARDSON *et al.*, 1986). Of these, the direct enzymatic digestion of the protoplast through the pores of the pollen grain seems to be the most likely mechanism in this case, as partially digested grains were present in the samples and pollen tubes and pollen grains that had obviously burst were not observed. If this is the case, then the percentage of empty pollen grains is likely to be an underestimate, as many grains that appeared intact under a light microscope may have been partially digested. This is supported by an earlier study which found that the apparent digestibility of *Eucalyptus* pollen nitrogen for *C. nanus* was higher than the proportion of empty pollen grains in its faeces (VAN TETS, 1996).

The importance of *Protea* pollen relative to other protein sources in the diet of these small mammals is even less clear. It is dependent on a number of variables including the quantity of pollen available, the foraging behaviour of the mammalian species and the ability of that species to absorb and retain the protein contained in the pollen. Unfortunately, very little data is available on any of these variables. However, as the pollen of *Eucalyptus* (the only pollen that has been looked at in detail in this respect) proved to be a very good source of nitrogen for three mammal species (SMITH & GREEN, 1987; LAW, 1992a; VAN TETS, 1996), it is likely that this is also true for *Protea* pollen.

The success of these four species in extracting the protoplasts from the pollen of *Protea*, taken in conjunction with the Australian data for mammals feeding on *Banksia* and *Eucalyptus*, suggests that small mammals may be able to extract nutrients from many pollen species. Pollen is often rich in protein, and in areas where flowers are seasonally abundant rodents may ingest it in large quantities. It should not be overlooked in dietary studies of rodents in such areas.

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REFERENCES

FERRARI, S.F. & K.B. STRIER (1992) – Exploitation of *Mabea fistulifera* nectar by marmosets (*Callithrix flaviceps*) and muriquis (*Brachyteles arachnoides*) in south-east Brazil. *J. Trop. Ecol.*, **8**: 225-239.

FLEMING, T.H. (1993) – Plant visiting bats. *Am. Sci.*, **81**:460-467.

GOLDINGAY, R.L., S.M. CARTHEW & R.J. WHELAN (1991) – The importance of non-flying mammals in pollination. *Oikos*, **61**: 79-87.

HUME, I.D. (1982) – *Digestive physiology and nutrition of marsupials*. Cambridge University Press, Cambridge, 256 pp.

LAW, B.S. (1992a) – The maintenance nitrogen requirements of the Queensland blossom bat (*Syconycteris australis*) on a sugar/pollen diet: Is nitrogen a limiting resource? *Physiological Zoology*, **65**: 634-648.

LAW, B.S. (1992b) – Physiological factors affecting pollen use by Queensland Blossom Bats, *Syconycteris australis*. *Funct. Ecol.*, **6**: 257-264.

NAGY, K.A & M.H. KNIGHT (1994) Energy, water and food use by springbok antelope (*Antidorcas marsupialis*) in the Kalahari Desert. *J. Mamm.*, **75**: 860-872.

RAVEN, P.H., R.F. EVERT & S.E. EICHHORN (1992) – *Biology of plants*. Worth, New York, 791 pp.

REBELO, A.G. (1995) – *Proteas; a field guide to the Proteas of Southern Africa*. Fernwood Press, Vlaeberg, 224 pp.

REBELO, A.G. & G.J. BREYTENBACH (1987) – Mammal pollination in the Cape flora. In: *A preliminary synthesis of pollination biology in the Cape Flora. South African National Scientific Programmes Report 141* (Ed. A.G. Rebelo) CSIR, Pretoria, 109-125.

RICHARDS, C.G.J., A.C. WHITE, E. HURRELL & F.E.F. PRICE (1984) – The food of the common dormouse, *Muscardinus avellanarius*, in South Devon. *Mamm. Rev.*, **14**: 19-28.

RICHARDSON, K.C., R.D. WOOLLER & B.G. COLLINS (1986) – Adaptations to a diet of nectar and pollen feeding in the marsupial *Tarsipes rostratus*. *J. Zool.* (London), **208**: 285-297.

SAUER, J.J.C. (1983) – Food selection by giraffes in relation to changes in chemical composition of the leaves. *S. Afr. J. Anim. Sci.*, **13**: 40-42.

SKINNER, J.D. & R.H.N. SMITHERS (1990) – *Mammals of the southern African subregion*. University of Pretoria. Pretoria, 771 pp.

SMITH, A.P. (1982) – Diet and feeding strategies of the marsupial sugar glider in temperate Australia. *J. Anim. Ecol.*, **51**: 149-166.

SMITH, A.P & S.W. GREEN (1987) – Nitrogen requirements of the sugar glider (*Petaurus breviceps*), an omnivorous marsupial, on a honey-pollen diet. *Physiol. Zool.*, **60**: 82-92.

TURNER, V. (1984) – *Banksia* pollen as a protein source in the diet of two Australian marsupials: *Cercartetus nanus* and *Tarsipes rostratus*. *Oikos*, **43**: 53-61.

VAN TETS, I.G. (1996) – *Pollen in the diet of Australian mammals*. PhD thesis. University of Wollongong, Wollongong, 146 pp.

VAN TETS, I.G. & R.J. WHELAN (1997) – Pollen in the diet of four flower feeding mammals. *Ecography*, **20**: 499-505.

WIENS, D., J.P. ROURKE, B.B. CASPER, E.A. RICKART, T.R. LAPINE, C.J. PETERSON & A. CHANNING (1983) – Non-flying mammal pollination of southern African proteas: a non-coevolved system. *Annals of the Missouri Botanical Garden*, **70**: 1-31.