Physiological and microbiological studies of nectar xylose metabolism in the Namaqua rock mouse, *Aethomys namaquensis* (A. Smith, 1834)

by

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Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Natural & Agricultural Sciences
University of Pretoria
Pretoria

(November 2005)
Declaration

I declare that the work contained in this thesis is my own original work and I have not previously submitted this thesis or any part of it for degree purposes at any other university.

Shelley Johnson

30 November 2005
Physiological and microbiological studies of nectar xylose metabolism in the Namaqua rock mouse, *Aethomys namaquensis* (A. Smith, 1834)

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**Degree:** Doctor of Philosophy (Zoology)

Xylose is an unusual nectar sugar found in the nectar of *Protea* and *Faurea* (Proteaceae). Since nectar composition is an important floral characteristic in plant strategies for ensuring reproductive success, the unexplained presence of xylose in *Protea* nectar prompted this study of the interaction between pollinators and *Protea* species. Among pollinators that visit *Protea* flowers in the south-western Cape Floral Kingdom, South Africa, insects and birds show an aversion to, and are poor assimilators of, xylose, whereas rodents such as Namaqua rock mice *Aethomys namaquensis* are the only pollinators so far shown to consume xylose willingly, and are able to obtain metabolic energy from this sugar. Mammalian tissues are not capable of catabolizing xylose efficiently, but certain gastrointestinal microflora are, through a process of fermentation which produces short-chain fatty acids used by host animals in oxidative metabolism. I explored mechanisms enabling *Aethomys namaquensis* to utilize xylose, in particular, the role of resident intestinal microflora in this process.

Chapter One discusses pollination syndromes and the definitions thereof, mammal pollination, with particular reference to Australia and South Africa, and explains the rationale behind the questions addressed in this thesis.
To assess xylose utilization in wild-caught mice with and without their natural gastrointestinal microflora, an antibiotic treatment was developed (Chapter Two). The veterinary antimicrobial agent, Baytril 10% oral solution, was found to be effective in significantly reducing gut microflora in animals on a four-day treatment protocol. The protocol developed here reduced the gut microflora sufficiently for subsequent experiments comparing xylose utilization in mice with and without intact microflora.

Xylose utilization was assessed using $^{14}$C-labelled xylose (Chapter Three). Rock mice were caught during *Protea humiflora* flowering and non-flowering seasons, and fed $^{14}$C-labelled xylose. Exhaled CO$_2$ and excreted urine and faeces were collected, and label recovery determined. These experiments showed that xylose-utilizing bacteria in the rock mouse gut are very important for xylose utilization. More efficient xylose utilization during the flowering season suggests that this component of the gut microflora is inducible.

Culturable gut microflora were then isolated from rock mouse faecal and caecal samples, assessed for xylose utilization to identify positive xylose-fermenters and classified by 16S rRNA based taxonomy (Chapter Four). Faecal isolates were *Lactobacillus murinus* and *Enterococcus faecium*, and caecal isolates were three *Bacillus* species, *Shigella boydii*, one *Arthrobacter* species and two fungal isolates from *Aspergillus* and *Penicillium* genera. The types and concentrations of short-chain fatty acids arising from xylose fermentation by caecal microflora were measured using gas chromatography. The fatty acid profile produced by rock mouse gut microflora is similar to that for other animals that rely on gut microbial fermentation to produce fatty acids then used in oxidative metabolism.

Chapter Five concludes with a discussion of possible explanations for the presence of xylose as a nectar sugar, its ecological significance, and the relevance of the fermentative capacity of pollinator digestive systems for xylose utilization in animals.
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Animals

$^{14}$C-xylose experiment

Statistics

Results

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Recovery as $^{14}$CO$_2$ in mice with natural gut flora in flowering vs non-flowering season

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