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.

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30 November 2005

Thesis Summary

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

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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.

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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 ¹⁴C-labelled xylose (Chapter Three). Rock mice were caught during *Protea humiflora* flowering and non-flowering seasons, and fed ¹⁴Clabelled 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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Acknowledgements

I would like to thank my supervisors: Sue Nicolson, for her inspiration and support during all my postgraduate studies and for introducing me to pollination biology; and Sue Jackson, for being a mentor and friend during the past five years, for all her help and guidance and for giving me a home at the University of Stellenbosch.

I would like to thank Riaan Conradie for his enthusiastic assistance in the field and laboratory, and for spending nights alone in the department doing tedious sample collections.

I would like to thank Val Abratt for teaching me the basics of microbiology and related laboratory techniques, and for helping me get my PhD research started in her laborartory. Carola Niesler is thanked for laboratory space and the use of a laminar flow cabinet. Gideon Wolfaardt is thanked for his advice and the use of his laboratory to perform anaerobic microbial studies. Ricardo Cordero-Otero is also thanked for laboratory space and for his help and advice relating to the fatty acid studies. I thank Bettine Jansen van Vuuren for her help in taxonomic classification of bacterial isolates. I would also like to extend my thanks to Steven Chown for providing me with laboratory and office space during the final year of my degree, and to Anton Pauw for his comments on Chapter One.

Lastly I would like to extend a special thank you to my family: to Bevil, for being so supportive of my decisions, for his patience, tolerance and help, and for always standing by me. To my parents, Basil and Mavis, for their support and understanding, and for being such loyal and enthusiastic field assistants and accompanying me on trips to Villiersdorp and nights at Varinghuisie. Also, thank you to Wendy and Kevin for their support.

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