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Dietary Selectivity in the Green Ringtail Possum *Pseudochirops archeri*: The Effect of Plant Secondary Metabolites on Food Preference

Thesis submitted by

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for the degree of Doctor of Philosophy

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Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. In all cases I am the senior author and principle contributor to the development of ideas, experimental design and execution and ecological interpretation. Chapters 2, 3 and 5 are co-authored with my supervisor Andrew Krockenberger who contributed to the original plan for this research and assisted in the development of ideas. Sarah Maclagan assisted with field-work and made helpful suggestions and is an additional author on chapter 3. Appendix 2 was written by A. Krockenberger, S. Maclagan and myself and is included because the majority of data was collected as an aside to my PhD research. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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Preface

Publications arising from this thesis:

Jones, K. M. W. & A. K. Krockenberger (in review) Determining the diet of folivores: An assessment of techniques using the green ringtail possum (*Pseudochirops archeri*) as a case study. Wildlife Research **(Chapter 2)**

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Jones, K. M. W. & A. K. Krockenberger (in prep.) Near infrared reflectance spectroscopy as a tool to investigate diet of a folivore. **(Chapter 4)**

Jones, K. M. W. & A. K. Krockenberger (in review) Plant secondary metabolites determine intraspecific feeding preferences in a specialist rainforest folivore. Journal of Animal Ecology **(Chapter 5)**

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Abstract

The feeding ecology of the green ringtail possum, *Pseudochirops archeri* was studied from 2003 to 2006 in a tropical rainforest fragment on the Atherton Tablelands, north Queensland. I investigated dietary preference at the level of tree species and individual trees within preferred species, the techniques for determining diet, the nutritional characteristics that were determinants of preference, and the use of a rapid, non-destructive technique for determining nutritional characteristics of rainforest foliage.

Three diet-determination techniques were tested. Faecal analysis, direct observation and tree-use each provided an indication of dietary preferences of *P. archeri*. Tree-use was selected as the most robust technique because preference could be statistically tested against availability of tree species at the site, it was the most time efficient method, and it provided information on individual tree use.

Green ringtail possums are highly specialised folivores, focussing foraging effort on only a few of the available trees at the site. Although there were 94 plant species identified in the canopy, over 50% of tree use was from only four tree species, *Aleurites rockinghamensis*, *Ficus fraseri*, *Arytera divaricata* and *F. copiosa*. These species were used significantly more frequently than would be expected if tree species were selected randomly in proportion to their relative abundance in the forest. Because there were few other social or behavioural reasons clearly contributing to preferential use of these species, I infer that they made up the majority of the diet. This was also supported by direct observation and faecal analysis. Possums also favoured particular individual trees within some of the preferred tree species, behaviour that has been well established in *Eucalyptus*-specialised folivores. In 91% of feeding observations, possums consumed mature leaves only. The availability of young leaves, flowers and fruit varied throughout the year, with a peak in availability of these resources during the early wet season. By primarily selecting mature leaves, green ringtail possums reduced their dependence on seasonally variable resources. I propose that this high degree of dietary specialisation might be due to advantages associated with limiting the number of plant secondary metabolites (PSM) in the diet and only secondarily minimising PSM intake within those species.

Near infrared reflectance spectroscopy (NIRS) was investigated as a potential tool for studying nutritional and defensive characteristics of rainforest leaves. Calibration equations developed in this study were successful in predicting chemical characteristics of rainforest leaves. NIRS is a particularly useful technique as once calibration equations are developed using samples of known composition, very large numbers of samples can be processed quickly and no chemical waste is produced. With further development, this technique has the potential to predict dietary intake by rainforest folivores and be used for broad-scale surveys of chemical characteristics of rainforest foliage.

Leaves from preferred and avoided plant species were tested for nitrogen concentration, digestible nitrogen concentration, nitrogen digestibility, dry matter digestibility, water content and cyanogenic glycoside concentration. Within the four preferred tree species, two different nutritional characteristics were associated with variation in individual tree use. Cyanogenic glycosides acted as a deterrent within the predominant preferred species, *A. rockinghamensis,* and within the remaining preferred species, *F. fraseri*, *F. copiosa* and *A. divaricata,* preference was positively related to nitrogen digestibility, but not gross nitrogen content, indicating an effect of nitrogen-digestibility reducing tannins. The preference for mature leaves over juvenile was explicable by the same factors determining tree preference, as juvenile leaves were either higher in cyanogenic potential or lower in nitrogen digestibility. Tree species preference was not related to the same factors that determined preference within species. It is likely that interspecific selectivity is driven by a complex interaction between distribution and abundance of trees, as well as nutrients and PSMs, including compounds that were not measured in this study, and each non-preferred tree species may be avoided due to a different undesirable combination of these factors. Interspecific choice may be based on the need to minimise the diversity of PSMs ingested, whereas intraspecific choice may be based on the need to minimise the quantities of those few PSMs.

Table of Contents

CHAPTER 1.DIET SELECTION IN ARBOREAL FOLIVORES: THE EFFECT OF PLANT CHEMISTRY ON FOLIAGE INTAKE

CHAPTER 2. AN ASSESSMENT OF DIET ANALYSIS TECHNIQUES

CHAPTER 3. A SPECIALIST FOLIVORE IN A DIVERSE FOREST

CHAPTER 4. NEAR INFRARED SPECTROSCOPY AS A TOOL TO INVESTIGATE DIET

CHAPTER 5. PLANT DEFENSES DETERMINE FEEDING PREFERENCES IN A SPECIALIST RAINFOREST FOLIVORE

CHAPTER 1.

Diet selection in arboreal folivores: The effect of plant chemistry on foliage intake

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Plant defences against arboreal folivores

Arboreal herbivores occupy a particularly harsh niche in the superficially plentiful ecosystems of temperate and tropical forests. Herbivore diets consist of any plant part including fruit, seeds, bark, flowers and petioles, but are often dominated by leaves. Although foliage appears to be an abundant resource, its availability to an herbivore is limited by several critical factors. Plant spines, thorns and hairs discourage herbivores through physical hindrance (Cooper & Ginnett, 1998) and provide a first line of defence at low initial outlay and no maintenance cost to the plant (Janzen, 1979). High levels of leaf toughness and fibre content reduce the digestibility of cell-wall polysaccharides and decrease the availability of a major source of energy for a folivore (Cork & Foley, 1991; Lowman & Box, 1983). Foliage with low concentrations of nutrients such as nitrogen and water are often avoided by folivores, as the cost of ingestion overrides the benefits (Cork, 1986). Plant secondary metabolites (PSMs) are thought to function as chemical defence of plant parts against herbivory through interference with feeding and post-ingestive processes (Cork *et al.*, 1991; Iason & Villalba, 2006; Marsh *et al.*, 2006; McArthur, Hagerman & Robbins, 1991a). PSM intake, even at subtoxic levels, can cause significant costs to an animal through nutrient dilution (Guglielmo & Karasov, 1996; Robbins *et al.*, 1991) or increased energetic demands of detoxification and excretion (Marsh, Wallis & Foley, 2005; Sorensen, Mclister & Dearing, 2005b).

PSMs are contained in all plants and are characterised by the absence of a clearly defined role in metabolic processes (Waterman and McKey 1989). The three main classes of secondary products, phenolics (eg. tannins, resin, formylated phloroglucinol compounds-FPCs), nitrogen-containing compounds (eg. alkaloids, cyanogenic glycosides) and terpenoids, are each produced via different biosynthetic pathways (Harborne 1991). Products of two or more pathways can act together to produce a particular anti-herbivore interaction. Phenolics, alkaloids, cyanogenic glycosides and terpenes are chemical metabolites commonly found in rainforest leaves (Coley & Aide, 1991) and are known herbivore deterrents (Cork *et al.*, 1991).

Theories of plant defence

Coevolution between plants and folivorous animals occurs as animals impose directional selection on plant populations, and plant chemical defences (PSMs) influence the fitness of individual animals (Freeland, 1991). For example, plants within the family Umbelliferae seem to have responded to selective pressure by specialised herbivorous butterfly larvae with a toxic ‘angular’ version of the common deterrent linear furanocoumarin (Berenbaum & Feeny, 1981). This interaction is driven by the animal choosing foliage that it is able to detoxify, thus selecting for chemical defence in the plant population as long as the fitness of the plant is reduced more by herbivory than by the cost of the defence (Janzen, 1978). The ‘growth-differentiation balance’ hypothesis (GDB) is based on the observation that plants must grow fast enough to compete with other plants for light and nutrients, but resources must equally be allocated to defence against herbivory (Herms & Mattson, 1992). This resource-based trade-off drives natural selection in both plants and herbivorous animals. Evolution-based cost/benefit analysis provides the basis for the original ‘apparency theory’ (Feeny, 1976; Rhodes & Cates, 1976) and ‘optimal defence theory’ (Rhodes, 1979) and many subsequent hypotheses. According to the apparency theory, late successional species are predictable in time and space and thus easily found by herbivores. Consequently they invest in costly defences such as polyphenols and fibre that are difficult for the herbivore to overcome. Unapparent, early successional species and plants with ephemeral or unpredictable distributions are more likely to escape herbivore discovery and therefore invest less in chemical defence (Feeny, 1976; Rhodes *et al.*, 1976). However this theory fails to predict the pattern found in lowland rainforest, where ephemeral pioneer plants actually suffer higher levels of herbivory than apparent species growing in the same light gaps (Coley, 1983b). Gap-colonizing species have less tough leaves, lower fibre and phenolic content, more nitrogen and moisture, are shorter lived, faster growing, and are grazed at six times the rate of shade-tolerant ‘climax’ species (Coley, 1983a). Coley (1983a) found that insect herbivory in this study was also negatively related to toughness and fibre and positively related to nutritive quality. She found no evidence supporting the apparency model (Feeny, 1976; Rhodes *et al.*, 1976) as the experiments did not show that unpredictable colonizing species were more likely to escape discovery. Differences in damage were attributed to the faster growth and lower defence in leaves of colonizers. Recent tropical forest experiments by Kursar and Coley (2003) showed leaf toughening (which occurs over a few days once the leaf is fully expanded) was the main factor reducing insect folivore and pathogen damage in mature leaves. Lowman and Box (1983) further showed that leaf damage was correlated more negatively with leaf toughness than with phenolics. They also reported that rainforest leaves were not grazed as heavily as eucalypts, and cited other factors such as leaf nutrients, the abundance and seasonality of herbivores, and predator evasion in rainforest compared to schlerophyll habitat as determinants of diet preference in folivores.

Bryant *et al*. (1983) proposed that the concentration of PSMs in plant tissues is controlled by the relative availability of carbon and nitrogen in the environment. Their theory has been termed the ‘carbon-nutrient balance hypothesis’ (CNB), and is based on the premise that plants in low nutrient environments are slow growing and nitrogen limited and therefore invest heavily in carbon-based secondary metabolites (such as phenolics and terpenes). Conversely, plants growing in nutrient rich soil, that may be light-limited, are predicted to produce nitrogen based defences including alkaloids and cyanogenic glycosides (Bryant, Chapin & Klein, 1983). The ‘resource availability hypothesis’ builds on the CNB hypothesis, stating that there should be a link between the type of defensive strategy and nutrient availability as well as length of leaf lifetime (Coley, Bryant & Chapin, 1985). According to this theory, plants will have a slower growth rate and be better defended in a nutrient limited environment, because resources lost to herbivores will be harder to replace. Similarly, in boreal systems high-resource adapted plants have rapid growth rates that allow quick regeneration of foliage with leaves that are only chemically defended in the juvenile phase (Bryant *et al.*, 1983).

Recently the utility of these theories of plant-herbivore interactions have been debated (Hamilton *et al.*, 2001; Koricheva, 2002; Lerdau & Coley, 2002; Nitao *et al.*, 2002), with some authors citing the many examples of studies where the theories have been unable to predict experimental outcomes (Herms *et al.*, 1992). The optimal defence theory of Rhodes (1979) hypotheses that plants that are never subjected to herbivores or pathogens should not be defended if defence has a cost. Critics of the widely used carbon-nutrient balance hypothesis believe that the omission of ‘probability of attack’ is the key failing of this and other theories, as probability of herbivory may be the over-riding factor in the benefit of defence (Hamilton *et al.*, 2001). Thus the optimal defence theory is currently the favoured explanation for the interaction between plants and herbivores because it allows for the costs as well as benefits of defence.

PSMs at a landscape scale

Nutritional and defensive characteristics of leaves vary among and within plant species in response to environmental conditions, and at the landscape scale, environmental gradients such as soil type, moisture availability and aspect create variation in foliage quality through differences in PSM allocation (Coley *et al.*, 1985; Gartlan *et al.*, 1980; Herms *et al.*, 1992; Lambert, Turner & Kelly, 1983). Resource patchiness for a folivore also occurs on a fine scale due to intraspecific variation in PSM concentrations and foliar nutrient availability (Lawler, Foley & Eschler, 2000; Scrivener *et al.*, 2004; Wallis, Watson & Foley, 2002). Spatial patterns of distribution and abundance of folivores are thought to be largely dependent on variations in PSM concentration (Cork & Catling, 1996; Iason *et al.*, 2006).

Arboreal folivorous marsupials and primates are more likely to be found and are more abundant in forest types growing on high nutrient soils (Braithwaite, 1984; Braithwaite, Dudzinski & Turner, 1983; Emmons, 1984; Johnson *et al.*, 2001; Oates *et al.*, 1990) and nutrient status has been reported as statistically the most powerful predictor of abundance (Pausas, Braithwaite & Austin, 1995). Research on species of *Lepilemur* in Madagascan rainforests has found that leaf nutrients predict folivore distribution and abundance. A strong correlation has been demonstrated between the average leaf quality of a forest (described by the ratio of protein to fibre concentration) and the biomass of folivorous primates (Ganzhorn, 1992). However, some authors suggest that the correlation may be too simplistic and perhaps other factors such as the presence of PSMs, leaf toughness, and leaf moisture content influence the individual trees chosen by primates as forage (Ganzhorn, 1992; Oates *et al.*, 1990; Waterman *et al.*, 1988). Cork and Catling (1996) reviewed mammalian distribution studies conducted in temperate eucalypt forests of eastern

Australia, and found that there was not a simple correlation between nutrients and population size. They argued that leaf PSM concentration is negatively correlated with soil nutrients and that total phenolics and the ratio of phenolics to nutrients (higher at infertile sites) also predict mammal abundance. The abundance of some rainforest-dwelling marsupial folivore species varies with geology and the proximate cause has been attributed to the effect of nutrient content of soils on the nutritional quality of foliage (Kanowski *et al.*, 2001).

Mammalian herbivores often discriminate between individual plants within a species (Chapman *et al.*, 2003; Hindell, Handasyde & Lee, 1985; Hindell & Lee, 1987; Jones, Maclagan & Krockenberger, 2006; Lawler *et al.*, 1998a; Milton, 1978; Pahl, 1987; Pahl & Hume, 1990; Scrivener *et al.*, 2004). However, while many studies examining herbivore feeding preferences have considered the effects of plant secondary metabolites at a landscape scale (Braithwaite *et al.*, 1983; Cork *et al.*, 1996; Oates *et al.*, 1990), intraspecific variations between individuals have often been ignored. Recent studies have shown that PSMs influence foraging on a local and landscape scale (Iason *et al.*, 2006; Moore & Foley, 2005a; Scrivener *et al.*, 2004; Wiggins *et al.*, 2006c), and studies of intra-specific feeding have found that marsupial folivores choose food items according to concentration of deterrent compounds (Lawler *et al.*, 2000; Marsh *et al.*, 2003a; Wallis *et al.*, 2002).

Problems with a herbivorous diet

Arboreal mammals with a highly folivorous diet face difficulties beyond overcoming the initial defences of a plant. While most small endotherms feed on high quality resources such as animal tissue, nectar, starch or lipid-rich seeds, the diet of arboreal herbivores is dominated by indigestible (or slowly digestible) plant fibre (Foley & Cork, 1992). Arboreal herbivores are characteristically small, primarily due to the energy required to move a large mass vertically and limitations of branch strength (Grand, 1978). In general, small herbivores are restricted in their capacity to utilize plant fibre (Parra, 1978), because they have higher mass-specific energy requirements but a smaller gut volume to body weight ratio compared with larger animals (Clutton-Brock & Harvey, 1977; Nagy, 1987). Therefore, these animals have high nutritional requirements per unit weight, but due to small gut volume, their rate of food intake is limited and subsequently their rate of energy extraction is low (Cork *et al.*, 1991). Further restrictions are placed on volume of leaf intake by problems associated with processing and excreting the large amounts of toxins ingested with a herbivorous diet (Dearing, Foley & McLean, 2005a). Animals have a limited capacity to biotransform and eliminate toxic PSMs (Foley, Iason & McArthur, 1999; Foley & McArthur, 1994; Freeland & Janzen, 1974) and the internal acid-base homeostasis of the animal can be disturbed by the metabolism and biotransformation of absorbed PSMs (Foley, 1992). Organic acids are a common byproduct of PSM biotransformation and they must be buffered and quickly excreted to avoid acidosis (Foley, McLean & Cork, 1995). PSM induced acidosis may result in diuresis (Dearing, Mangione & Karasov,

2002) and decreases the ability of an animal to maintain water balance. Acidosis is particularly threatening to herbivores with restricted dietary access to nitrogen and water. An acidotic animal produces high levels of ammonium and little urea, this reduces the capability of the animal to conserve nitrogen through urea recycling (Foley, 1992; Foley & Hume, 1987b), which is an important part of nitrogen metabolism in some small folivores. Despite the significant implications of PSMs for the nitrogen balance of herbivores, the absorption and detoxification of these metabolites may be more important for restricting intake in generalist herbivores (Dearing, Mclister & Sorensen, 2005b).

Herbivore feeding strategy: generalists

Two main hypotheses exist to explain the prevalence of generalist feeding strategy in mammalian herbivores; the detoxification limitation hypothesis (Freeland and Janzen, 1974) and the nutrient constraint hypothesis (Westoby, 1978). The nutrient constraint hypothesis suggests that no single food source can comprehensively provide a herbivore with its nutrient and energy needs (Westoby, 1978). According to this theory, herbivores will feed from a variety of sources to maximise nutrient content and diversity in the diet. On the other hand the detoxification limitation hypothesis (DLH) proposes that herbivore foraging will be influenced more by avoidance of toxic plant defences. The DLH, reviewed by Marsh *et al*. (2006), suggests that most herbivores cannot detoxify large amounts of any particular toxin type and must forage from a variety of plants to avoid overloading any individual detoxification pathway and suffering toxicity as a consequence. The detoxification capacity of the mammalian liver is thought to limit the amount of any specific toxin that an herbivore can ingest (Foley *et al.*, 1999; Freeland, 1991), but different PSMs are detoxified via separate biochemical pathways (Dearing & Cork, 1999; Freeland *et al.*, 1974), although see (Boyle & Mclean, 2004). Marsh *et al*. (2005) tested the theory that herbivore detoxification rate influences rate of feeding and found that brushtail possums recognize changes in their detoxification state and consequently altered their feeding. As the rate of detoxification influences rate of feeding, PSMs do not necessarily have a predetermined effect, but depend on the detoxification state of the herbivore (Marsh *et al.*, 2005). The type and amount of plant material that a herbivore can eat, may be influenced by its physiological state, which in turn may be affected by other factors such as climate, nutritional status, and reproductive state (Stapley *et al.*, 2000; Villalba, Provenza & Bryant, 2002; Wang & Provenza, 1996). These hypotheses are supported by studies that show that many herbivores are able to increase intake when offered a choice of dietary species (Dearing *et al.*, 1999; Wiggins *et al.*, 2006b; Wiggins *et al.*, 2003), and increase feeding efficiency when the spatial scale of plant heterogeneity is small (Wiggins *et al.*, 2006c). Due to this detoxification limitation, specialisation on a single food-plant species is rare in mammals (Freeland, 1991).

Folivores: specialist herbivores

In contrast with the expected strategy of generalist herbivory, a small proportion of herbivores are specialists, selecting few species of plants, and often choosing to feed on specific individual trees within the preferred species (Bennett *et al.*, 1991; Dearing, Mangione & Karasov, 2000; Ganzhorn, 1989; Hladik, 1978; Pahl, 1984; Sorensen, Turnbull & Dearing, 2004). This inter- and intra-specific selectivity by folivores probably minimises energy costs associated with the biochemical effects of ingesting certain combinations of PSMs (Sorensen *et al.*, 2005b) and allows the animals to efficiently metabolise and excrete toxins (Boyle *et al.*, 1999). It has recently been suggested that specialist folivores should use detoxification pathways that are less energetically expensive than those used by generalists (Dearing *et al.*, 2005a). Biotransformation of toxic PSMs is achieved through either energetically expensive conjugation (glucuronidation) pathways or less costly functionalization, and it seems that specialists rely more on the latter (Boyle, McLean & Davies, 2000; Boyle *et al.*, 2001; Lamb *et al.*, 2004). Dearing *et al*. (2005a) further predict that because functionalization pathways are more substrate, and thus toxin, specific than conjugation pathways, specialists may have more difficulty processing novel toxins than do generalists. This is consistent with the greater effects of novel PSMs on specialist woodrats, *Neotoma stephensi*, than on their generalist congeners (Sorensen, Mclister & Dearing, 2005a).

The high capacity of specific detoxification pathways in specialists is presumably related to evolutionary exposure to those toxins. Variation in detoxification capacity between populations of herbivores within a species is related to evolutionary exposure to toxins, and herbivores can evolve higher detoxification capacities to overcome constraints of some detoxification systems (Sorensen 2003). For example, quokka, *Setonix brachyurus* populations that had previously been exposed to fluoroacetate (“1080”) toxins in their diet had higher detoxification ability for pesticides containing this toxin than populations with no previous exposure (Mead et al. 1985).

Even with a high capacity for detoxification of specific PSMs, and less energetically costly pathways, PSMs will still have effects on specialist herbivores. Consequently, even though their intake of specific PSMs may be high compared with that of a generalist herbivore, specialists would still benefit from minimising their PSM intake (Sorensen, 2003). Specialists may behaviourally limit their intake of toxins through discerning choice of food items (Freeland, 1991). Ruffed grouse (*Bonasa umbellus*) feed preferentially on aspen (*Populus tremuloides*) flower buds with relatively low levels of toxic coniferyl benzoate (Guglielmo *et al.*, 1996; Hewitt & Kirkpatrick, 1997). Although detoxification costs for ruffed grouse are substantial, they are reduced by selective foraging. Pika (*Ochotona princeps*) manipulate the PSMs in their diet by storing high phenolic leaves for consumption in the winter. Over time the PSMs decay to tolerable levels and actually preserve better than leaves with a low concentration of phenolics (Dearing, 1997). Similarly, beavers (*Castor canadensis*) use water to leach PSMs from their food (Muller-Schwarze *et al.*, 2001). The *Eucalyptus*-specialised koala selectively feeds within preferred food species, choosing individual trees based on size and concentration of formylated phloroglucinol compounds (FPCs) (Moore *et al.*, 2005a).

Deterrents of feeding in marsupial folivores

A deterrent PSM disrupts normal physiological function of the herbivore, and often induces an emetic response (nausea and sometimes vomiting) (Provenza, 1995). Because toxic PSMs are unavoidable in a folivorous diet, animals must respond to some sort of feedback that signals them to reduce, or cease feeding (Lawler *et al.*, 1998b; Provenza, Pfister & Cheney, 1992). Herbivore strategy commonly combines pre-ingestive and post-ingestive feedback to first identify PSMs before consumption and then to limit intake if toxicosis, digestibility reduction or disruption of acid-base balance occurs (Lawler, 1998). Herbivores may develop a conditioned food aversion (CFA) that associates a particular smell and/ or taste of a food (pre-ingestive) with a negative post-ingestive feedback caused by a PSM in that food (Provenza, 1995; Provenza *et al.*, 1992). The flavour cue that the animal responds to may not be directly associated with the PSM causing post-ingestive feedback, but the concentrations of the cue and the active PSMs must be correlated (Lawler, 1998). In this way the animal can regulate PSM intake without experiencing the negative effects of repetitively ingesting too much of the metabolite. Lawler (1998) partially suppressed the deterrent effects of jensenone in common brushtail and ringtail possums by administering a serotonin receptor antagonist (andansetron), which probably reduced the nausea associated with that particular CFA. However, results derived from captive animals do not necessarily extrapolate to free-ranging herbivores (Duncan & Young, 2002). Duncan and Young (2002) found that in a controlled feeding experiment where a single food type was offered at a time, goats learnt to associate foods with particular post ingestive effects and when subsequently offered all foods at once, adjusted their feeding choices according to learned effects of each food type. However, when the test foods were offered simultaneously during the learning phase (as they would be in the wild), goats selected food from more than one food type, thus reducing their capacity to learn about particular post-ingestive effects of each food type. Thus, although these animals had the capacity to learn which foods were more palatable by selecting one at a time (and therefore develop a strong CFA), in the wild they are more likely to select a mixed diet. A huge diversity of PSMs influence folivore feeding strategies. Two particular groups of compounds recur throughout the literature as strong deterrents to folivore feeding, tannins, and in the case of the eucalypt-specialised marsupial folivores, formylated phloroglucinol compound (FPCs), these PSMs are discussed below.

Tannin

Tannins are plant polyphenols whose defining characteristic is that they bind and precipitate proteins. Tannins adversely affect folivores by forming complexes with proteins in the gut, causing negative effects on appetite and nutrient utilisation, particularly of protein, forcing the animal to excrete large quantities of valuable nitrogen in the faeces (Robbins *et al.*, 1987; Silanikove *et al.*, 1996; Zucker, 1983). The digestibility of nitrogen contained within a leaf is likely to reflect the activity of tannins, which precipitate plant proteins and complexing and denaturing digestive enzymes, thereby reducing digestibility of protein and cell walls (Mole & Waterman, 1987; Robbins *et al.*, 1991; Zucker, 1983). High tannin concentration and/or activity can lead to a net loss of nitrogen from ingesting a food item due to losses of enzymes and other gut proteins (Mole *et al.*, 1987; Robbins *et al.*, 1991; Zucker, 1983). Tannins can also reduce the digestibility of plant fibre, probably as a result of precipitation of digestive enzymes, although the effects are not as clear as those on protein digestibility (Robbins *et al.*, 1991). As early as 1970, researchers were suggesting that tannins and other phenolics were likely to be involved in plant defence against herbivores by reducing available foliar nitrogen (Feeny, 1970). Tannins are important deterrents for feeding in folivorous primates. Chimpanzees in Guinea, Bossou spent most time feeding in tree species within the plant family Moraceae, which includes the genus *Ficus* (Takemoto, 2003), and these animals preferentially selected foliage with low condensed tannin content. Similarly, colobus and rhesus monkey leaf preference was significantly inversely correlated with tannin content (Marks *et al.*, 1988; Oates, Swain & Zantovska, 1977). Marsupial folivores are also affected by tannin concentration in food plants (Cork, 1986; Marsh *et al.*, 2003a; Marsh, Wallis & Foley, 2003b).

The actual effect of tannin on a folivore is subject to the chemical characteristics of the tannin and any physiological mechanisms the consumer employs to reduce the effects of the tannin (McArthur *et al.*, 1991a; Robbins *et al.*, 1991). Tannins are hugely diverse in structure and activity so it is difficult to chemically define tannin levels (Zucker, 1983). Browsers often consume high tannin diets and may be better adapted to counteracting tannins than animals such as grazers living on a tannin-free diet (McArthur & Sanson, 1991b). Tannin-binding salivary proteins (TBSP) are produced by many browsers and are thought to prevent tannins interacting with other proteins, thus minimizing tannin absorption and reducing tannin toxicity (Austin *et al.*, 1989; Shimada, 2006). However high levels of secretion of TBSPs cause protein loss in the faeces, affecting nitrogen digestibility (Skopec, Hagerman & Karasov, 2004) and the rate of TBSP secretion in some species is so low that it is unlikely they mitigate any potential effects of tannin (McArthur, Sanson & Beal, 1995). It is currently unknown how important TBSPs are to tannin detoxification in marsupial folivores (Marsh *et al.*, 2003b).

The eucalypt specialist marsupial folivores consume foliage high in tannins and demonstrate several mechanisms of adaptation or tolerance to the effects of tannins. Common ringtail possums (and possibly koalas and greater gliders) conserve nitrogen in the gut by absorbing and processing tannin using caecal microbes to dissociate protein complexes (McArthur *et al.*, 1991b). Recent experiments by Marsh *et al*. (2003a) and Marsh *et al*. (2003b) showed that while common brushtail possums have a greater tolerance than common ringtail possums for the FPC phenolics (see below), tannins limit their food intake. These experiments used polyethylene glycol (PEG) to bind with tannins and block their deterrent effects on possum feeding. By coating foliage with PEG, the amount of tannin that was free to bind with gut-protein was reduced. Any effects of tannins on ringtail possum feeding may have been obscured by the overriding deterrence of FPCs (Marsh *et al.*, 2003a). Ringtails fed leaves from trees of the FPC-free eucalypt subgenus *Monocalyptus* showed a small (10%) but significant increase in feeding when the leaves were coated in PEG (Marsh *et al.*, 2003b) suggesting that tannins are a deterrent in common ringtail possums.

Total protein has often been reported as important for defining nutritional quality of food plants and for interpreting their selection by wild herbivores (DeGabriele, 1983; McKey & Gartlan, 1981; Milton, 1979). Indeed, past studies have found relationships between nitrogen content of leaves and herbivore density on a landscape scale (Braithwaite *et al.*, 1983). However it does not necessarily follow that nitrogen determines herbivore preference at the scale of the individual tree. As discussed above, nitrogen digestibility of leaves is inhibited to various extents by tannins that bind dietary proteins and digestive enzymes (Mole *et al.*, 1987; Robbins *et al.*, 1991; Zucker, 1983). Nitrogen digestibility and digestible nitrogen concentration may be superior measures to that of total nitrogen content when assessing the relationship between folivore food preference and dietary nitrogen, because they incorporate a more ecologically relevant measure of nitrogen availability. In addition, we now know that intraspecific variation in plant chemistry provides a more powerful predictor of herbivore preference, and particular PSMs show greater intra and inter specific variation than do amounts of nutrients (Moore & Foley, 2000).

Terpenes

Eucalyptus foliage often contains large quantities of terpenes (also known as essential oils) (Cork & Sanson, 1990; Foley & Hume, 1987a). Terpenes are considered an important part of eucalypt defense against herbivory (Boyle *et al.*, 2004; Dearing *et al.*, 1999; Pass & McLean, 2002) and may also provide marsupial folivores with a cue to indicate palatability (Hindell *et al.*, 1985; Lawler *et al.*, 1998a; Zoidis & Markowitz, 1992). Variation in terpene concentration within eucalypt species may be directly related to FPC concentration, and FPCs may actually directed preference in the eucalypt-specialised marsupial folivores (Lawler *et al.*, 1998a).

FPCs

Koalas, greater gliders, common brushtail possums and common ringtail possums respond to a set of phenolic plant secondary metabolites in their *Eucalyptus*-dominated diet known as formylated phloroglucinol compounds (FPCs) (Lawler *et al.*, 2000; Marsh *et al.*, 2003a; Moore *et al.*, 2005a; Pass & Foley, 2000; Wallis *et al.*, 2002). In Pass and Lawler's experiments (Lawler *et al.*, 1998a; Lawler *et al.*, 1999; Pass, Foley & Bowden, 1998) more than 70% of the variation in foliage intake by marsupial folivores was explained by FPCs. Lawler (1998) reported that intraspecific selectivity by *Eucalyptus*-specialised folivores correlated inversely with concentration of terpenes and FPCs, but only FPCs were actually deterrent. Terpenes had a secondary role due to their strong pre-ingestive feedback (taste and smell) being associated with the post-ingestive emesis caused by FPCs.

Within ecosystems dominated by *Eucalyptus*, common brushtail possums have shown preference for individual trees within species, and preference has been correlated with low formylated-phloroglucinol compound (FPC) concentrations (Scrivener *et al.*, 2004). This study found a polygonal rather than linear relationship between FPC content and tree-use by possums at the site. Trees frequently visited never had high FPC concentrations, but there were also trees with low FPC concentrations that were never visited, highlighting the issue that while some aspects of folivore preference can be observed in field studies, factors other than plant palatability (e.g. territory, threat of predators) influence food-plant choice by folivores in the wild. Similarly, a population of wild koalas visited individual eucalypts predominantly based on tree size, but at the same time avoided trees with high foliar concentrations of FPCs and low concentrations of nitrogen (Moore *et al.*, 2005a). As a result of these studies, it has been suggested that the distribution and abundance of common brushtail possums, common ringtail possums and koalas are influenced by foliar FPC concentrations at a landscape scale.

Digestive physiology

Apart from metabolic and behavioural characteristics of folivores that enhance their ability to deal with PSMs, folivores display an array of highly complex digestive tracts (Bauchop, 1978) that serve to enhance their extraction of nutrients from nutrient poor foliage (Cork and Foley 1992). This complexity has been achieved through enlargement of either the foregut (stomach) or hindgut (caecum/colon) and establishment of symbiotic associations with gut microorganisms (Bauchop, 1978). Both foregut and hindgut fermenting folivores including sloths (Foley, Vonengelhardt & CharlesDominique, 1995; Montgomery & Sunquist, 1978), primates (Caton *et al.*, 1996; Caton, Lawes & Cunningham, 2000; Milton, 1981), and marsupial folivores (Foley & Hume, 1987c; Martin & Handasyde, 1999; Wellard & Hume, 1981) appear to employ a common strategy of retaining long retention times for digesta. Small mammals are expected to eat high quality, low fibre diets because they require more energy each day relative to their body mass, than do large animals (Demment & Van Soest, 1983; Parra, 1978). However the arboreal folivores are a group of small mammals that appear to violate the 'allometric rules' by choosing foods high in fibre (Foley *et al.*, 1992). Hindgut fermentation with selective retention of fluid digesta and rapid excretion of indigestible is a common strategy of small folivores (Cork *et al.*, 1991), including most of the marsupial folivores. Among the folivorous primates, strategies for coping with a high fibre diet include hindgut fermentation, in the *Lepilemur*, *Propithecus, Avahi, Indri* and *Alouatta* monkeys, and foregut fermentation using a sacculated stomach with bacterial digestion and selective absorption as the digesta moves down the gut in the *Presbytis, Colobus* and *Nasalis* genera (Hladik, 1978)

Foregut fermentation

Foregut fermentation occurs in a diverse range of arboreal folivores including tree-kangaroos (Hume, 1999), colobine primates (Dasilva, 1992; Oates, Waterman & Choo, 1980; Waterman *et al.*, 1980) and sloths

(Montgomery *et al.*, 1978). Foregut fermentation is advantageous because microbial cells (containing high quality protein and B vitamins), synthesised in the stomach are digested in the small intestine, whereas microbial cells synthesised in the hindgut are lost in the faeces (Hume, 1999) and can only be recovered by caecotrophy. Therefore foregut fermenters are less dependent on the nitrogen concentration of their food (Hume, 1999). Stomachs of foregut fermenters are alkaline with a diverse bacterial and protozoan flora that have been shown to degrade PSMs such as alkaloids, cyanogenic glycosides, essential oils and phenols (Freeland *et al.*, 1974; Oates *et al.*, 1980). In many ways hindgut fermentation is a superior strategy for small mammals faced with a high fibre diet. Production of carbon dioxide and methane during foregut fermentation can lead to energy loss (up to 20% of that ingested) from easily digestible and fermentable foods (Demment & Van Soest, 1985). In addition, hindgut fermenters have more rapid passage of digesta than foregut fermenters of similar size (Duncan *et al.*, 1990; Illius & Gordon, 1992), an important strategy when the diet consists of large amounts of gut-filling fibre.

Hindgut fermentation

Hindgut fermentation in an enlarged caecum and/or colon, facilitates efficient digestion and excretion of fibre and PSMs (Cork *et al.*, 1991; Cork & Foley, 1997). Folivores of the temperate *Eucalyptus* forests of south-eastern Australia such as koalas, greater gliders, common ringtail and common brushtail possums, and tropical north-eastern rainforest species such as coppery brushtail, lemuroid ringtail, Herbert River ringtail, Daintree ringtail and green ringtail possums all perform hindgut digestion (Strahan, 1995). Although little research has been conducted on the digestive physiology of the rainforest folivores, all Pseudocheirids (folivorous possums from the temperate and tropical forests) may have similar strategies because gut morphology is an architectural feature generally conserved at family level (although Crowe and Hume (1997) and Hume *et al*. (1993) did find some fine-scale variation within the Pseudocheirids). Amongst these species, the strict folivores have developed mechanisms to efficiently process high fibre leaves while preserving nitrogen from a protein-poor diet (Hume, 1999). This process was reported in the caecum-colon of common ringtail possums and koalas, where nitrogen-rich solutes are selectively retained, while indigestible fibre is quickly excreted to reduce gut-filling bulk (Chilcott & Hume, 1985; Cork & Warner, 1983b). The common ringtail possum (and perhaps other Pseudocheirids, koala and greater glider) is able to further preserve nitrogen by recycling endogenous urea to its hindgut where it is used in microbial protein synthesis (Chilcott & Hume, 1984b; Rubsamen *et al.*, 1984), as well as dissociating tannin-protein complexes in a specialized caecum (McArthur *et al.*, 1991b). Caecotrophy, the ultimate small folivore strategy for nutrient conservation, involves reingestion of ‘soft faeces’ allowing additional extraction of nitrogen and microbes. Caecotrophy has been observed in rabbits, *Lepilemur* (Hladik, 1978), common ringtail possums (Chilcott, 1984; Chilcott *et al.*, 1985) and perhaps green ringtail possums (Crowe & Hume, 1997; Goudberg, 1990; Winter & Goudberg, 1995).

Rainforest folivores

In comparison with the extensive recent literature on eucalypt-specialist marsupial folivores (Lawler *et al.*, 2000; Marsh *et al.*, 2003a; Moore *et al.*, 2005a) there have been very few studies of the effects of leaf chemistry on dietary selectivity by the 89% of arboreal marsupial folivores that inhabit highly diverse rainforest (Flannery, 1995; Strahan, 1995). There are many differences between eucalypt-dominated woodland/forests and rainforest. These differences include plant species diversity, rainfall, soil characteristics, availability of particular nutrients and plant defensive strategies. Plant species complexity and therefore PSM diversity in eucalypt forest is relatively low in comparison to a rainforest ecosystem. A *Eucalyptus*-specialized folivore may be choosing between individuals of as few as two to four tree species in a community, whereas a rainforest folivore may select from more than 90 species. Foliage of rainforest trees tends to be more nutrient-rich than *Eucalyptus* (Braithwaite, 1996; Cork, 1996). Furthermore, total phenolics (which include the tannins and FPCs, among other PSMs) are generally higher and more variable in temperate *Eucalyptus*-dominated forests compared with those in tropical forest plants (Cork, 1996). Procter-Gray (1984) and Goudberg (1990) conducted seminal work on the feeding ecology of coppery brushtail, green ringtail, lemuroid ringtail and Herbert river ringtail possums and Lumholtz's tree-kangaroo. Interestingly, these studies both showed that rainforest resources were partitioned between herbivore species present at the sites. Procter-Gray (1985) found that Lumholtz tree-kangaroo, the largest folivore present, had the poorest quality diet (low nitrogen, high fibre), the generalist folivore/herbivore coppery brushtail had the highest quality diet (high nitrogen, low fibre), while the green ringtail possum which as a Pseudocheirid belongs to a specialist family with physiological adaptations to a high fibre low nitrogen diet selected intermediate quality foliage. These findings support the theory proposed by Cork and Foley (1991) that larger folivores can process a lower quality diet, as can specialist folivores with selective solute retention, urea recycling and caecotrophy, while generalist folivores rely on a higher quality diet to meet energy requirements. However these studies were conducted in the 1980s when less was understood of the specific effects of PSMs on the diet of eucalypt-specialist marsupial folivores. More recent eucalpt-folivore studies have used bioassay techniques and generally concentrated on the factors determining intraspecific foliage selection, greatly expanding our understanding of determinants of diet in folivores (Lawler *et al.*, 1998a; Moore *et al.*, 2005b; Pass *et al.*, 1998). This study proposes to examine determinants of foliage choice in a rainforest marsupial folivore, the green ringtail possum (*Pseudochirops archeri*: Pseudocheiridae) (Strahan, 1995) in the light of these recent findings of the direct influence of PSMs on selection of foliage within plant species.

Green ringtail possum

The green ringtail possum *Pseudochirops archeri* is restricted to rainforest in the uplands of north-eastern coastal Queensland, between Townsville and Cooktown (Flannery, 1994). As a pseudocheirid, *P. archeri* is a member of a specialist folivore family with features that assist them to live close to the physiological threshold of a small folivore, such as hindgut fermentation, urea recycling, selective retention of fluid digesta and caecotrophy (Cork *et al.*, 1991). Crowe and Hume (1997) found that pseudocheirids (7 species) had lighter small intestines, heavier caecums and longer proximal colons than the six generalist folivore/herbivore phalangerid species in the study (3 brushtail, 2 cuscus and the scaly tailed possum). In addition total nitrogen concentrations of stomach, caecum and proximal colon (but not distal colon) were higher in the Pseudocheiridae than the Phalangeridae. These differences reflect lesser emphasis on enzymatic digestion in the foregut, a greater reliance on hindgut microbial fermentation and a mechanism to selectively retain solutes in the hindgut of the Pseudocheirids (Crowe *et al.*, 1997) compared with the other more generalist possums. In addition the green ringtail has been reported as caecotrophic (Winter *et al.*, 1995), although Crowe and Hume's (1997) finding that the caecum was less well developed than that of the common ringtail possum, a known caecotroph, must cast some doubt on the importance of caecotrophy to *P. archeri*. Kingdon (1990) interpreted hyper development of the parotid salivary gland complex, poor mastication of food (especially compared to other rainforest pseudochierids) and enlarged absorptive area of the hind-gut in *P. archeri* as specific adaptations to buffer immediate impacts of PSMs.

At Curtain Fig State forest, (type 5b (Webb, 1959), Regional ecosystem 7.8.3 (Sattler & Williams, 1999) green ringtail possums ate only 1.4% non-leaf material, and specialised on primary rainforest species (Procter-Gray, 1984). Procter-Gray (1985) conducted preliminary chemical analyses of the preferred and avoided food plants of *P. archeri*, Lumholtz's tree-kangaroo (*Dendrolagus lumholtzi*) and coppery brushtail possum (*Trichosurus vulpecula johnsonii*). As discussed above, she found that the leaves eaten by the three folivores differed in chemical composition. *P. archeri* selected food species with relatively low nitrogen and high fibre content. However some potential food items such as *Castanospermeum australe* and young leaves of *Aleurites rockinghamensis* may have been ignored due to particularly high fibre contents (Procter-Gray, 1985). No other PSMs were tested in this study. In a different forest type (5a (Webb, 1959)) in Longlands Gap State forest, the feeding ecology of *P. archeri* was compared with two sympatric possums, the lemuroid ringtail (*Hemibelideus lemuroides*) and (*Pseudocheirus herbertensis*) (Goudberg, 1990). *P. archeri* was found to be the most specialised of the three species and fed extensively on young and mature fig leaves.

The lack of a den tree (and thus lack of associated limitations inherent in travelling and returning to a specific den each night) may enable *P. archeri* to forage more widely and hence be more selective than other rainforest possums (Russell, 1980). However, higher energetic demands associated with constant exposure to ambient temperature may incur additional energetic costs.

The studies discussed above have suggested that green ringtail possums are specialist folivores. They have assessed the nutrient content and presence of PSMs in food items selected and found that the possums may select resources on the basis of foliage quality. However, no attempt has been made to investigate the dietary selectivity of green ringtails within preferred plant species based on foliar chemistry.

PSMs, diet selection and climate change

Investigation of the chemical basis for diet selection by *P. archeri* will aid in understanding the importance of PSMs in determining distribution of rainforest marsupial folivores. The relationship between dietary ecology and distribution of the rainforest folivores is of particular interest due to indications that these animals are limited in their distribution to uplands and highlands by thermal constraints (Kanowski, 1999; Winter, 1997) and limitations on water intake, for evaporative cooling, imposed by PSMs (Krockenberger & Kanowski, in review). Both temperature and rainfall are strongly associated with altitude in Australia's wet tropics (Nix & Switzer, 1991) (temperature negatively and rainfall positively), and the small endemic folivores of the Wet Tropics are most abundant in cool, moist forests at altitudes above 700 – 800m (Kanowski *et al.*, 2001). Changes associated with global warming that may negatively affect the marsupial folivores include increases in temperature, especially during extreme events, and the effects of increases in atmospheric carbon dioxide.

Temperature

It is widely accepted that global climate warming is already affecting endemic vertebrate distributions in the wet tropics region of northern Queensland (Williams, Bolitho & Fox, 2003) and elsewhere around the globe

(Thomas *et al.*, 2004), and it has been predicted that with global warming, maximum temperatures in northern Queensland will increase by about 3.5°C before the year 2100 (Walsh *et al.*, 2001). Bioclimatic models have estimated that a temperature increase of 3.5 °C will result in a dramatic decline in distribution of all 65 regionally endemic vertebrate species, with 30 of these species completely losing climatically suitable habitat (Williams *et al.*, 2003). High altitude forests (complex notophyll vine forests, and simple notophyll and simple microphyll forests) may be especially susceptible to global warming due to the increase in altitude of the cloud base and associated mist with increasing temperatures (Hilbert, Ostendorf & Hopkins, 2001). Mist is thought to provide an important source of moisture in a high altitude forest and therefore may be a limiting factor in the distribution of rainforest possums (Kanowski *et al.*, 2001). Wet Tropics rainforests are believed to be derived from refugia formed during Pleistocene glacial periods about 18000 years ago (Nix *et al.*, 1991; Winter, 1984, 1997). Extant rainforest folivore species are therefore relatively cold-adapted and will be particularly susceptible to any increase in ambient temperatures. Green ringtails are restricted to the cooler rainforest above 300m altitude (Winter *et al.*, 1995), perhaps due to thermal intolerance (Kanowski, 2004). These animals appear to have a ‘heat storing’ capacity that effectively reduces water loss through evaporative cooling for short periods over 30°C (Krockenberger *et al.*, in review). In effect, *P. archeri* may be limited, especially in the late dry/ early wet season, by an interaction between duration and severity of extreme temperatures (over 30°C), the water content of foliage and toxic PSMs (Cork *et al.*, 1991; Krockenberger *et al.*, in review). According to this theory, the influence of plant defensive compounds on green possum distribution and abundance will become increasingly crucial with the rise in extreme heat events predicted by climate change models (Easterling *et al.*, 2000; IPCC, 2001). The role of plant secondary metabolites on *P. archeri* diet selection and intake form an important component of physiological limits to distribution. High thermoregulatory costs and low nutritional value of the diet during the late dry and early wet season will particularly affect rainforest possums (Kanowski, 1999; Krockenberger *et al.*, in review). At this time of year, water may be a limiting nutrient and concentrations of PSMs may be higher relative to nutritional qualities of leaves. While all other species of rainforest possum spend daylight hours insulated in a den, *P. archeri* rests curled-up on a branch and is therefore constantly exposed to the weather (Winter *et al.*, 1995). Exposure to a wide thermal range is energetically demanding for a small mammal (Withers, 1992), therefore *P. archeri* may require more energy and/or water for thermoregulation than a denning species.

Carbon dioxide

If PSMs are currently limiting folivore food intake, an increase in atmospheric CO_2 is likely to exacerbate the problem through increased temperatures and the secondary effects of carbon enrichment of foliage. Atmospheric concentrations of CO_2 are expected to double within the next 50-100 years (IPCC, 2001). Generally, elevated CO_2 levels affect foliar chemistry by reducing concentrations of nitrogen, while increasing content of carbon-based PSM compounds such as phenolics (Curtis & Wang, 1998; Koricheva *et* *al.*, 1998). Recent experiments applied treatments of elevated CO_2 (about 800 p.p.m., ambient 350 p.p.m.) to rainforest seedlings and saplings (Coley *et al.*, 2002; Kanowski, 2001; Wurth, Winter & Korner, 1998). The authors found that elevated CO_2 caused leaves to be tougher, have significantly higher phenolic content and increased carbon:nitrogen, particularly under conditions of low soil fertility, deep shade and high temperatures. In an elevated CO_2 environment, insect folivores consume more leaves per capita than at ambient CO_2, probably due to lowered nitrogen concentrations (Stiling *et al.*, 2003). Despite the carbon fertilization effect (increased leaf production under elevated CO_2) plants may experience increased folivore pressure and thus defend leaves more intensely. Folivores with high mass specific metabolic requirements, such as the green ringtail possum, will be limited in their ability to utilize low nutrient, highly defended foliage in a CO_2 rich environment (Crawley, 1983), especially if their intake is limited by PSMs, preventing them adjusting for lowered nutrient concentrations by increasing intake.

Thesis structure

This thesis examines the determinants of diet selection in the green ringtail possum in four stages Each result chapter has been written as, or is derived from a manuscript for submission to a refereed journal.

Chapter 2, “An assessment of diet analysis techniques” examines the techniques required to determine diet in a cryptic, secretive, nocturnal rainforest marsupial folivore and is currently under review with the journal Wildlife

Research as Jones K. M. W. and A. K. Krockenberger, Determining diet of folivores: a review of techniques.

Chapter 3, “A specialist folivore in a diverse forest” establishes the level of dietary selectivity in these possums in the context of their highly diverse rainforest habitat, establishes preferred tree species, individual trees and plant parts. The results of this chapter are published as Jones, K. M. W., S. J. Maclagan and A. K. Krockenberger (2006) Diet selection in the green ringtail possum (*Pseudochirops archeri*): a specialist folivore in a diverse forest. *Austral Ecology* **31**(7) 799-807.

Chapter 4, “Near infrared spectroscopy as a tool to investigate diet” provides a detailed description of the use of near infrared spectroscopy (NIRS) in this thesis. It documents the first use of NIRS to predict nutritional characteristics relevant to marsupial folivores in foliage of rainforest species, but is not currently under review with a journal.

Chapter 5, “Plant defenses determine feeding preferences in a specialist rainforest folivore” examines the determinants of foliage selectivity by *P. archeri* within and among canopy tree species. A manuscript derived from this chapter is currently under review in the Journal of Animal Ecology as “Jones, K. M. W. and A. K. Krockenberger, Plant defences determine feeding preferences in a specialist rainforest folivore”.

Chapter 6 is a general discussion that synthesises the results of this study, summarises the major findings of this thesis and considers their relevance to theory of dietary specialisation and the effects of PSMs on distribution and abundance of folivores.

CHAPTER 2.

An assessment of diet analysis techniques

Abstract

The dietary requirements of folivores affect many aspects of their ecology including home range, potential for social interaction, abiotic/climatic requirements and habitat choice at the landscape and local scale. The purpose of this study was to investigate techniques commonly used to determine the diet of folivores. Numerous studies detailing various methods of diet determination have been published as part of efforts to understand the feeding ecology of herbivores. However many folivores are arboreal, nocturnal and cryptic, causing a variety of problems for determining their diet. The largely folivorous green ringtail possum (*Pseudochirops archeri*) is all of these, inhabiting complex rainforest and often displaying particularly cryptic or evasive behaviour. The advantages and disadvantages of three techniques currently used in the literature (feeding observation, faecal analysis and a selectivity index 'tree use') are discussed in this paper. No single technique produced a comprehensive dietary list for *P. archeri* and each had different biases in the diet composition determined. We suggest that methods of dietary analysis should be selected according to the behaviour of the study species and the time and resources available and the nature of the specific research question. The outcomes of this research are particularly relevant to studies of folivorous primates, sloths, and all herbivorous marsupials of Australia and New Guinea.

Introduction

Dietary studies are vital to understanding the survival and productivity of individual herbivores and the distribution and abundance of populations. Determining diet of arboreal folivores is particularly important in studies of the co-evolved interactions between plants and herbivores and the factors limiting population density and distribution. For example, global climate change is predicted to cause increased atmospheric concentrations of CO_2, increase drought intensity and raise annual mean maximum temperatures (IPCC, 2001). This can affect nutrient and PSM content of food plants and thus may alter the suitability of large areas that are currently folivore habitat (Kanowski, 1999, 2001). In order to investigate the dietary ecology of arboreal herbivores, it is important for diet to first be established using reliable and repeatable techniques.

A common limitation of dietary studies on rare arboreal, herbivorous mammals (such as rainforest possums, tree-kangaroos, koalas, gliders, herbivorous primates and sloths) is the inability to directly measure diet selection. It is generally not feasible to catch animals regularly for assessment of mouth contents or fistula samples, and their rarity and ethical constraints prevent destructive techniques such as examination of gut contents. In many forest types, particularly tropical rainforest, observation of animals feeding in the canopy is made difficult by dense interlocking tree crowns and lianas, and impenetrable understory shrubs and scrambling vines. In this study we aimed to test three non-invasive techniques (direct feeding observations, faecal analysis and a selectivity index, ‘tree selection’) for determining diet of a folivorous marsupial, *Pseudochirops archeri* (green ringtail possum), endemic to tropical rainforest in far north Queensland.

Feeding observations

Direct observation of animal feeding continues to be a common diet analysis method (Chiarello, 1998; Dwiyahreni *et al.*, 1999). This technique involves locating marked animals and making behavioural observations through binoculars. Direct observation of arboreal herbivore feeding is valuable because the plant part consumed (leaf blade, petiole, flower, ripe or unripe fruit) can often be identified. Scan-spotlighting searches along transects are commonly used to locate arboreal animals for observations (Goudberg, 1990; Pahl, 1987), however some animals may be more easily located than others depending on individual behaviour, colouration and home range location in relation to forest edges. Biases associated with opportunistic observations may be avoided by radio-tracking animals, a technique that is particularly useful for cryptic, arboreal species located high in the canopy (Procter-Gray, 1984). By recording the behaviour of radio-collared individuals, it is possible to systematically avoid repeatedly observing the same easy-to-view animals, and ensure that equal effort is allocated to all study animals across their entire home range. The greatest difficulty associated with the feeding observation technique is identification of food species. In cases where the animal is nocturnal, species composition is complex, reproductive parts (fruit and flowers) unavailable, or the local flora poorly known, identification to the species level is not always possible (Chiarello, 1998).

Faecal analysis

Faecal analysis is a widely used technique that relies on species specificity of the plant cuticle, a waxy, non-cellular layer that protects the leaf from desiccation. Cuticles carry the imprint of leaf surface characteristics such as size, shape and alignment of cell patterns, stomates and trichomes, and can be identified to species level (Hinnant and Kothmann 1988; Christophel and Rowett 1996). Faecal analysis is commonly used in rangeland studies to assess grazing on grasses and herbs (De Boer *et al.*, 2000; Horsup & Marsh, 1992; Mussa *et al.*, 2003; Norbury, 1988a) and for studies of arboreal herbivores (Ellis *et al.*, 1999; Fitzgerald, 1984; Gaylard & Kerley, 1997; Pahl, 1987). Oesophageal fistula, analysis of gut content and faecal analysis all employ the microhistological technique of cuticle identification. These applications of cuticle identification have been compared in the literature with most authors concluding that faecal analysis is a relatively reliable technique (Anthony & Smith, 1974; Ellis *et al.*, 1999; Henley, Smith & Raats, 2001; Holechek, 1982; Todd & Hansen, 1973), although others consider it lacking in precision (Gill *et al.*, 1983; Johnson & Wofford, 1983). The main disadvantage of cuticular analysis is differential digestion, which results in plant species with fragile cuticles being under-represented or missing from the sample (Norbury, 1988b). Nevertheless, the faecal analysis technique was used for this study because it is non-invasive and allows easy access to large samples. To our knowledge this technique has not previously been published as a tool for investigating the diet of rainforest folivores.

Tree selection

The presence of a possum in a tree (tree selection) is a useful proxy of feeding, particularly when study animals are cryptic or easily disturbed and therefore rarely observed actually feeding. This is a currently accepted diet analysis method (Jones *et al.*, 2006; Martin *et al.*, 1999; Moore *et al.*, 2005a; Scrivener *et al.*, 2004), which relies on the premise that diet selection, rather than simply diet composition, must be considered to compare intake of various plant types in relation to their relative abundance in the field (Sprent & McArthur, 2002). A variety of selectivity indices have been used in previous studies of this sort (Dwiyahreni *et al.*, 1999; Goudberg, 1990; Oates *et al.*, 1977; Petraitis, 1979; Procter-Gray, 1985). In this study we used a randomisation procedure to identify whether plants are used more, less, or in proportion to their availability, so that tree species use could be adjusted for species abundance at the site (Jones *et al.*, 2006). For this reason, the tree selection technique is more powerful as a measure of habitat preference than for simple diet composition. Some problems are encountered when this technique is used to determine diet composition. For example, observations of animals in trees that are not preferentially selected and are either used as expected or avoided may not actually be trees that are being consumed (particularly when that tree species is abundant at the site). Furthermore, in a complex forest of vines, epiphytes and overlapping tree canopies, an animal may be feeding from any one of a number of different plant species, not necessarily the tree in which it is observed. However, other studies of marsupial folivores have shown that tree selection can be strongly related to feeding activity (Hindell *et al.*, 1985; Martin *et al.*, 1999; Moore *et al.*, 2005b), and that it can be used successfully to determine underlying causes for food selection (Scrivener *et al.*, 2004), so is a useful technique to consider in this study.

In this study we determined diet using all three techniques on the same set of study animals at the same study site, and compared the results to determine the practical strengths and weaknesses of each technique. There is no known technique available to field-based ecologists that allows true diet (measured as dry-matter intake) to be unequivocally measured. Therefore, the aim of this paper is to consider and compare the advantages and disadvantages of the methods available. The outcomes of this research are particularly relevant to studies of folivorous primates, sloths, and all herbivorous marsupials of Australia and New Guinea.

Methods

This study was conducted about 4 km south of Atherton on the Atherton Tablelands, Queensland (145°30′E, 17°18′S), elevation 750m, in a 33 ha fragment of complex notophyll vine forest (type 5b (Webb, 1959), Regional ecosystem 7.8.3 (Sattler *et al.*, 1999)). This forest type is characterised by a large number of deciduous and semi-evergreen trees, a tendency for heavy leaf-fall during times of drought stress and a dense shrub and scrambling vine layer. Ninety-four plant species were recorded as part of a vegetation survey at the site (Jones *et al.*, 2006).

Data were collected over five years, beginning in May 2000, with the majority of field observations made between September 2003 and April 2005. Twenty-five green ringtail possums were caught, collared, released and radio-tracked during this time. Possums were sighted opportunistically from within the fragment and along the forest edge using 35-watt spotlights. Animals in suitable positions were captured using a sedative (30mg ml^{-1} Zoletil, Virbac (Australia) Pty Ltd, 0.15ml at 200mg ml^{-1}) in 0.5ml tranquilliser darts fired from a gas powered dart gun (Black Wolf, Tranquil Arms Company, VIC). Anaesthesia usually occurred within 10 minutes. When they fell, the possums were caught in a tarpaulin. Each animal was weighed, measured, sexed, PIT tagged (Life Chip, Destron Technologies, USA) and fitted with a collar-mounted two-stage radio-transmitter (Sirtrack Limited, NZ). Possums were released into the capture tree when able to climb (after about two hours). After release, possums were located using a hand-held radio-receiver (Regal 2000 receiver and Yagi antenna; Titley Electronics Pty Ltd, NSW). Once a collared possum was located, observations including date, time, tree species, tree number, height, behaviour, weather, presence of other animals and whether the possum was feeding were recorded.

Feeding observations

Feeding was recorded throughout this radio-tracking study whenever it was observed. Possums were observed feeding on 104 occasions during almost 1000 hours of fieldwork. Binoculars were used to determine the plant part (young or mature leaves, petioles, fruits or flowers) consumed.

Faecal analysis

Faecal pellets were collected opportunistically over all months as possums were captured for radio collaring. Twenty-one faecal samples from 17 individuals were analysed. Each sample of pellets was homogenised and sub-sampled for each possum.

Approximately 1g of faeces was placed in a beaker of 35% w/w hydrogen peroxide (H_2O_2) and boiled gently for approximately 6 hours, until the cuticle pieces in the faeces were clear of cellular debris. The resulting strew was then rinsed and stained with crystal violet in a fine mesh sieve (150 microns). Approximately 0.15g (wet weight) of fragments were mounted on a large glass slide (5.0 x 7.5cm) in glycerine jelly and the coverslip edges sealed with nail polish to prevent mould and desiccation.

Reference slides were made for all trees, shrubs, vines and epiphytes within the rainforest fragment. A one cm^2 section was cut from the edge of the leaf, placed in a small test-tube of H_2O_2 and boiled until mesophyll tissue was digested and the lower and upper epidermal surfaces separated (3 to 12 hours depending on the plant species) (Christophel & Rowett, 1996). The open cuticular folder was cleared of cellular debris using water and small paint brushes, and stained with crystal violet. The cuticles were then mounted on glass slides with glycerine jelly, and the coverslip edges sealed with nail polish.

The strew slides were analysed using a compound microscope on x10 magnification. Once a cuticular fragment was encountered, a x40 lens was used to investigate surface features (stomata, trichomes, cell shape and cell wall characteristics) that were particular to the abaxial cuticle. Fifty randomly selected fragments were identified from each slide. Abaxial cuticular fragments in the faecal strews were matched with the reference slides and the cuticles were identified to species. We visually estimated the area covered by each cuticle type, as this method has been found to be more precise than frequency of occurrence for approximating relative amounts of species in faecal strews (Norbury, 1988b; Storr, 1968). This is largely because the cuticle of some species fragment more than others and therefore would be over-represented when recording frequencies of cuticles fragments (Dearden, Pegau & Hansen, 1975). This data was presented as the mean (± standard error) proportion of total cuticular area within each scat sample covered by each plant species. Only abaxial cuticular surfaces were used in this analysis because adaxial surfaces have few or no stomates making identification difficult.

Tree selection

A total of 524 observations of tree use were documented. Possum observations were included in this analysis only if they met criteria as described in Jones *et al*. (2006). A 20m x 20m grid was overlaid on the site without reference to topographic features. Each grid intersection was used as the centre of a 5m radius quadrat and all plants within the quadrat were identified to the level of species. Within the 60 quadrats sampled, 6374 individual plants were recorded from 94 species. Only plants with diameter at breast height (DBH) ≥ 3 cm were used in the analysis, as possums were never observed in plants any smaller than this. The DBH was recorded for all plants surveyed and the rank of plant species abundance was compared with rank of total basal area (calculated from DBH) for each species using Spearman's rank correlation (Jones *et al.*, 2006). The presence of a possum in a tree (tree selection) was used as an indication of feeding behaviour. Tree species selectivity was determined using a randomisation procedure. Five hundred and twenty four records (corresponding with number of observations) were randomly selected (with replacement) from the 6374 records of trees, and the tree species were recorded. This procedure was repeated 20 000 times to produce an expected distribution of randomly selected tree species. The number of sightings of possums in a specific tree species was compared with the randomly generated distribution and the probability of the result determined directly from the randomised distribution (Jones *et al.*, 2006). Only tree species that comprised more than 5% of the observations or abundance in the forest were analysed independently (n=8). All remaining tree and shrub species were combined in the group 'other' (n=86).

Results

Feeding observations

Four species comprised the majority of the 104 direct feeding observations of *P. archeri* at the study site (*A. rockinghamensis* (35%), *F. fraseri* (21%), *F. copiosa* (5%) and *A. divaricata* (4%)) (Table 1 and Figure 1). Possums were never observed feeding on juvenile leaves from the four preferred tree species. Mature leaves were the only items consumed, except a single observation of a ripe *F. fraseri* fruit (1%), *Solanum mauritianum* fruit (2%) and occasionally the petioles of *F. copiosa* (2%) and *D. photinophylla* (2%). Leaves from the vines *Cissus antarctica* (3%), *Melodorum lichardtii* (3%), *Elaeagnus triflora* (2%) and Glycine spp. (1%) were all consumed, and possums were also observed chewing vine stems. In the canopy, at night, a complex mix of tree, vine and epiphyte leaves sometimes made it impossible to identify the species of leaf being consumed. Therefore the group ‘unidentified vines’ probably includes some of the named vine species as well as unnamed species.

Faecal analysis

Of the 94 cuticle reference slides that were prepared in this study, 30% of species were unidentifiable due to degradation of the cuticle during preparation (Table 2). *Aleurites rockinghamensis* was the dominant cuticle type found in the faecal strews (51% of total cuticle area identified) (Figure 1). No *Ficus* species were identified. *Acronychia acidula, Aglaia sapindina, Cryptocarya triplinervis, Euroschinus falcata, Endiandra insignis* and *Melia* *azedarach* were found in the strews between 1 and 6 times each, and were combined in the group ‘other’ (Figure 1). Two species could not be identified using the cuticles in the reference collection (94 species of tree, shrub, vine and epiphyte, with all known genera at the site included).

Tree selection

Four tree species (*Aleurites rockinghamensis* (21%), *Ficus fraseri* (17 %), *Arytera divaricata* (11%) and *F. copiosa* (6%) accounted for over half (55%) of all trees selected by *P. archeri* (Figure 1). Each of these species was visited more frequently than expected (P<0.00005 of randomly generating a greater number than observed). Two species, *Castanospermum australe* (1% of use) and *Castanospora alphandii* (9%), were selected less than expected (P>0.99995 of randomly generating a greater number than observed). Selection of *D. photinophylla* (7%) and *Melia azedarach* (8%) was not different from expected (P=0.2869 and P=0.9127 respectively), although there was a tendency for *M. azedarach* to be avoided. When combined, all other tree and shrub species available at the site (n=86) were selected less than expected (avoided) (P>0.99995 of randomly generating a greater number than observed).

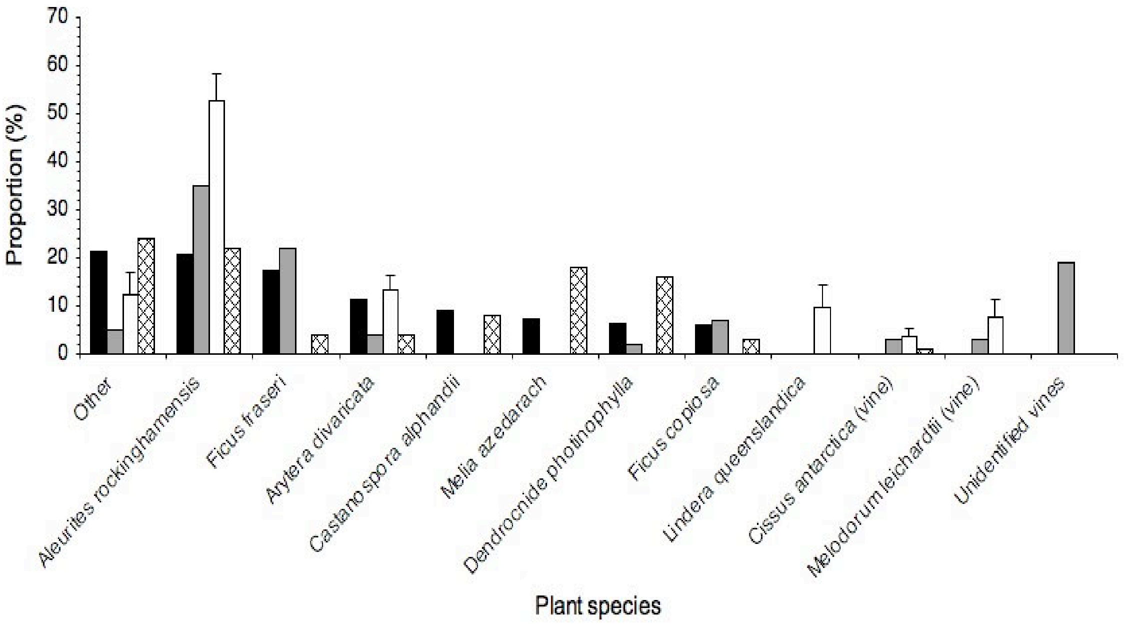

Figure 2.1: Proportion of plant species eaten or used by green ringtail possums as determined by three techniques; tree selection (■), direct observation(▒) and faecal analysis (□) as compared to tree availability represented by proportion of basal area (☒). Species encountered in less than 5% of cases by all 3 techniques (combined) were pooled in the group ‘other’. Errors on faecal analysis bars are standard errors.

Table 2.1: Proportion of food items consumed by *Pseudochirops archeri* according to direct observation of feeding (N=104)

Plant species	Habit	Leaf	Fruit	Petiole
Aleurites rockinghamensis	tree	35		
Arytera divaricata	tree	4		
Ficus copiosa	tree	5		2
Ficus fraseri	tree	21	1	
Dendrocnide photinophylla	tree			2
Other tree species	tree	4		
Solanum mauritianum	shrub		2	
Cissus antarctica	vine	3		
Melodorum leichardtii	vine	3		
Elaeagnus triflora	vine	1		
Glycine spp.	vine	1		
Dendrophthoe spp.	epiphyte	1		
Unidentified vines	vine	17		2

Table 2.2: Relative abundance within three categories of 'cuticle toughness' for cuticle reference slides of 94 plant species. Fragile cuticles were unidentifiable after boiling in H_2O_2 and cleaning with water and paintbrushes, moderate cuticles were fragmented but identifiable after boiling in H_2O_2 and cleaning with water and paintbrushes and robust cuticles were complete and undamaged after boiling in H_2O_2 and cleaning with water and paintbrushes. The important species listed were either preferentially selected, used as expected, or avoided (but abundant at the site) according to the tree selection technique.

Cuticle toughness	Proportion (%)	Important species
Fragile	30	Ficus fraseri (preferentially selected) Ficus copiosa (preferentially selected) Dendrocnide photinophylla (used as expected)
Moderate	21	
Robust	49	Aleurites rockinghamensis (preferentially selected) Arytera divaricata (preferentially selected) Melia azedarach (used as expected) Castanospora alphandii (avoided, common) Castanospermum australe (avoided, common)

Discussion

Feeding observations

Direct observation of feeding was the most time consuming of the techniques. About 1000 hours were spent over the course of this study, catching, radio-tracking and observing green ringtail possums, but possums were sighted feeding only 104 times (one data point per 10 hours of fieldwork). Although animals were radio-tracked for this study, possums were usually easier to view from the edge of the forest where trees were smaller, vines more readily identified and pioneer species more prevalent. In addition, plant species varied in their ease of identification at night in the forest canopy. The direct outcome of this disadvantage can be seen in the feeding observation results (Table 1), where in 17% of observations, possums fed on vines that could not be identified. Due to the huge investment of time required and biases associated with animal and foliage visibility, we propose that the direct observation method used in isolation is not particularly well suited to arboreal, nocturnal, cryptic or evasive animals. However, this technique did allow identification of plant parts consumed, valuable information that was not available using the other two methods tested in this study.

Faecal analysis

Faecal analysis was the most suitable technique to avoid biases caused by complexity of habitat or nocturnality, and was the only method that allowed us to estimate variance between individual possums (Figure 1), however this method had disadvantages. Differential digestion of cuticles is widely discussed in the microscopic diet-analysis literature and was evident in this study (Table 2). Some authors have found that digestion does not significantly alter species composition in faecal strews (Johnson *et al.*, 1983; Norbury, 1988a; Todd *et al.*, 1973), although differential digestion of plant material in the animal gut means that the amount of cuticle of each plant species present in the faecal strew is rarely proportional to that ingested (Holechek, 1982; Norbury, 1988b). Over-represented species generally have robust cuticles, while species with fragile cuticles will be underestimated or completely missing from the faecal strew. *A. rockinghamensis* and *A. divaricata* have particularly robust cuticles, they dominated the faecal strews (Figure 1 and Table 2), and thus their importance in the diet may be over-estimated when using this technique. Conversely, according to the results of the tree use and direct observation techniques, we know that *Ficus fraseri* and *F. copiosa* were significant components of the green ringtail diet at this site, but they were not present in any faecal strews. When preparing reference slides for *Ficus* species the cuticle was so fragile it disintegrated and tore easily even when treated with a very weak hydrogen peroxide solution (Table 2). It is therefore likely that *Ficus* cuticle is completely digested in the possum gut. A complete absence of species with fragile cuticles, caused by differential digestion, is a significant disadvantage of faecal analysis as a technique for assessing folivorous diets. Differential digestion may be accounted for using correction terms, developed from regression equations that compensate over or underestimation of cuticle types (Dearden *et al.*, 1975). Correction factors are produced by feeding captive animals known quantities of forage and then assessing the relative quantity of each species present in their faeces. This requirement enormously increases the cost and logistic difficulty of a dietary study, and still can not correct for a total loss of the identifiable fragments of important species as documented here. In addition, individual trees or parts of the tree being used can not be identified using faecal analysis, and considering the large amount of variability within species this information is particularly important.

Tree selection

The tree selection technique provides a statistical test for possum preference of tree species. Selected trees are chosen more often than would be expected if animals were choosing trees at random and should not be confused with principal or important species which may be eaten in proportion to availability (Petrides, 1975). The randomisation technique was used to predict the number of times a possum would be expected to choose a particular species if selection was random. From this distribution we determined which species were preferentially selected, which were avoided and which were selected as expected. Green ringtail possums do not actively defend territories, nor did observations correspond with concentrated periods of other social behaviour, so bias due to non-feeding activities was minimized (Jones *et al.*, 2006; Scrivener *et al.*, 2004). Furthermore, our behavioural observations show that, although these possums spent much of the night sleeping, they usually chose to rest in the tree from which they had just finished feeding (Jones *et al.* unpublished data). However, it is likely that the four preferentially selected tree species (*A. rockinghamensis, A. divaricata, F. fraseri* and *F. copiosa*) identified using the tree selection technique made up a much greater proportion of the diet than suggested from the observations (55%), because most of the observations of animals in avoided species were probably not associated with feeding (Jones *et al.*, 2006). This theory is supported by the feeding observation and faecal analysis methods, which found the four preferentially selected species comprised 68 % and 66 % of the diet respectively (Figure 1). Furthermore, tree species used at the 'expected' level (*D. photinophylla* and *M. azedarach*) were rarely or never present in the faecal analysis and direct observation results, and all 88 'avoided' species were completely absent from the faecal analysis (despite having robust cuticles, Table 2) and direct observation results. This data provides additional evidence that the 'as expected' and 'avoided' (for example *C. alphandii* and *C. australe*) species are actually not being eaten, but instead the animals were probably just moving through these common species to access preferred trees.

Studies of *Eucalyptus*-specialised folivores have isolated the chemical basis of feeding deterrence within trees of preferred species (Lawler *et al.*, 2000; Lawler *et al.*, 1998a; Moore *et al.*, 2005a; Pass *et al.*, 1998). The advantage of the tree selection technique is that it can be used to systematically identify patterns of intraspecific tree preference. This is a vital feature of a diet analysis technique to be used in studies that aim to determine underlying factors that drive diet choice (Scrivener *et al.*, 2004).

An inherent problem in our tree selection technique was the assumption that a possum observed in a tree was feeding only on tree leaves and not associated vines and epiphytes. The faecal analysis and direct observation revealed at least four species of vines (*C. antarctica, M. leichardtii, Glycine* spp and *E. triflora*) and an epiphyte (*Dendrophthoe falcata*) in the diet, so this assumption is at least partially wrong. The difficulty of discerning whether the animal is feeding from the tree in which it is sitting or a nearby tree, vine or epiphyte presents the key weakness of this otherwise robust diet-determination technique. However, animals feeding from vines are unlikely to bias the selectivity indices for tree species unless certain vines are disproportionately associated with specific tree species. In this case, the tree species accounting for the greatest number of observations, *Aleurites rockinghamesis*, is a rapidly growing pioneer species and tends not to support a high biomass of vines, so it is unlikely that the preference for that species at least was confounded by animals feeding from vines.

We therefore suggest that the nature of the research question should be considered when choosing a dietary analysis technique. In forest types where there is no overlap between potential food species in the canopy, the tree selection technique provides a reliable and relatively efficient method for identifying plants important in the diet of arboreal herbivores. It also allows identification of intraspecific plant preference. However, for rainforest studies there is value in combining the techniques as ‘tree selection’ can identify tree species, as well as individual trees selected, while faecal analysis also recovers vines and epiphyte cuticles, except those that are fragile (for example Glycine spp) or only rarely eaten (for example *Dendrophthoe falcata*). In this study these fragile and rare dietary items were identified using opportunistic feeding observations. Direct observation is also advantageous when the study animals are not strict folivores, as food items such as flowers and fruit are more difficult to identify using faecal analysis or the tree selection technique. Thus, a study aimed at identifying the full diversity of the diet should use a number of techniques. Single techniques may be suitable in some cases, for example, if field-time is limited (faecal analysis) or as part of a long-term behavioural study when field-hours are not restricted (feeding observations).

CHAPTER 3.

A specialist folivore in a diverse forest

Abstract

This study investigated the feeding ecology of the green ringtail possum, *Pseudochirops archeri* (Pseudocheiridae) in a tropical rainforest with 94 plant species in the canopy. Over 50% of tree use was from only four tree species, *Aleurites rockinghamensis*, *Ficus fraseri*, *Arytera divaricata* and *F. copiosa*. These species were used significantly more frequently than would be expected if tree species were selected randomly in proportion to their relative abundance in the forest. Conversely, 88 other tree species present were used less frequently than expected. Possums also favoured particular individual trees within some of the preferred tree species. In 91% of feeding observations, possums consumed mature leaves only. The availability of young leaves, flowers and fruit varied throughout the year, with a peak in availability of these resources during the early wet season. By primarily selecting mature leaves, green ringtail possums reduce their dependence on seasonally variable resources. We suggest that green ringtail possums should be considered as specialist folivores, focussing on only a few of the tree species available, possibly due to advantages associated with limiting the number of plant secondary metabolites (PSM) in their diet. Furthermore, they favour certain individual trees within species, perhaps due to intraspecific variation in PSM or nutrient content, behaviour that has been well established in eucalypt folivores.

We conclude that green ringtail possums are highly specialised in their feeding ecology, limiting their diet to a small number of continuously available food items.

Introduction

Plant secondary metabolites (PSMs), function as chemical defence of plant parts against herbivory through interference with feeding and post-ingestive processes (Cork *et al.*, 1991; McArthur *et al.*, 1991a). Freeland and Janzen (1974) proposed that most herbivores should be dietary generalists because different classes of PSMs must be detoxified via separate metabolic pathways. Herbivores should then feed from a variety of sources to avoid overloading any particular detoxification pathway. Dietary studies have found that most herbivores are generalists (for example (Freeland, 1991; Ganzhorn, 1988; Kerle, 1984)) and detoxification limitation at least partially explains this feeding strategy (Dearing *et al.*, 1999). Alternatively, herbivores may be specialists in order to concentrate on plants with a certain group of PSMs and avoid others (Ganzhorn, 1989), thus concentrating on a single or limited set of detoxification pathways.

As specialists within a low diversity forest, eucalypt folivores (the koala, greater glider, common brushtail and common ringtail possums) exploit mature and young leaves, buds, fruit and flowers of three *Eucalyptus* subgenera to varying degrees depending on their ability to procure and digest the food items (Cork *et al.*, 1997; Moore *et al.*, 2000). Most interestingly, these folivores base much of their selectivity on a group of deterrent phenolics in their *Eucalyptus*-

dominated diet known as formylated phloroglucinol compounds (FPCs). The concentration of these compounds can explain more than 70% of variation in foliage intake, although there is some variation between folivore species (Lawler *et al.*, 1998a; Lawler *et al.*, 1999; Moore *et al.*, 2005a; Pass *et al.*, 1998; Pass *et al.*, 2000; Wallis *et al.*, 2002). For example, common brushtail possums have a greater tolerance than common ringtail possums for the FPC phenolics, but their food intake is limited by tannins (Marsh *et al.*, 2003a; Marsh *et al.*, 2003b).

The *Eucalyptus*-specialised folivores have little opportunity to be generalists due to the limited diversity of the forests they inhabit. However rainforest marsupial folivores occupy habitat that is highly diverse in both nutritional possibilities and PSMs (Cork, 1996), and thus provide a unique insight to the origins and advantages of dietary specialisation. All eucalypt and rainforest specialised folivores have their evolutionary origin in the southern cool temperature rainforests shared with Antarctica and South America prior to the separation of Australia from Gondwanaland about 60 million years ago (Archer, 1984). A rich arboreal rainforest marsupial fauna deposited at Riversleigh in north-western Queensland about 20 million years ago included a giant koala, 16 species of possums and a greater diversity and wider distribution in the genera *Pseudochirops* than is presently found in north Queensland rainforests (Flannery, 1994). Ancestors of the eucalypt-feeding folivores are thought to have already been specialists on eucalypts within this ancient rainforest (Archer, Hand & Godthelp, 1991). As Australia dried out, eucalypt-dominated forests expanded and rainforests contracted, while the ranges of *Eucalyptus* and rainforest specialist folivores expanded and contracted accordingly (Archer *et al.*, 1991; Kershaw, 1981). Climate change during the Pleistocene glaciations, which ended 15, 000 years ago, caused further rainforest contractions resulting in the restriction of rainforest mammals to refugia in the Wet Tropics of far north Queensland (Winter, 1988, 1997).

The green ringtail possum, *Pseudochirops archeri* (Pseudocheiridae) has been previously described as a fig-foliage specialist with a high fibre diet (Goudberg, 1990). As a small (~1kg) folivore, the green ringtail possum faces stringent energetic limitations posed by a high fibre diet (Cork *et al.*, 1991). Furthermore, as a small folivore inhabiting a rainforest environment, this possum provides an excellent model in which to examine the diet of a folivore in a diverse forest. This study quantifies selectivity at the level of tree species, individual tree and food item within a tree (fruit, flowers, young and mature leaves) in the green ringtail possum diet.

Methods

Study Site

This study was conducted at Nasser’s Nature Refuge, a privately owned 33 ha fragment of complex notophyll vine forest (type 5b (Webb, 1959), Regional ecosystem 7.8.3 (Sattler *et al.*, 1999)), located about 4 km south of Atherton on the Atherton Tablelands, Queensland (145°30¢E, 17°18¢S), elevation 750m. The forest is relatively dry with the average annual rainfall about 1400 mm (Atherton: Bureau of Meteorology data), most of which falls during the ‘wet’ season between December and April. This forest type is associated with fertile basaltic soils and is characterised by an uneven canopy (25–45m) with a large number of deciduous and semi-evergreen trees, a tendency for heavy leaf-fall during times of moisture stress and a dense shrub and scrambling vine layer.

Radio-collaring animals

Data were collected over five years, beginning in May 2000, with the majority of field observations made between September 2003 and April 2005. Twenty-five green ringtail possums were caught, collared, released and radio-tracked during this time. Possums were sighted opportunistically from within the fragment and along the forest edge using 35-watt spotlights. Animals in suitable positions were captured using a sedative (30mg ml^{-1} Zoletil, Virbac (Australia) Pty Ltd, 0.15ml at 200mg ml^{-1}) in 0.5ml tranquilliser darts fired from a gas powered dart gun (Black Wolf, Tranquil Arms Company, Seymour, Vic., Australia). Anaesthesia usually occurred within 10 minutes. When they fell, the possums were caught in a tarpaulin. Each animal was weighed, measured, sexed, PIT (passive integrated transponder) tagged (Life Chip, Destron Technologies, South Saint Paul, Minnesota, USA) and fitted with a collar-mounted two-stage radio-transmitter (Sirtrack Limited, Havelock North, New Zealand). Possums were released into the capture tree when able to climb (after about two hours). After release, possums were relocated regularly during monthly five-day field trips using a hand-held radio-receiver (Regal 2000 receiver and Yagi antenna; Titley Electronics Pty Ltd, Ballina, NSW, Australia). When a collared possum was located, observations including date, time, plant species, plant number, height, behaviour, weather, and presence of other animals were recorded. If the animal continued to feed in an undisturbed manner, it was observed using binoculars and the part of the plant (young or mature leaves, petioles, fruits or flowers) consumed was also noted. Young leaves were not fully expanded and distinguished from mature based on their lighter colour and in some species the covering of fine brown hairs. Furthermore, the possums often fed by breaking off a leaf at the petiole, eating part of the leaf and dropping the remainder, allowing us to examine the discarded, partly eaten leaves.

Vegetation

A 20m x 20m grid was overlaid on the site without reference to topographic features. Each grid intersection was used as the centre of a 5m radius quadrat and all plants within the quadrat were identified to the level of species. Within the 60 quadrats sampled, 6374 individual plants were recorded from 94 species. Only plants with diameter at breast height (DBH) ≥ 3 cm were used in the analysis, as possums were never observed in plants any smaller than this. The DBH was recorded for all plants surveyed and the rank of plant species abundance was compared with rank of total basal area (calculated from DBH) for each species using Spearman's rank correlation. The phenologies of the 12 most common tree species were recorded at the site monthly from May 2004 to April 2005.

Tree species selection

Observations of tree use were used as the indicator of diet in this study, largely because it allowed both interspecific and intraspecific selectivity to be determined statistically, in a shorter time than required for direct observations, and without the artifactual errors associated with faecal analysis when the figs *Ficus fraseri* and *F. copiosa* were important parts of the diet (Jones & Krockenberger, in review).

Possum observations were included in this analysis only if they were made between 6pm and 6am (active feeding hours) and the individual had been sighted and positively identified (524 observations). All observations were made at least 2 hours apart (more than 50% of possums moved tree within 2 hours). The presence of a possum in a tree (tree use) was used as an indication of feeding behaviour. Tree species selectivity was determined using a randomisation procedure. Five hundred and twenty four records (corresponding with number of observations) were randomly selected (with replacement) from the 637 records of trees, and the tree species were recorded. This procedure was repeated 20 000 times to produce an expected distribution of randomly selected tree species. The number of sightings of possums in a specific tree species was compared with the randomly generated distribution and the probability of the result determined directly from the randomised distribution. Only tree species that comprised more than 5% of the observations or abundance in the forest were analysed independently (n=8). All remaining tree and shrub species were combined in the group ‘other’ (n=86).

Individual tree selection

Intraspecific tree preference was tested by comparing the frequency of use of individual trees within a species with that expected from a truncated Poisson distribution (Cohen, 1960). This method accounts for the lack of zeros (individual trees could not be identified as ‘never visited’).

Results

Vegetation survey

The dominant tree species were *Castanospora alphandii* (17% of all plant species surveyed DBH≥3cm), *Castanospermum austral*e (8%), *Aleurites rockinghamensis* (8%) and *Dendrocnide photinophylla* (6%) (Figure 1). The most common plants also contributed most basal area, as the rank of abundance and basal area of plant species were strongly correlated (Spearman’s rank correlation P<0.001).

Tree species use

Four tree species (*Aleurites rockinghamensis* (21%), *Ficus fraseri* (17 %) and *Arytera divaricata* (11%) and *F. copiosa* (6%) accounted for over half (55%) of all trees used by *P. archeri* (n=524 observations in total) but together accounted for only 10 % of trees at the site (Figure 1). These species were used more frequently than expected (P<0.00005 of randomly generating a greater number than observed; 0/20000 of the random distribution were equal or greater). Two species, *Castanospermum australe* (1% of use) and *Castanospora alphandii* (9%), were used less than expected (P>0.99995 of randomly generating a greater number than observed; 20000/20000 of the random distribution were greater). Use of *D. photinophylla* (7%; P=0.2869; 5738/20000 of the random distribution were equal or greater) and *Melia azedarach* (8%; P=0.9127; 18252/20000 of the random distribution were equal or greater) were not different from expected, although there was a trend for *M. azedarach* to be avoided. When combined, all other tree and shrub species available at the site (n=86) were used less than expected (P>0.99995 of randomly generating a greater number than observed).

Individual tree use

Within the preferred tree species, certain individual trees tended to be preferred (Figure 2). The frequency distribution of use of individual trees within the most commonly used species, *A. rockinghamensis*, was significantly different from the expected Poisson ($c^2 = 10.5$, $P = 0.02$, $df = 3$). The frequency distribution of use of *F. fraseri* trees tended to be different from expected, but this was not significant (c^2 = 6.8, P = 0.08, df = 3). The distribution of use in *A. divaricata* and *F. copiosa* was not significantly different from expected (c^2 = 3.47, P = 0.34, df = 3 and c^2 = 1.53, P = 0.47, df = 2 respectively).

Within tree availability and use

The phenologies of tree species at the study site were highly variable between months (Table 1). Only 50% of trees at the site had new leaves during March - May, while 87% had new leaves in the preceding three months of the wet season. Flowers and fruit were found on less than half of the tree species during the dry season (June - August), but 87% of species during December - February. Variation in flowering/fruiting and leafing was even more pronounced among the four preferred food species (Table 1). For example, only 25% of preferred species had flowers or fruit during June – August, compared to 74% in the following 3 months. Furthermore, almost all preferred trees had new growth from December – February but fewer than 50% were leafing in the following 3 months. Undisturbed feeding behaviour was observed on 103 occasions (Table 2). Ninety-one percent of food items were mature leaves and nine percent were petioles and fruit.

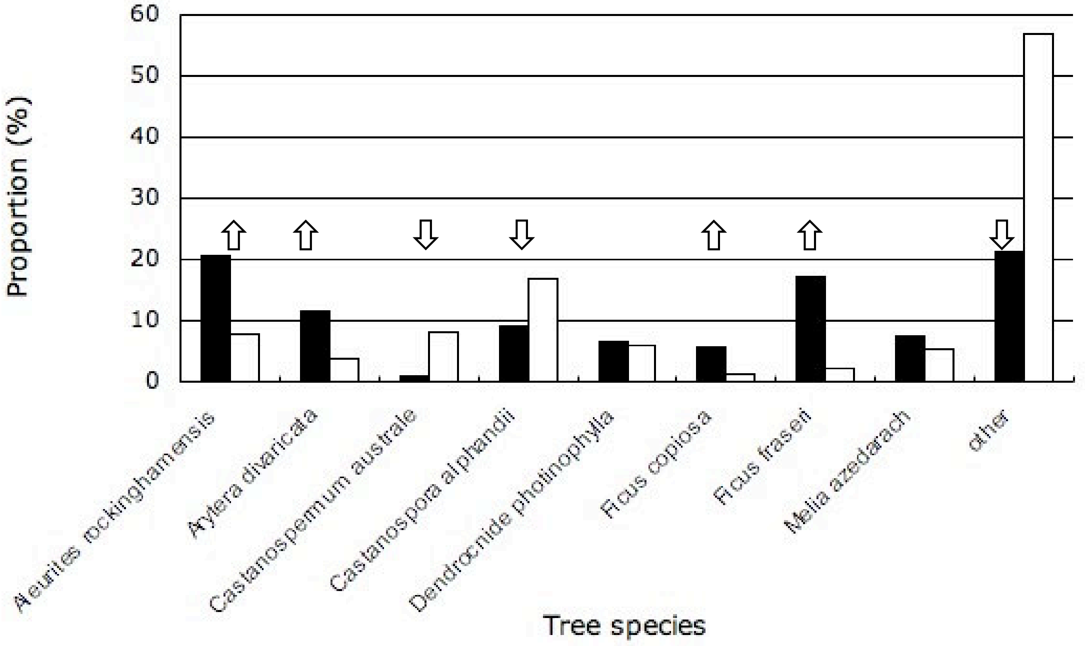

Figure 3.1: A comparison of the proportion of tree species used by green ringtail possums (☐) and proportion of available tree species (■) at the study site. Species used significantly more frequently (P<0.00005) than expected (⇧) and significantly less frequently (P>0.99995) than expected (⇩).

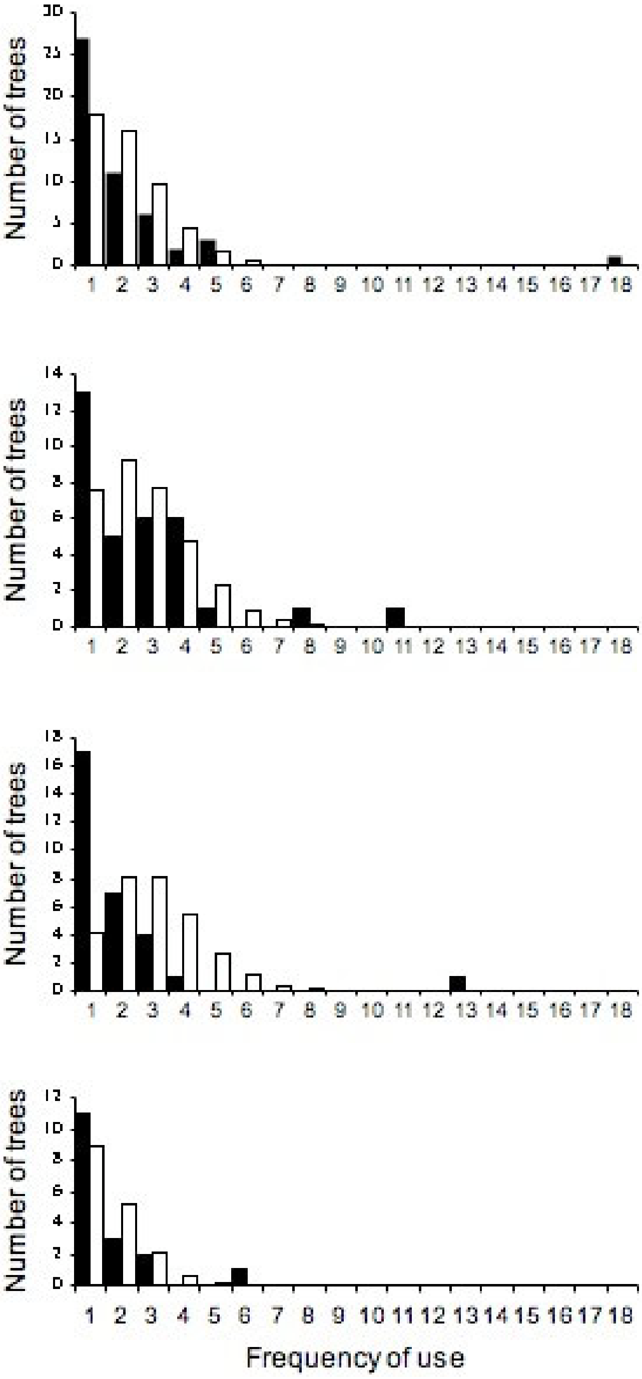

Figure 3.2: Frequency of individual tree use by green ringtail possums (■) and expected frequencies from the truncated Poisson distribution (□), within the tree species (a) *Aleurites rockinghamensis*, (b) *Ficus fraseri*, (c) *Arytera divaricata* and (d) *F. copiosa.*

Table 3.1: Phenology of the dominant tree species at the study sight, showing proportion of tree species fruiting/ flowering and leafing during the dry season 2004 (June-August and September-November) and wet season 2004-2005 (December-February and March-May). Preferred species are *Aleurites rockinghamensis*, *Ficus fraseri*, *Arytera divaricata* and *F. copiosa*.

	Preferred species		All Species	
	Flowers/fruit(%)	New growth(%)	Flowers/fruit(%)	New growth(%)
June-Aug	25	75	47	73
Sept-Nov	74	50	78	64
Dec-Feb	67	92	87	87
March-May	50	42	57	50

Table 3.2: Direct feeding observations of the rainforest arboreal folivores green ringtail possum, coppery brushtail possum (CBTP), Herbert River ringtail possum (HRRT), Lumholtz's tree-kangaroo (LTK) and lemuroid ringtail possum (LRTP) from the present study (103 observations), Procter-Gray, (1985) (PG) (141 observations) and Goudberg, (1990) (G) (118 observations). Mature leaves (L), fruit (F), petioles (P), young leaves (Y), leaf bud (B) and fruit or flower (R).

Family	Tree species	Green ringtail possum feeding			Other species feeding
		This study	Procter-Gray	Goudberg	Procter-Gray, Goudberg
Euphorbiaceae	A. moluccana /rockinghamensis	35L	37L	0	CBTP 1R (PG), HRRT 1L (G)
Moraceae	F. fraseri	21L 1R	8L	0	CBTP 3F (PG)
Moraceae	F. copiosa	5L 2P	5Y	0	
Sapindaceae	A. divaricata	4L	0	0	LTK 1L (PG), HRRT 1L 1F (G)
Urticaceae	D. photinophylla	2P	24L 1B	2L	LTK 1L (PG)
Meliaceae	M. azedarach	0	0	0	CBTP 36L 4F 1Y (PG)
Fabaceae	C. australe	0	0	0	CBTP 3R, LTK 2Y (PG)
Sapindaceae	C. alphandii	0	0	0	HRRT 5L, LRTP 63L 3B (G)
Other plant species		29L 2P 2R	60L 5Y 2B	91L 6F	

Discussion

Like the eucalypt-specialised temperate forest folivores (the koala, greater glider, common ringtail and common brushtail possums), green ringtail possums appear to be dietary specialists. Of 94 tree species available at the study site, the possums used only four species (*Aleurites rockinghamensis, Ficus fraseri, Arytera divaricata* and *F. copiosa*) more often than would be expected considering the relative abundance of each species, and these four species comprised 55% of tree use. Furthermore, green ringtail possums are selective at an intraspecific level in their favourite tree species, *Aleurites rockinghamensis,* and possibly also in *F. fraseri*, another significantly preferred species. In the specialist eucalypt-folivores, intraspecific selection is based on a group of deterrent PSMs called FPCs (Lawler *et al.*, 1998a; Pass *et al.*, 1998). We suggest that intraspecific variation in PSMs may also be responsible for intraspecific tree selection by green ringtail possums.

Both proto-koalas and eucalypts are relatively rare in rainforest fossil deposits from Riversleigh, leading Archer *et al.* (1991) to suggest that eucalypt-specialisation evolved in these rainforests of the Oligo-Miocene. Thus specialisation may have evolved concurrently in the ancestors of extant rainforest folivores through niche separation within the ancient rainforest. The similarity in feeding ecology of a rainforest possum with its eucalypt-specialised relatives is particularly significant because unlike temperate forests, rainforests contain a greater diversity of plant species and plant defences (Coley *et al.*, 1991; Cork, 1996). We suggest that green ringtail possums are dietary specialists either to minimise PSM intake of any sort and thus reduce the energy expenditure associated with PSM detoxification and elimination, or to limit the diversity of PSMs to which they are exposed. The detoxification limitations hypothesis predicts that herbivores in general should select a diverse diet to avoid overloading any single detoxification pathway (Freeland *et al.*, 1974). However, ingesting PSMs incurs energy costs in both specialist and generalist herbivores (Dearing *et al.*, 2000; Sorensen *et al.*, 2005b) so specialisation in this case could reflect avoidance or minimisation of PSMs in general (Freeland *et al.*, 1974). Alternatively, specialisation may arise when particular diet items high in PSMs are easily and consistently available (Freeland *et al.*, 1974). Recent studies show that specialist herbivores are able to maintain a higher intake of PSMs from within their normal diet, but are more strongly impacted by novel PSMs than are generalists (Dearing *et al.*, 2000; Sorensen *et al.*, 2005a). Specialists are thought to be able to maintain a higher intake of PSMs through increased detoxification ability (Dearing *et al.*, 1999), decreased absorption of toxins through increased excretion of unmetabolised toxins (Sorensen, 2003; Sorensen *et al.*, 2004) and adjustments to their energy budgets (Sorensen *et al.*, 2005b). In laboratory experiments, specialist herbivore woodrats absorbed less and excreted more PSM per unit ingested than generalist herbivore woodrats (when offered a PSM present in the natural diet of both species), which allowed the specialist to consume greater quantities of PSMs from their natural diet (Sorensen, 2003; Sorensen *et al.*, 2004). However, when novel PSMs were ingested, specialists lost body mass, reduced food intake and had one-sixth the energy available for energy dependent activities compared with generalists, which were able to maintain body weight and food intake on a novel diet (Sorensen *et al.*, 2005a).

Although green ringtail possums prefer to feed on a small proportion of the available tree species, preferences vary greatly between sites (Table 3.2). We therefore suggest that although this possum is a specialist, it is able to alter the primary species in its diet according to the tree species available at the site and the presence of potential competitors. It appears that dietary resources are partitioned among sympatric folivores (Lumholtz's tree-kangaroo, lemuroid ringtail possum, Herbert River ringtail possum and coppery brushtail possum), possibly based on PSM tolerance (Kanowski, Irvine & Winter, 2003). A link between interspecific diet partitioning and plant chemistry has been established among Malagasy primate species, where major differences in their food choice could be related to protein concentrations, condensed tannins and alkaloids (Ganzhorn, 1988). In previous studies, the green ringtail diet was higher in fibre and lower in protein, compared with sympatric possums, but similarly low in condensed tannins (Goudberg, 1990; Procter-Gray, 1985). One species preferred by green ringtail possums in the present study (*Aleurites rockinghamensis*) has relatively low concentrations of total phenolics and condensed tannins (<2.4% and <1.7 % DW respectively), whereas an avoided species *Castanospora alphandii* contains much higher proportions of these PSMs (11.8% and 10.8 % DW respectively) (Kanowski, 1999). However, the nutrient and PSM composition of the tree species preferred and avoided in this study would need to be better understood before we could test our suggestion that diet specialisation in green ringtail possums functions to limit the quantity or diversity of PSMs ingested, as found in specialist woodrats (Sorensen *et al.*, 2005a).

Ninety-one percent of food items consumed by green ringtail possums during this study were mature leaves. This is contrary to expectations because mature leaves are generally thought to be a lower quality resource than young leaves (which generally have higher nitrogen and lower fibre content) and fruits (which contain much less fibre, few PSMs and have a higher concentration of non-structural carbohydrate) (Hume, 1999). Small folivores should be limited by dietary fibre because as body size decreases, energy requirements per unit mass increase, without a proportional increase in gut capacity (Foley *et al.*, 1992). It has been suggested that green ringtail possums have so little available energy that they can not afford to search for unpredictable resources (Procter-Gray, 1984). In the Wet Tropics there is a wet season peak in abundance of new leaves and a late dry season peak in fruit and seed (Crome, 1975). Our phenological survey revealed the highest proportion of flowers, fruit and new leaves at the study site during the late dry to early wet season. Furthermore, only one of the possums’ four preferred tree species produced flowers or fruit during the early dry season and less than 50% flushed new leaves during the early wet. Clearly then, flowers, fruits and new leaves would not constitute a reliable food source at the study site. In contrast, mature leaves were available throughout the whole year, making them a reliable, continuously available resource. The green ringtail possum’s preference for mature foliage may be due to the fact that it is more continuously available throughout the year (Freeland and Janzen 1974), or alternatively that mature leaves are less defended than new leaves of the food species (Coley, 1983a). Mature foliage of *Aleurites*, a preferred food, has lower levels of nitrogen and ratio of nitrogen to fibre, but unusually also lower fibre than young leaves which are not consumed (Procter-Gray, 1985). However, both Goudberg (1990) and Procter-Gray (1985) found that the diet of green ringtails had higher fibre levels than diets of other sympatric folivores, making it unlikely that diet selection in this species is primarily driven by fibre avoidance. This also suggests that diet selection in this species is driven more by PSMs than nutrient content, otherwise it is difficult to explain why they would not opportunistically use higher nitrogen new leaves of the preferred species when they were available.

It is believed that small folivores cope with a high fibre diet through adaptive modifications to their digestive physiology, such as selective retention of fluid digesta and rapid passage of fibrous material through the gut (Cork *et al.*, 1991). The green ringtail’s highly specialised gut morphology may be the key allowing it to specialise on mature leaves despite their high fibre content. The most folivorous of the arboreal mammals (koalas, greater gliders, ringtail possums and sportive lemurs) are all caecum or caecum-colon fermenters with colonic specializations for selective retention of small particles of digesta (Cork, 1996). The ability of green ringtail possums to live close to the minimum size threshold for a folivore may be due to hindgut fermentation, rapid through-put of indigestible fibre and possibly caecotrophy (Cork *et al.*, 1991; Winter *et al.*, 1995). Compared with the Phalangeridae (dietary generalist brushtail and scaly tail possums and cuscuses), Pseudocheirids have a shorter small intestine and a larger caecum, as well as higher nitrogen concentrations in the stomach, caecum and proximal colon, suggesting the presence of a colonic separation mechanism (Crowe *et al.*, 1997).

One possible limitation of this study was the inability to measure diet selection more directly. It was not feasible to catch possums regularly for assessment of mouth contents or fistula samples and their rarity and ethical constraints prevent stomach content examination. Thus presence of a possum in a tree (tree use) was used as a proxy of feeding. We used collared animals to prevent biases associated with opportunistic observations where individual animals may vary in detectability, or where the visibility of animals varied between tree species. Studies on other marsupial folivores have concluded that tree visitation is an acceptable and useful measure of foraging (Martin *et al.*, 1999; Moore *et al.*, 2005a; Scrivener *et al.*, 2004). There may be reasons other than feeding for possums to be observed within particular trees, such as day-time roosts and movement between feeding locations. In this study we excluded day-time roost trees from the analysis, and there is no known behavioural reason, such as territorial defence or mating for these possums to occupy specific trees. Some of our observations would certainly reflect animals moving between feeding locations, however we would expect those observations to match the frequency of occurrence of tree species in the forest. Consequently, we have used a measure of selectivity to determine if tree species use was significant and out of proportion with the tree species occurrence within the forest. A further unavoidable bias associated with this approach was the inability to discern between trees or vines and epiphytes as the reason for an animal to occupy a tree. Green ringtail possums in previous dietary studies ate vines and epiphytes, but tree foliage comprised the majority of their diet (Goudberg, 1990; Procter-Gray, 1985) and the most commonly used preferred species in this study, *Aleurites rockinghamensis*, and *Ficus fraseri* are rapidly growing pioneers that rarely support vines or epiphytes within their canopy at this site. Therefore we are confident that the results of the study accurately reflect preferences of canopy species even though vines especially may also be important. Because we have compared tree use with tree availability, trees not actively used would be included in observations at the randomly expected level. We observed few species at the randomly expected level and most were strongly selected or avoided, so we believe it is reasonable to assume a close relationship between feeding behaviour and our observations (Scrivener *et al.*, 2004). With this in mind, it is possible that the four preferred tree species in this study made up a greater proportion of the diet than suggested from the observations (55%), because observations of animals in avoided species may not have been associated with feeding. Our estimation of selectivity compared frequency of use with density of tree species and did not account for plant biomass, which can affect feeding preference (Moore *et al.*, 2005a). Nevertheless, the rank abundance of tree species and biomass (measured as basal area) of those species were strongly correlated and tree species were so strongly preferred ($P<0.00005$ of randomly generating a greater number than observed) or avoided (P<0.99995 of randomly generating a greater number than observed) that we have confidence the results reflect real preferences.

In conclusion, green ringtail possums are strongly selective specialist folivores. We predict that, like eucalypt folivores, diet selection in the green ringtail possum will be driven by avoidance of PSMs, or limitation of PSM diversity in the diet, and operate at an intraspecific level. Furthermore, we expect this to be common among marsupial folivores even in the diverse rainforests, especially those with seasonal variation in phenology, and suggest that study of these folivores will provide valuable insight into the characteristics that lead to the evolution of dietary specialisation.

CHAPTER 4

Near Infrared Spectroscopy as a Tool to Investigate Diet

Abstract

Near infrared reflectance spectroscopy can be used to predict nutritional characteristics of rainforest leaves. The NIRS technique has only recently begun to be applied in the field of ecology and has not previously been used in any study of taxonomically and chemically diverse rainforest foliage. This technique allows cheap and rapid measurement of integrative and ecologically relevant attributes such as dry matter and nitrogen digestibility on a large scale, as many samples can be rapidly processed once calibration equations have been established. Near infrared spectroscopy was successful in predicting nutritional characteristics from the scanned spectra of rainforest tree leaves. Models for water content, nitrogen digestibility and dry matter digestibility were particularly accurate**.** However, this technique was unable to accurately predict possum preference between tree species or frequency of use of individual *Aleurites rockinghamensis* and *Ficus fraseri* trees, probably due to limitations inherent in the methods used to determine frequency of tree use in the field. The value of NIRS in predicting nutritional characteristics of rainforest foliage means that airborne NIRS remote sensing may be a valuable future tool for cheaply and rapidly determining landscape scale measures of habitat quality

and heterogeneity of rainforest canopies that are physically difficult to access by traditional methods and would require analysis of thousands of samples.

Introduction

When selecting food items, marsupial folivores must balance nutrient and water intake with avoidance of indigestible fibre and toxic plant secondary metabolites (PSMs) (Cork *et al.*, 1991; Foley *et al.*, 1994; Hume, 1999). While some potential food-plants may contain high concentrations of nutrients (for example nitrogen and water), PSMs such as tannins may also be present and thus potentially reduce palatability (Foley *et al.*, 1994). Mammalian herbivores are expected to be generalists, feeding from a variety of sources to avoid toxicosis or 'overloading' any particular detoxification pathway (Dearing *et al.*, 2000; Sorensen *et al.*, 2005b). However, green ringtail possums (*Pseudochirops archeri*), inhabiting a fragment of species-rich rainforest on the Atherton Tablelands, are specialist folivores, preferentially selecting foliage at an inter- and intra-specific level (Jones *et al.*, 2006). In order to understand the chemical basis of leaf selection by this species of rainforest possum, we conducted chemical analyses for nitrogen content, water content, dry matter digestibility, nitrogen digestibility, digestible nitrogen concentration and cyanogenic glycoside concentration (Jones *et al.*, in review)(Chapter 5). This 'wet chemistry' was determined for tree species that were preferred (*Aleurites rockinghamensis, Arytera divaricata, Ficus copiosa* and *Ficus fraseri*), used in proportion to availability (*Dendrocnide photinophylla* and *Melia azedarach)* and that were common at the site but not used by the possums (*Acronychia acidula, Castanospermum australe, Castanospora alphandii, Ficus septica* and *Mallotus philippensis*).

Traditional chemical analysis techniques are extremely time consuming, expensive to run, destroy samples during analysis and often require the use of hazardous chemical reagents that produce waste (Foley *et al.*, 1998). Ecologists are increasingly embracing near infrared spectroscopy (NIRS) as an effective, efficient and environmentally friendly analytical method for determining chemical constituents in plant tissues. This technology is commonly used in agricultural industry to assess the protein, fibre and *in vitro* digestibility of large quantities of pasture and forage samples (Baker, Givens & Deaville, 1994; Clark & Lamb, 1991; Norris *et al.*, 1976; Ronalds & Miskelly, 1985; Smith & Flinn, 1991). Recently NIRS has been used successfully in studies of the feeding ecology of marsupial herbivore species in temperate forests, for example; wombats (Woolnough & Foley, 2002), koalas (Moore, 2004), greater gliders and common ringtail possums (McIlwee *et al.*, 2001; Wallis & Foley, 2003), but to date no studies have used NIRS to determine nutritional characteristics of taxonomically and chemically diverse rainforest foliage. Foley *et al.* (1998) published a comprehensive review of the ecological applications and basis of the NIRS technique. Briefly, NIRS measures absorbance or reflectance spectra that reflect the underlying chemical bonds within plant material. The absorbance peaks in a near infrared spectrum are not as directly related to specific chemical bonds as true infra-red spectroscopy, but represent the sum of a number of chemical bonds, and consequently calibration equations are required to describe the relationship between representative samples with known chemical constituents (nutrients and PSMs) and their spectra. These equations can then be used to predict the same constituents from the spectra of samples that are unknown (Batten, 1998; Foley *et al.*, 1998).

In the past, wet chemistry techniques were used to investigate the basis of herbivore food selection by measuring chemical constituents from preferred or avoided plant species without taking into account intraspecific variation in plant chemistry. Recent studies have identified significant chemical variation within and between individual trees, and this variation has been shown to strongly affect mammalian browsing (Chapman *et al.*, 2003; Lawler *et al.*, 2000; Milton, 1978; Moore *et al.*, 2005a; Scrivener *et al.*, 2004; Stolter *et al.*, 2005; Wallis *et al.*, 2002). Another long-term problem has been that links between compositional analyses and nutritional quality are not always well defined (Foley, 1992; Hjalten, Danell & Ericson, 1996; Robbins, 1993). For example, the total nitrogen concentration of a leaf (as measured under laboratory conditions) is unlikely to reflect its value to an herbivore, because foraging is affected by the physiology of the animal, the state of N in the leaf and any PSMs or digestibility reducers (such as tannins or fibre) present (Stapley *et al.*, 2000; Villalba *et al.*, 2002; Wang *et al.*, 1996). Thus, ecologists are now viewing the traditional analysis methods as less robust and are advocating more intensive sampling and nutritionally relevant analyses (Foley *et al.*, 1998; Ganzhorn, 1988; Hjalten *et al.*, 1996; Lawler *et al.*, 1998a; McIlwee *et al.*, 2001; Pass *et al.*, 1998). NIRS has provided a solution to many of these problems by supplying a technique that can measure more ‘integrative attributes’ such as digestibility and palatibility. In addition, NIRS is fast, with low cost in maintenance and consumables, allowing large numbers of samples to easily be analysed once calibration equations are established (Aragones, 1997; Marten, Shenk & Barton, 1989).

The aim of this study was to determine whether near infrared spectroscopy (NIRS) could be used to predict ecologically relevant, nutritional characteristics in foliage of taxonomically and chemically diverse rainforest trees. I also investigated the ability of NIRS to predict inter- and intra-specific forage preference by the green ringtail possum, *Pseudochirops archeri*, for use as a tool to rapidly determine habitat quality.

Methods

Field

Foliage was collected in association with a broader study of feeding ecology in *P. archeri* (Jones et al. 2006), from Nasser’s Nature Refuge, a 33 ha fragment of complex notophyll vine forest (type 5b (Webb, 1959), Regional ecosystem 7.8.3 (Sattler *et al.*, 1999)), located about 4 km south of Atherton on the Atherton Tablelands, Queensland (145°30′E, 17°18′S), elevation 750m. The soils were fertile, derived from basalt parent material. The vegetation was characterised by an uneven canopy (25–45m) with a large number of deciduous and semi-evergreen trees, a tendency for heavy leaf-fall during times of moisture stress and a dense shrub and scrambling vine layer. Eleven tree species (of 94 canopy species present) were sampled according their use by *P. archeri* as determined in (Jones *et al.*, 2006). *Aleurites rockinghamensis, Arytera divaricata, Ficus fraseri* and *Ficus copiosa* were sampled as preferred forage species of *P. archeri*, *and Acronychia acidula, Castanospermum australe, Castanospora alphandii, Dendrocnide photinophylla, Ficus septica, Mallotus philippensis and Melia azedarach* were sampled as the most common, non-preferred tree species at the site (Jones et al. 2006). Mature leaves were collected from all species in September (dry season), 2005. Mature leaves were also collected from *A. divaricata* and *F. copiosa* during the wet season (February 2005). Juvenile (not fully expanded) and mature-new (fully expanded but not as tough or dark in colour as mature) leaves were collected from *A. rockinghamensis* and *F. fraseri* when they were available, which was mostly during the wet season (February 2005) (Table 4.1). Half of each sample (35 to 50g) was immediately weighed into a paper bag, then dried for 24 hours at 100°C and reweighed to determine water content. The remaining half of each sample was frozen in the field on solid CO_2, then stored in the laboratory at -20°C. The frozen samples were freeze-dried and ground to uniform particle size in a sample mill (Cyclotec 1093 sample mill, Foss Pacific Pty Ltd, North Ryde, Australia), then stored in sealed plastic bags with desiccant.

Chemical analyses

Nitrogen concentration, nitrogen digestibility and digestible nitrogen concentration

The nitrogen content of samples before and after *in vitro* digestion was determined using wet oxidation in sulphuric acid and hydrogen peroxide with a selenium catalyst. Lithium sulphate was added to raise the digestion temperature (Anderson & Ingram, 1989). Nitrogen was determined colorimetrically in the digest by the salicylate-hypochlorite method (Baethgen & Alley, 1989). Nitrogen digestibility was calculated by subtracting the nitrogen content of each sample post in vitro digestion from the original nitrogen content of that sample. Digestible nitrogen concentration was calculated by multiplying the nitrogen digestibility by the original nitrogen concentration of the sample.

In vitro digestibility of dry matter and nitrogen

Dry matter and nitrogen digestibility was measured in duplicate using a pepsin/cellulase *in vitro* procedure (modified from Choo *et al*. 1981). 0.6000 ± 0.05 g of dry ground leaf material was weighed into an oven-dry pre-weighed filter bag (F57 Ankom Technology, Macedon, U.S.A.). The bags were heat-sealed and duplicates placed together in individual small beakers to which was added a pepsin solution (40 mL per bag/ 80ml per beaker) containing 2.0 g of Pepsin (1:10,000 from porcine stomach mucosa, MP Biomedicals Inc, Solon, U.S.A.) per litre of 0.1N HCl (pH 1.0). The beakers were then placed within an agitating incubator at 36ºC for 60 hours. Upon removal, the samples were washed five times in distilled water and then incubated (36ºC) for 75 h in a 0.05 M cellulase-acetate buffer solution (40 mL per bag/80 ml per beaker). The cellulase-acetate buffer solution contained 6.8 g of sodium acetate, 2.9 mL of glacial acetic acid (99%) and 6.25 g of cellulase (from *Aspergillus niger,* MP Biomedicals Inc, Solon, U.S.A.) per litre of solution (pH adjusted to 4.6). At the end of this incubation, the samples were washed as above, dried to constant mass at 60ºC, and then reweighed.

Cyanogenic glycoside content

The Fiegl-Anger paper method was used to determine cyanogen presence/absence (Brinker & Seigler, 1989) across the whole range of canopy species sampled. Approximately 3 grams of freeze-dried, ground leaf was weighed into a screw cap vial and a few drops of water added. The indicator paper was suspended above the tissue and held in place by screwing the lid over the attached paper handle. The vials were incubated at 37 °C for 24 h. A strongly cyanogenic sample resulted in the indicator paper turning purple within minutes. A weakly cyanogenic sample turned the paper (or just the lower edge of the paper) purple over several hours. The papers were left for 24 h but no longer to avoid interference by bacterial cyanogenesis. *Prunus turneriana* was used as a positive control and *Alstonia scholaris* as a negative control (Miller, Jensen & Woodrow, 2006a).

Cyanogenic glycoside content was quantified in the only species that tested positive for presence of cyanogen, *Aleurites rockinghamensis*. The cyanogenic glycoside concentration (µg CN released/g dry tissue) was measured by hydrolysing the glycoside and trapping the resultant hydrogen cyanide in a NaOH well (Brinker *et al.*, 1989). Hydrolysis was achieved by adding 1 mL of 0.1 mM citrate buffer (pH 5.5) to a known amount of freeze-dried material (10-15 mg) in a sealed glass vial, and incubated for 20 h at 37 °C. Excess of a non-specific cyanogenic b-glucosidase enzyme (emulsin from *Prunus amygdalus* EC 3.2.1.21, Sigma-Aldrich, Castle Hill, Australia) was added to ensure the assay quantified the full cyanogenic potential of the tissue sample. Cyanide in the NaOH well was determined using a miniaturized cyanide assay, which was adapted from Brinker and Seigler (1989) for use with a photometric microplate reader (Labsystems Multiskan Ascent with incubator, Vantaa, Finland). The absorbance was measured at 590nm using NaCN as the standard (Goodger, Capon & Woodrow, 2002).

NIRS

Spectral collection

All (118) samples collected were scanned using NIRS to produce predictive models of the nutritional parameters measured above. All spectra were obtained with a Fourier Transform Near Infrared spectrometer - Multi Purpose Analyzer (MPA) (Bruker Optics Inc, Clayton, Australia), in a darkened room maintained at approximately 25°C and 55% RH. We used a 30-position sample wheel attached to the MPA to scan each sample of dried, ground leaf.

This unit measured spectral reflectance at wavelengths between 780 - 2780 nm.

Developing a calibration model

The data were processed using the software package OPUS version 5.5. (Bruker Optics, Ettlingen, Germany). Calibration models were developed using the statistical Quant software within the OPUS package. This program produced approximately 340 possible calibration models using various combinations of pre-processing treatment, rank (number of factors of the matrix used to analyse the internal test samples) and wavenumber frequency regions. The data was transformed using partial least-squares regression (PLS) and cross-validated to prevent over-fitting. OPUS employs PLS to extract relevant information from the complex NIR spectra. Full cross-validation was used to determine the optimal number of PLS factors used for prediction, termed “rank”. The accuracy of the calibration model is described by the squared correlation coefficient (r^2). The optimal rank indicates the maximum value for r^2 and the minimum value for the root mean standard error of the cross-validation (RMSECV) and thus prevents over-fitting of the model. The cross-validation procedure used Monte Carlo simulation where the population was arbitrarily divided into a small number of groups, which could include as few as one sample per group. A prediction model is developed based on the values from one group and then tested using the remaining groups. This procedure is repeated until all groups have been used in the prediction model. The resulting model is more robust because all available chemical data can be used in the calibration rather than having to maintain separate validation and calibration sets (Foley *et al.*, 1998; Shenk & Westerhaus, 1993). Mahalanobis distances were calculated to quantify outliers. During the PLS calculation the Mahalanobis distances of each calibration spectrum were determined. From these values the threshold of the Mahalanobis distance was derived. Spectra of unknown samples can be reliably analyzed using a calibration function if their Mahalanobis distance is within this threshold. The model was further refined by manually removing any outlying spectra for which predictions were significantly different from the observed 'wet chemistry' value. The model that offered the best predictive capability for each chemical constituent was then used as a predictive tool for spectra from samples with unknown chemical profiles.

Results

Nutrient content of foliage samples across the 11 species varied up to 10-fold (Table 4.2 and Appendix 1). For example, nitrogen digestibility ranged from -63.2 ± 6.8% to 45.9 ± 2.9%.

Near infrared spectroscopy was successful in predicting unknown chemical constituents from the scanned spectra of rainforest tree leaves (Figures 4.1 to 4.6 and Table 4.3). The predictive precision of the NIRS models ranged from 63% for cyanogenic glycoside concentration to 95% for dry matter digestibility. The RMSECV values effectively give error rates for the predictive models and the errors for nitrogen digestibility and cyanide were relatively high

(Table 4.3). For five of the six chemical components the optimal pre-processing mathematical transformation and smoothing function was the 1st derivative. Spectra for calibration of cyanogenic glycoside concentration were pre-processed using min-max normalization. Calibration models for nitrogen concentration, nitrogen digestibility, digestible nitrogen concentration and dry matter digestibility were developed using the regions 1333 – 1640 and 2173 – 2355 nm. The wavelength region for cyanogenic glycoside was slightly different at 1638 – 1836 nm and 2173 – 2355 nm, and for water 1333 – 2175 nm. It was necessary to remove outliers from all calibration equations except cyanogenic glycoside concentration. In particular nitrogen digestibility and digestible nitrogen concentration required 17 and 19 spectra respectively to be removed to produce optimal calibration equations (Table 4.3). The error in the cyanide equation was too large ($R^2 = 63.2$) for this model to be useful.

Predicting tree use by green ringtail possums

Although the predictions of NIRS models for interspecific tree species preference and intraspecific use of individual trees were statistically significant (interspecific: P<0.001, t=8.7 Figure 4.7 and Table 4.3, *A. rockinghamensis*: P=0.043, t=2.1 Figure 4.8 and Table 4.3, *F. fraseri*: P<0.001, t=5.1 Figures 4.9a and b and Table 4.3), their low precision (R^2 values) and frequent misclassification meant they were not useful as a practical ecological tool.

Table 4.1: Sample sizes used to develop NIRS predictive models.

Tree species	# Samples
Achronychia acidula	6
Aleurites rockinghamensis	33
Arytera divaricata	8
Castanospermum australe	5
Castanospora alphandii	5
Dendrocnide photinophylla	5
Ficus copiosa	8
Ficus fraseri	32
Ficus septica	6
Mallotus philippensis	5
Melia azedarach	5
Total	118

Table 4.2: Nutrient composition[1] of foliage from the eleven most common species in complex mesophyll vine forest at the Nasser's nature reserve study site (means ± SEM) as determined using traditional 'wet chemistry'.

Species (n)	Proportion basal area at site (%)	Nitrogen (mg/g)	Nitrogen digestibility (%)	Digestible nitrogen (mg/g)	Dry matter digestibility (%)	Water content (%)
Acronychia acidula (6)	3	23.4 ± 1.0	45.4 ± 1.2	10.6 ± 0.4	61.8 ± 1.1	70.8 ± 0.3
Aleurites rockinghamensis (33)	22	20.1 ± 0.4	-4.3 ± 1.8	-0.7 ± 0.4	47.5 ± 1.0	72.0 ± 0.8
Arytera divaricata (8)	4	18.5 ± 0.3	3.7 ± 2.0	0.7 ± 0.4	32.6 ± 0.7	51.0 ± 1.5
Castanospermum australe (5)	6	22.8 ± 1.0	45.9 ± 2.9	10.4 ± 0.6	42.8 ± 1.6	61.7 ± 1.4
Castanospora alphandii (5)	8	17.6 ± 0.6	22.3 ± 3.7	4.0 ± 0.7	41.0 ± 0.7	54.4 ± 1.3
Dendrocnide photinophylla (5)	16	32.4 ± 0.9	-63.2 ± 6.8	-20.4 ± 2.1	74.7 ± 0.6	82.5 ± 1.3
Ficus copiosa (8)	3	14.9 ± 0.7	29.2 ± 2.6	4.4 ± 0.4	62.8 ± 0.9	70.3 ± 1.6
Ficus fraseri (32)	4	23.4 ± 0.7	32.7 ± 1.6	7.6 ± 0.4	74.0 ± 0.6	69.7 ± 0.8
Ficus septica (6)	1	19.7 ± 1.6	-36.4 ± 3.4	-7.0 ± 0.5	73.4 ± 1.2	74.7 ± 1.4
Mallotus philippensis (5)	1	17.5 ± 0.9	4.4 ± 1.3	0.8 ± 0.3	32.6 ± 0.5	48.3 ± 0.4
Melia azedarach (5)	18	25.6 ± 0.7	-24.0 ± 7.1	-6.2 ± 1.6	72.1 ± 1.1	71.9 ± 1.3

[1] All nutrient content except water were calculated on a dry matter basis

Table 4.3: Near infrared spectroscopy modelling of nutrient content of foliage from the eleven most common species[1] found in complex mesophyll vine forest at Nasser's nature reserve.

Nutrient[2]	N (outlier)	Pre-processing[3]	Frequency region[4] (nm)	Rank[5]	RMSECV[6]	R^2
Nitrogen (mg/g)	117 (2)	1st derivative	1333 – 1640 2173 – 2355	10	1.49	88.7
Nitrogen digestibility (%)	102 (17)	1st derivative	1333 – 1640 2173 – 2355	8	7.63	87.1
Digestible nitrogen concentration (mg/g)	100 (19)	1st derivative	1333 – 1640 2173 – 2355	9	1.76	86.7
Dry matter digestibility (%)	115 (4)	1st derivative	1333 – 1640 2173 – 2355	8	3.46	95.1
Water (% ww)	116 (3)	1st derivative	1333 – 2175	10	2.8	90.1
Cyanogenic glycosides (ug/g)	87 (0)	min-max normalization	1638 – 1836 2173 – 2355	8	8.55	63.2
Interspecific tree use	111 (5)	Multiplicative scattering correction	1639 – 1836 2173 – 2355	4	1.29	47.2
Aleurites rockinghamensis tree use	39 (1)	min-max normalization	1835 – 2355	1	1.08	9.34
Ficus fraseri tree use	28 (0)	Multiplicative scattering correction	1333 –1836 2173 - 2260	6	1.3	46.4

[1] Species used in calibration are *Acronychia acidula, Aleurites rockinghamensis, Arytera divaricata, Castanospermum australe, Castanospora alphandii, Dendrocnide photinophylla, Ficus copiosa, Ficus fraseri, Ficus septica, Mallotus philippensis* and *Melia azedarach*.

[2] All nutrient content except for water are calculated on the basis of dry matter

[3] Pre-processing technique of spectral data applied before regression analysis as described in Woolnough and Foley (2002).

[4] Spectrum of wavelength used by each model, to nearest nm

[5] Number of factors of the matrix used to analyse the internal test samples

[6] Root-mean-square error of cross-validation.

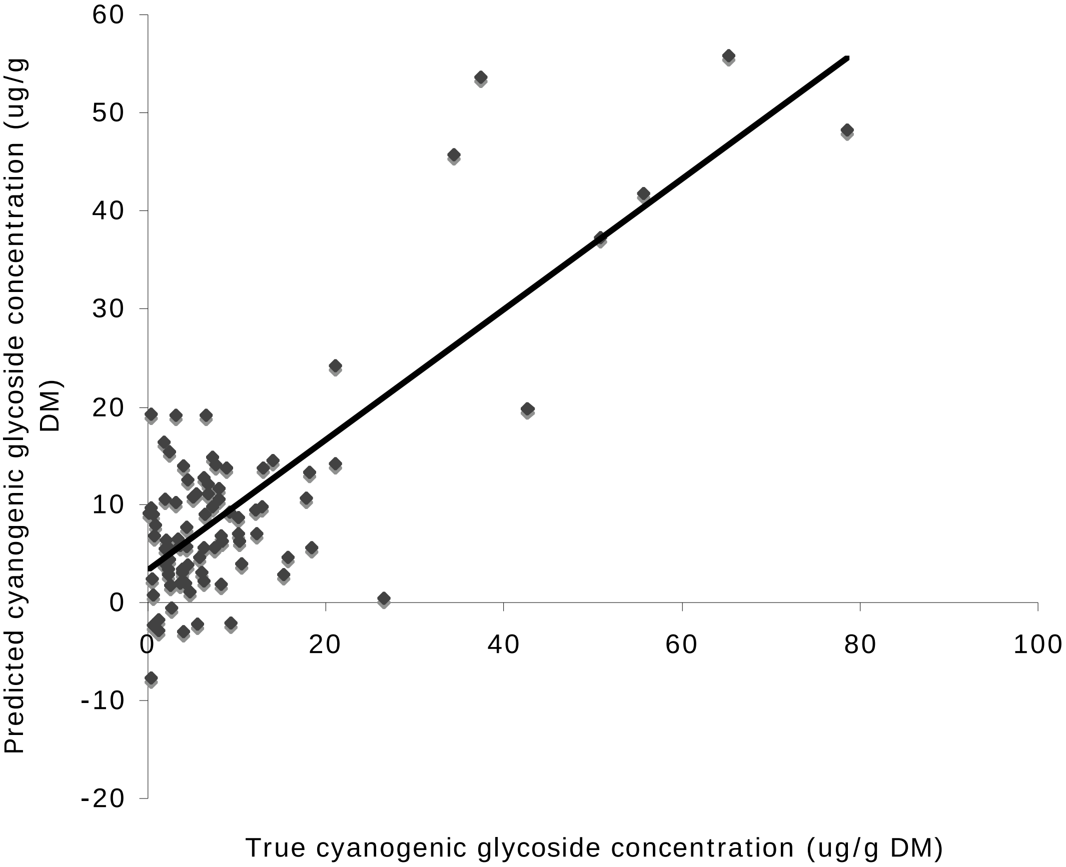

Figure 4.1: Relationship between cyanogenic glycoside concentration (ug/g DM) measured in the laboratory and the value predicted by near-infrared spectroscopy (R^2 = 63.2).

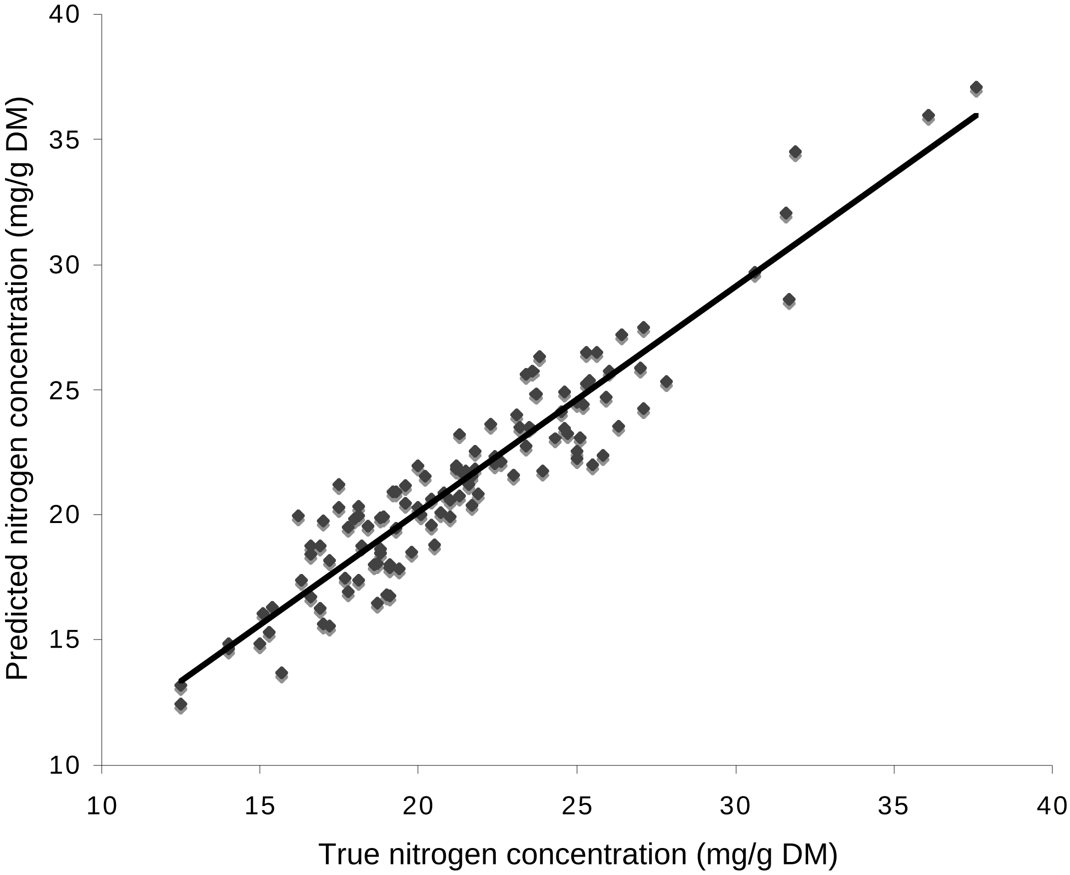

Figure 4.2: Relationship between nitrogen (mg/g DM) measured in the laboratory and the value predicted by near-infrared spectroscopy (R^2 = 88.7).

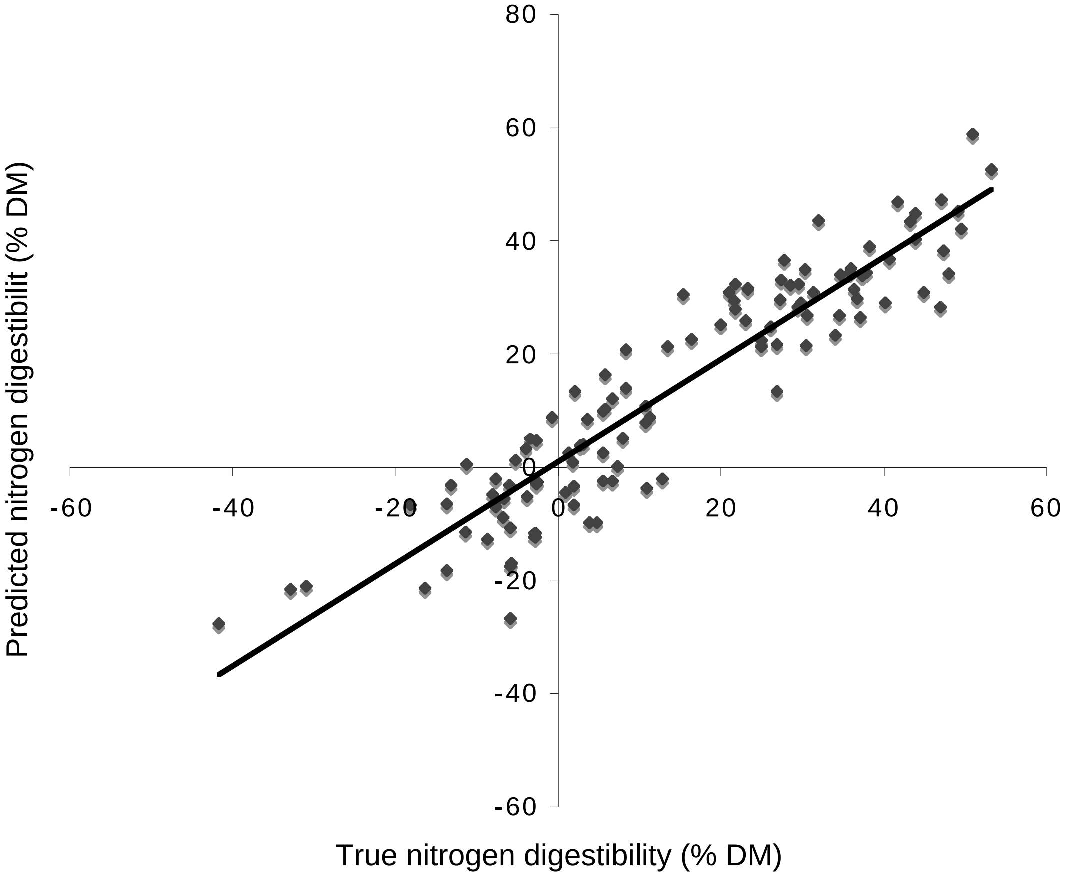

Figure 4.3: Relationship between nitrogen digestibility (% DM) measured in the laboratory and the value predicted by near-infrared spectroscopy (R^2 = 87.1).

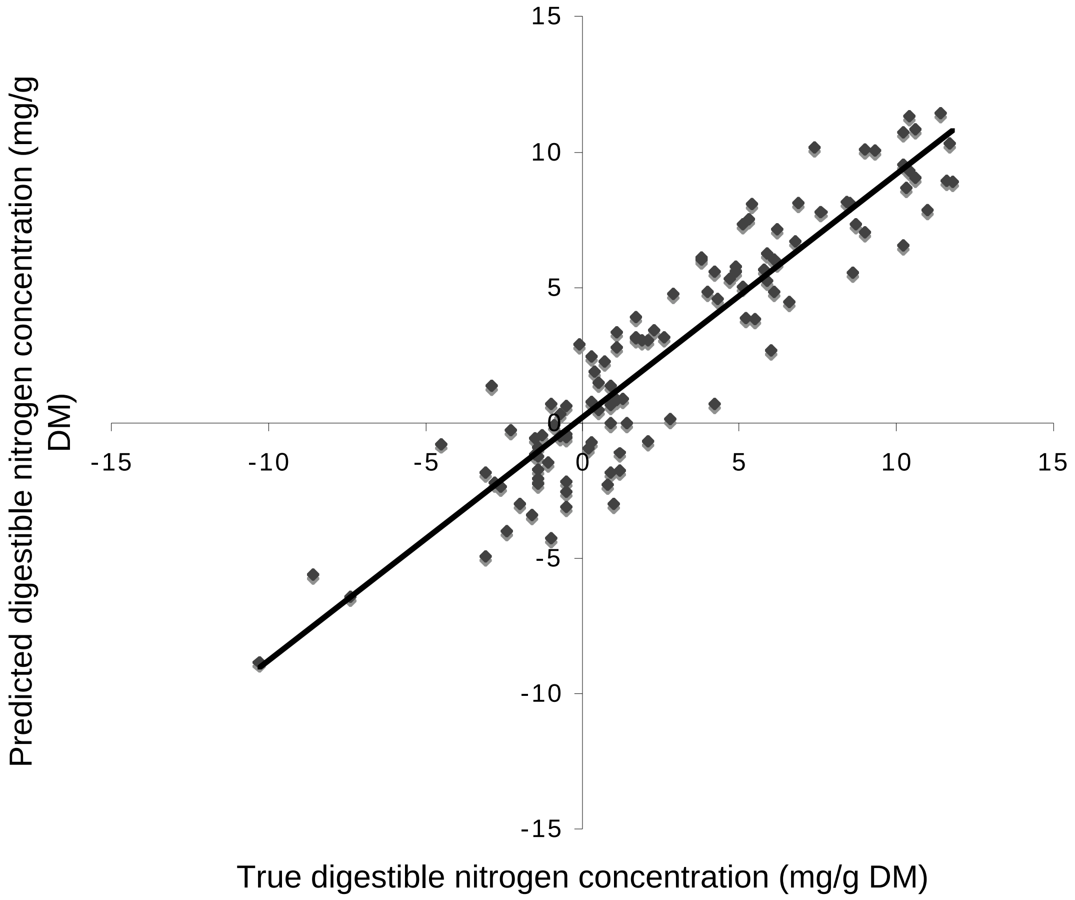

Figure 4.4: Relationship between digestible nitrogen concentration (mg/g DM) measured in the laboratory and the value predicted by near-infrared spectroscopy (R^2 = 86.7).

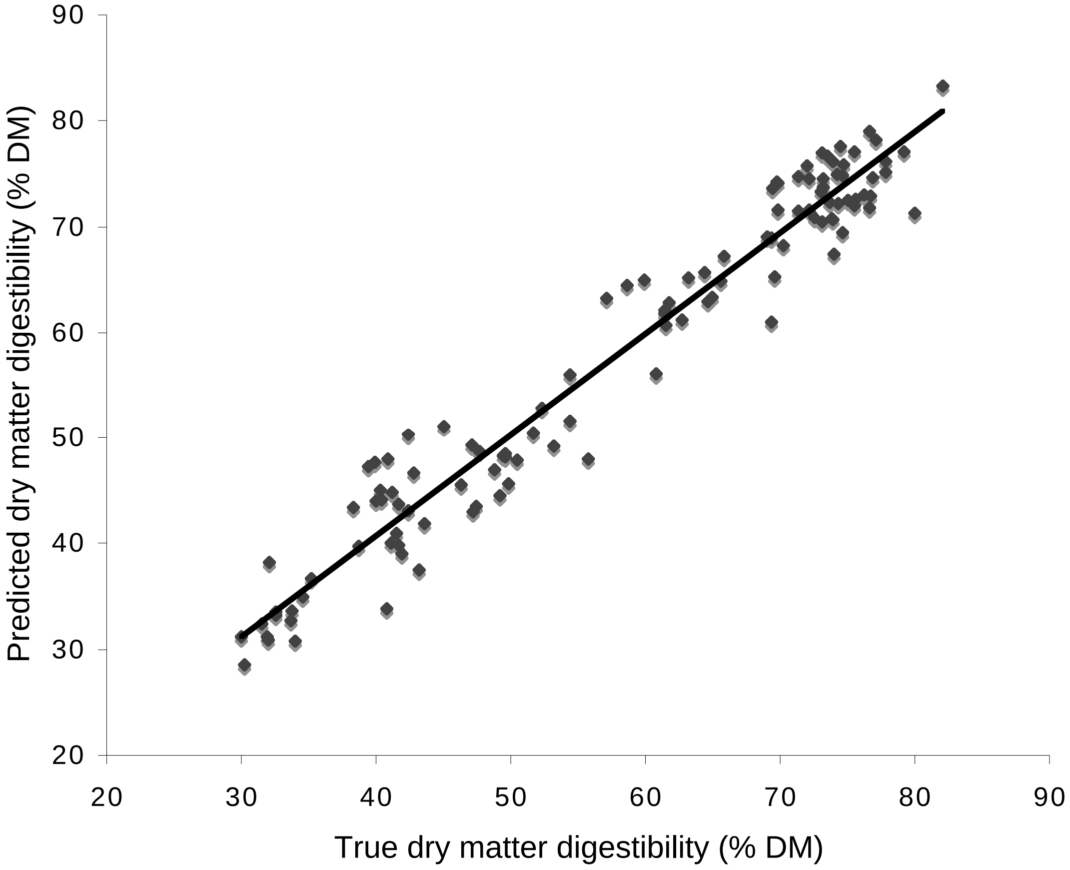

Figure 4.5: Relationship between dry matter digestibility (% DM) measured in the laboratory and the value predicted by near-infrared spectroscopy (R^2 = 95.1).

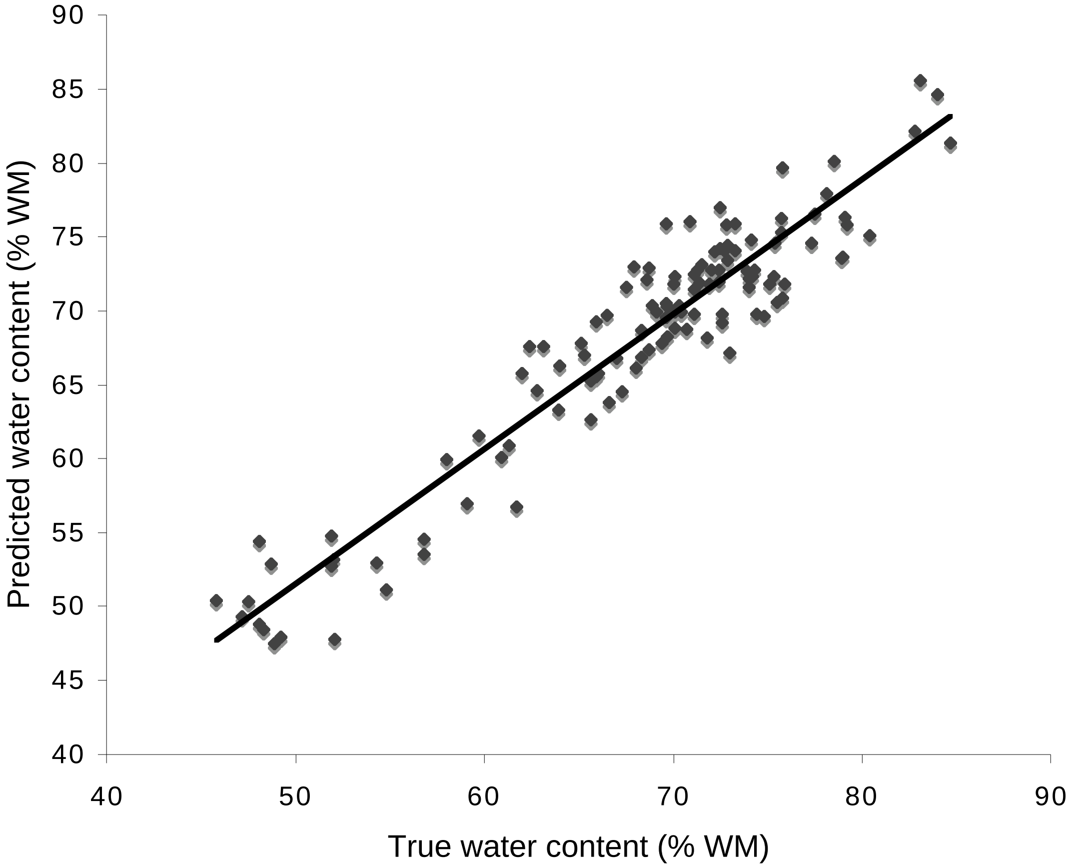

Figure 4.6: Relationship between water content (% WM) measured in the laboratory and the value predicted by near-infrared spectroscopy (R^2 = 90.1).

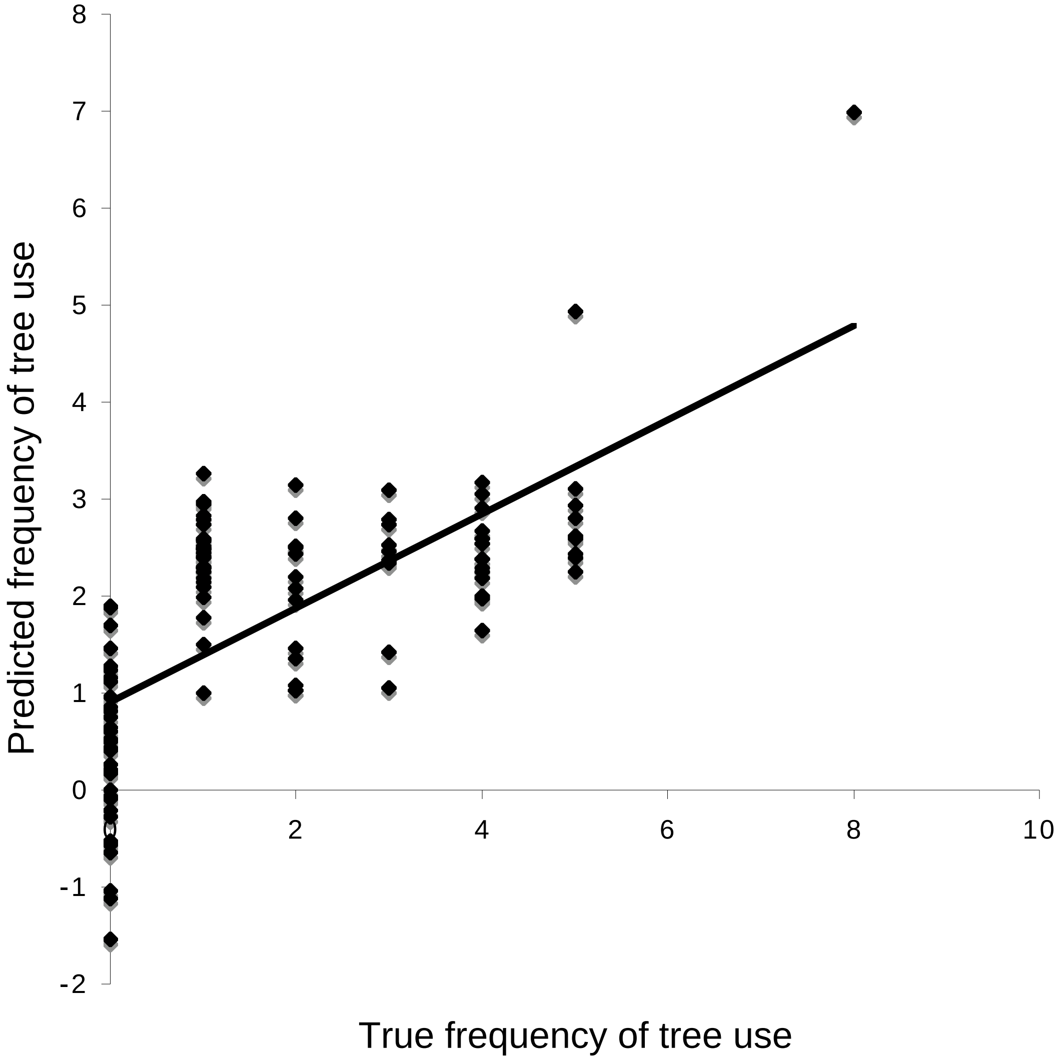

Figure 4.7: Relationship between frequency of tree use by *P. archeri* (individual trees within 11 species) recorded in the field and the value predicted by near-infrared spectroscopy (R^2 = 47.2).

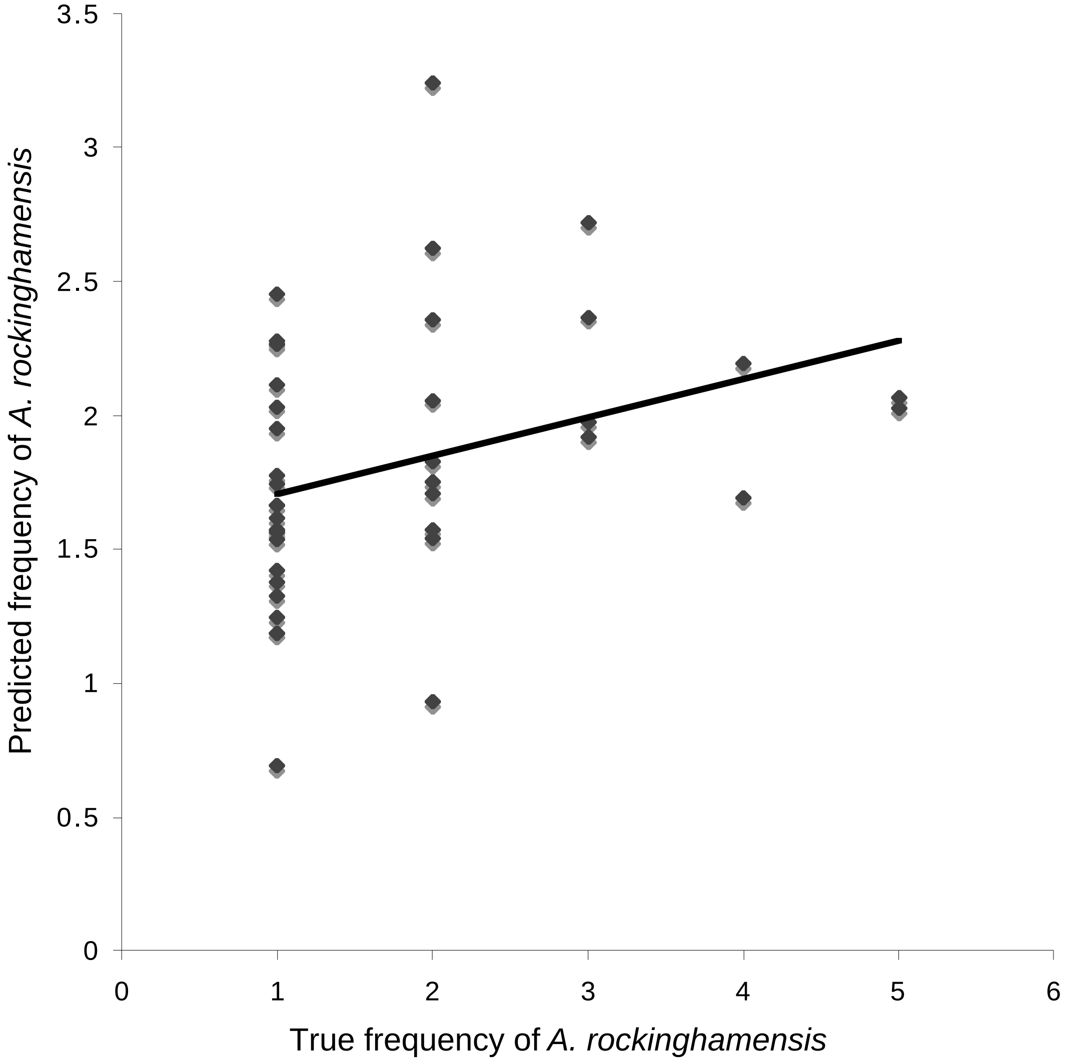

Figure 4.8: Relationship between frequency of tree use by *P. archeri* (individual trees within *Aleurites rockinghamensis*) recorded in the field and the value predicted by near-infrared spectroscopy (R^2 = 9.34).

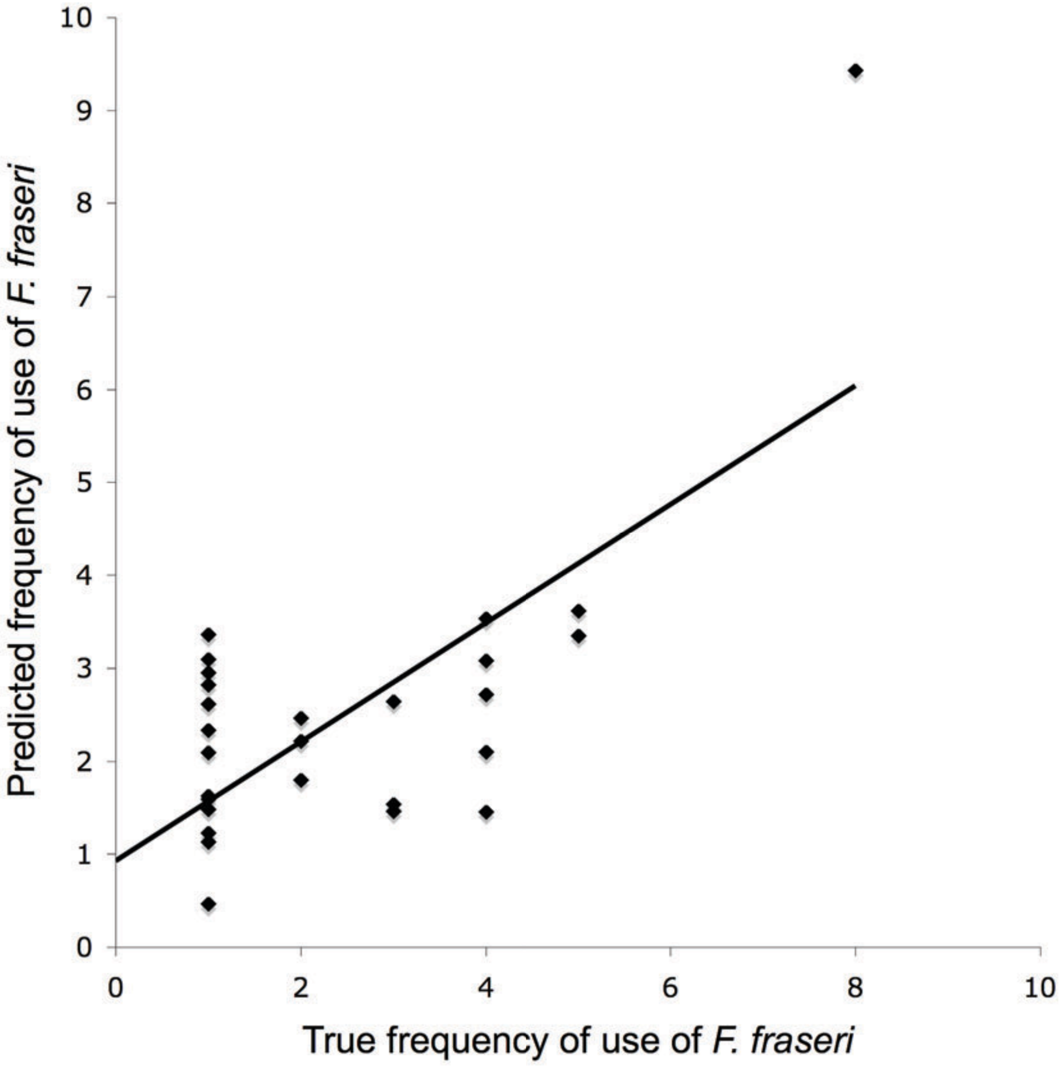

Figure 4.9a: Relationship between frequency of tree use by *P. archeri* (individual trees within *Ficus fraseri*) recorded in the field and the value predicted by near-infrared spectroscopy (R^2 = 46.4).

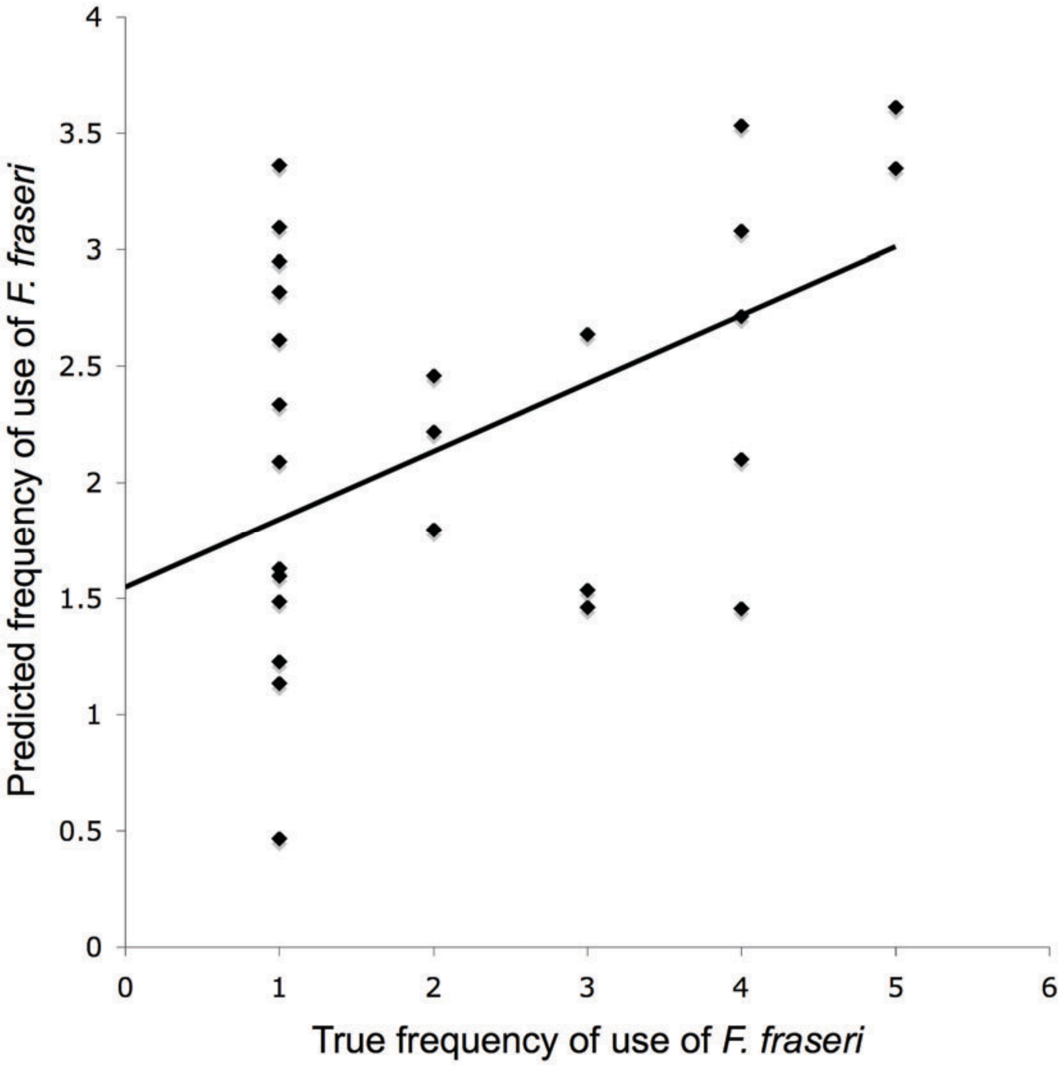

Figure 4.9b: Relationship between frequency of tree use by *P. archeri* (individual trees within *Ficus fraseri*) recorded in the field and the value predicted by near-infrared spectroscopy, (as above, but with the outlier (8, 9.4) removed) (R^2 = 21.8).

Discussion

Predicting chemical constituents in rainforest leaves

Prediction models developed for the nitrogen content, nitrogen digestibility, digestible nitrogen concentration, dry matter digestibility and water content were all relatively accurate. Cyanogenic glycoside content could not be accurately predicted using NIRS.

One outstanding advantage of this technique is the speed with which samples can be processed. We only had the time and resources to determine traditional ‘wet chemistry’ on 118 samples, but by using NIRS we could expand that sample size within the species included in the calibrations rapidly and at very little cost, for most of our nutritional measures except cyanogenic capacity. This meant that we were able to make comparisons between seasons and leaf age for the preferred species *Aleurites rockinghamensis* and *Ficus fraseri* (Chapter 5), which would have otherwise been logistically impossible. Many researchers investigating the dietary ecology of herbivores have recognised the value of NIRS because it allows a large number of samples to be analysed quickly with minimal cost or waste (Andre & Lawler, 2003; Aragones *et al.*, 2006; Lawler *et al.*, 2000; Stolter *et al.*, 2005; Stolter, Julkunen-Tiitto & Ganzhorn, 2006; Woolnough *et al.*, 2002). The advantages associated with being able to process many more samples than has been possible using traditional analyses should not be underestimated. For example, studies looking to define the chemical basis for herbivore selectivity require detail at the intraspecific plant level (Lawler *et al.*, 1998a), thus the number of samples that need to be analysed are dramatically increased.

Variation in the precision of the wet chemistry, and the complexity of the chemical structures, for each chemical component directly affected the quality of the corresponding calibration model. For example, the predictive model for cyanogenic glycoside concentration had comparatively low precision and high error rates, perhaps due to the complex and variable structures of cyanogenic glycosides (Harborne, 1998; Miller *et al.*, 2006a), or because CN bonds are unlikely to have strong harmonic resonances in the NIR, so are unlikely to have been detected directly (Coates, 2000). Cyano compounds have intense absorption in the infrared between wavelengths 4386 and 4545 nm (Coates, 2000), which is outside of the spectrum measured by NIRS. It is thus possible that the model developed was based on a correlated constituent rather than the actual glycosides. Conversely, predictive accuracy of *in vitro* dry matter digestibility was high, probably due to the relative simplicity of bonds relating to the digestible/ indigestible components of plant dry matter (mainly cell contents/ cell wall polysaccharides and lignin/ fibre) respectively) (Choo *et al.*, 1981).

The overall accuracy of these predictions is perhaps surprising considering that up to 11 species from seven families were used in each calibration equation. However, for this technique to be a practical leaf-analysis option for ecologists, it is important that broad-based calibration models are accurate when used with many different plant species and leaf ages, collected during different seasons (Foley *et al.*, 1998). Woolnough and Foley (2002) also found that NIRS calibrations developed with many plant species were just as useful as those developed for a single species. Furthermore, these authors concluded that calibration equations developed using 25 potential food species of the northern hairy-nosed wombat were effective in predicting seven attributes of foliage quality (Woolnough *et al.*, 2002). Similarly, 10 species of seagrass (Lawler *et al.*, 2006), 85 species of *Eucalyptus* (McIlwee *et al.*, 2001), and 36 meadow grass and dicotyledon species (Duru, 1997) were used to produce robust calibration equations for herbivore dietary studies. The accuracy of predictions developed in the current study could be further improved by increasing the number of species and samples in the calibration model to reduce the effect of outliers. In this way the NIRS model could be continuously updated and enhanced to meet the needs of field-based ecological research. The robust calibration equations developed during this study will be extremely useful to future studies on the nutritive value of rainforest foliage. This is particularly true, as NIRS calibration models for tropical rainforest foliage have never been previously published. This technique would be especially useful for investigating the nutritional or defensive basis of the diet of tropical rainforest herbivores (such as primates, marsupials and sloths) because large numbers of samples can be processed from a wide variety of species.

Predicting tree use by green ringtail possums

Food intake by herbivorous marsupials is closely linked to the chemical composition of foliage (Lawler *et al.*, 1998a; Moore *et al.*, 2005b) (and Chapter

5). This chemical information is ‘summarised’ by near infrared spectroscopy (NIRS), which produces representative spectra that can be used to predict intake or palatability without knowing the chemical constituents involved (Foley *et al.*, 1998; McIlwee *et al.*, 2001). NIRS calibration models for food intake are usually developed with captive animals under controlled conditions. For example, McIlwee (2001), Wallis & Foley (2003) and Moore (2004) were able to produce NIRS models that explained more than 90% of the variation in dry matter intake by greater gliders, common ringtail possums and koalas.

The NIRS models developed to predict tree use by green ringtail possums were not as accurate as those developed for temperate forest folivores (only 9-47% of variation explained). Overall, the model have some ability to separate preferred and non-preferred trees within the 11 most common species at the site, but there was substantial overlap (R^2 = 47.2, Figure 4.7). Although the model for frequency of visits within *F. fraseri* appeared relatively robust (R^2 = 46.4, Figure 4.9a), when the outlying, highly preferred tree was removed the relationship broke down (R2 = 21.8, Figure 4.9b). These results may be explained by one or more differences between the current study and those conducted on the temperate, eucalypt-specialised folivores. Firstly, the basis of dietary selectivity by the temperate-forest folivores has been well documented, with a specific class of plant secondary metabolites, formylated phloroglucinol compounds (FPCs), acting as strong deterrents within *Eucalyptus* foliage (Lawler *et al.*, 2000; Moore *et al.*, 2000; Pass *et al.*, 1998). In contrast, the diet of the rainforest folivore, *Pseudochirops archeri* is probably defended by a diverse array of PSMs Chapter 5, (Jones *et al.*, in review), making this a far more complex situation than the *Eucalyptus* dominated forests. The rainforest site contained a complex mix of up to 100 species in the canopy, with presumably an equally complex array of associated defensive chemicals Chapter 5, (Jones *et al.*, in review; Jones *et al.*, 2006). In comparison a large proportion of the diet of temperate-forest folivores consists of trees from within a single genus, *Eucalyptus* (Chilcott & Hume, 1984a; Moore *et al.*, 2005b), which also dominates their forest habitat.

Secondly, as part of this entirely field-based study it was impossible to measure dry matter intake by the possums, so frequency of tree-use was used instead. The tree-use technique (as described in Chapter 2) is a relatively robust and accurate method for determining dietary selectivity of wild folivores, however some biases may have been present when attempting to define intraspecific tree use. For example, a possum may avoid a palatable tree because it is at the edge of its range, more exposed to predators or due to some other unmeasurable factor, thus some trees within the NIRS model will be “under-used” in relation to their chemical profile. It appears that the defensive compounds and nutritional characters measured in this study set limits to tree use, so that although possums will only ever rarely visit individual trees (within preferred species) that have high concentrations of deterrent compounds, individual trees with low concentrations of deterrent compounds may be visited either often or rarely Chapter 5, (Jones *et al.*, in review). Similarly, when the simple linear relationship between FPC phenolics and intake determined under laboratory conditions, of eucalypt-specialised folivores, was tested in the field, it was complex and non-linear (Moore *et al.*, 2005a; Scrivener *et al.*, 2004) and deterrent compounds functioned to set limits on tree use rather than directly determining tree use. In the wild many other unquantified factors have the potential to influence food intake from a specific tree such as variation in exposure to predation risk, choice between alternative food species, reproductive state and competition for dietary resources with conspecifics (Moore *et al.*, 2005a; Scrivener *et al.*, 2004). Despite difficulties encountered when attempting to determine food intake in the field, calibration equations developed in this way do have advantage over laboratory results. Models derived using no-choice food intake of animals under laboratory conditions, as developed for eucalypt-specialised folivores (Lawler *et al.*, 2000), provide relative measures of palatability under laboratory conditions but are not applicable to wild animals which are subject to a wide variety of influences (Wallis *et al.*, 2003). I suggest that because the tree use technique is better suited to defining limits than linear relationships with nutrient composition it may not provide a field-based alternative for actual dry matter intake that is accurate enough to be used to produce NIRS calibration models. Future work including no-choice captive feeding trials for *P. archeri,* combined with feeding preferences determined under realistic circumstances in the field, would provide the data necessary to produce accurate calibration models so that NIRS could be used to predict tree use and food intake. With further development, the NIRS models in this study which measure functionally relevant parameters, such as water content, digestible nitrogen content and digestibility, could form a highly useful tool to rapidly determine habitat quality and heterogeneity (Moore *et al.*, 2004; Stolter *et al.*, 2006; White *et al.*, 2000). Recent developments in portable NIR instruments allow real-time analyses of plant composition in the field and make way for the possibility of combining traditional airborne remote sensing with fine resolution prediction of suitability of habitat (Foley *et al.*, 1998; Green *et al.*, 1998; White *et al.*, 2000). This would also circumvent the substantial difficulty of physically sampling foliage from the canopy (Stork, Samways & Eeley, 1996; Stork, WrightStephens & Mulkey, 1997). The value of NIRS in predicting nutritional characteristics of rainforest foliage is that airborne NIRS remote sensing may be a important future tool for cheaply and rapidly determining landscape scale measures of habitat quality and heterogeneity of rainforest canopies, that are physically difficult to access by traditional methods and would require analysis of thousands of samples.

CHAPTER 5.

Plant defenses determine feeding preferences in a specialist rainforest folivore

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Abstract

Specialist folivores are predicted to make foraging choices that minimise diversity and quantity of plant secondary metabolites (PSMs) in their diet. This study established that PSMs determine intraspecific dietary preference by the specialist folivore green ringtail possum (*Pseudochirops archeri*), which preferred mature foliage from just 4% of the canopy species in a diverse tropical rainforest. Within the four preferred tree species, specific nutritional parameters were responsible for variation in individual tree use. Within the predominant preferred species, *Aleurites rockinghamensis,* cyanogenic glycosides acted as a deterrent, limiting tree use. Within the remaining preferred species, *Ficus fraseri*, *F. copiosa* and *Arytera divaricata,* nitrogen digestibility set the lower limit to tree use. Trees with low nitrogen digestibility (high levels of deterrent tannins) were never highly used. The preference for mature leaves over juvenile was consistent with the factors determining tree preference, as juvenile leaves were either higher in cyanogenic potential or lower in nitrogen digestibility. The relationship between tree species preference and nutritional parameters was not related to the same factors that determined preference within species. We suggest

that interspecific choice was based primarily on the need to minimise the diversity of PSMs ingested, whereas intraspecific choice was based on the need to minimise the quantities of that limited set of PSMs.

Introduction

In their seminal paper, Freeland and Janzen (1974) proposed that most herbivores should be dietary generalists because different classes of PSMs must be detoxified via separate metabolic pathways. This became known as the detoxification limitation hypothesis, predicting that herbivores should feed from a variety of sources to avoid overloading any particular detoxification pathway (Marsh *et al.*, 2006; Wiggins, McArthur & Davies, 2006a). Dietary studies show that most herbivores are generalists (Freeland, 1991; Ganzhorn, 1988; Kerle, 1984) and detoxification limitation at least partially explains this feeding strategy (Dearing *et al.*, 1999). However, a minority of herbivores are dietary specialists, concentrating on plants with a certain group of PSMs and avoiding others (Ganzhorn, 1989), and thus focussing on a single or limited set of detoxification pathways (Lamb *et al.*, 2004).

Although specialist folivory is generally rare, mammals as diverse as the sloth (Chiarello, 1998), koala (Moore *et al.*, 2000) and sportive lemur (Hladik, 1978) manage folivory with a highly selective diet, probably resulting from the evolution of physiological adaptations to cope with a smaller range of plant secondary metabolites (PSMs). Ingesting PSMs incurs energy costs in both specialist and generalist herbivores (Dearing *et al.*, 2000; Sorensen *et al.*, 2005b) so specialisation could reflect avoidance or minimisation of PSMs in general (Freeland *et al.*, 1974). Alternatively, specialisation may arise when particular diet items high in PSMs are easily and consistently available (Freeland *et al.*, 1974), providing a competitive advantage to a herbivore able to cope with those PSMs. Recent studies show that specialist herbivores are able to maintain a higher intake of PSMs from within their normal diet, but are more strongly impacted by novel PSMs than are generalists (Sorensen *et al.*, 2005a). Specialists are thought to be able to maintain a higher intake of PSMs through increased detoxification ability (Dearing *et al.*, 1999), decreased absorption of toxins through increased excretion of unmetabolised toxins (Sorensen *et al.*, 2004) and adjustments to their energy budgets (Sorensen *et al.*, 2005b).

Recently, relationships between specific plant secondary metabolites (PSMs) and selective foraging behaviour by mammalian herbivores has received extensive research effort (Burgess & Chapman, 2005; Chapman *et al.*, 2003; Lawler *et al.*, 1998a; Moore *et al.*, 2005a). The majority of these studies have focussed on either the eucalypt-specialist marsupial folivores of Australia’s temperate forests or the folivorous primates of Asian and African rainforest. Food intake and diet selection by folivorous marsupials in the temperate *Eucalyptus*-dominated forests of Australia can be influenced by leaf nitrogen, fibre, phenolic constituents and tannin (Cork & Pahl, 1984; Foley *et al.*, 1987b;

Kavanagh & Lambert, 1990; Pass *et al.*, 1998). However, more recently it has become clear that the temperate-forest marsupial folivores (the koala, greater glider, common brushtail and common ringtail possums) base much of their selectivity on a group of deterrent phenolics in their *Eucalyptus*-dominated diet, known as formylated phloroglucinol compounds (FPCs). In captive trials the concentration of this single group explained more than 70% of variation in foliage intake, although there is some variation between folivore species (Lawler *et al.*, 1998a; Moore *et al.*, 2005a; Pass *et al.*, 1998). For example, common brushtail possums have a greater tolerance than common ringtail possums for the FPC phenolics, but their food intake is then limited by tannins (Marsh *et al.*, 2003a; Marsh *et al.*, 2003b). This variability in plant defence leads to folivore selectivity even at the intraspecific scale, and much of the advance in understanding determinants of feeding in marsupial folivores came from focusing on the chemical variability and determinants of preference within individual forage species (Lawler *et al.*, 2000; Scrivener *et al.*, 2004; Wallis *et al.*, 2002). Intraspecific variation in selectivity has also been demonstrated in other, more complex plant:folivore systems (Chapman *et al.*, 2003; Milton, 1978). However, in general, the determinants of folivore feeding preferences are less well understood in diverse systems such as rainforests where the high diversity and low abundance of many species (Coley & Kursar, 1996b) would seem to provide less advantage to a specialist mammalian folivore (Freeland *et al.*, 1974).

In comparison with the extensive recent literature on eucalypt-specialist marsupial folivores (Lawler *et al.*, 2000; Marsh *et al.*, 2003a; Moore *et al.*, 2005a) there have been very few studies of the effects of leaf chemistry on dietary selectivity by the 89% of arboreal marsupial folivores that inhabit highly diverse rainforest (Flannery, 1995; Strahan, 1995). This study investigates the chemical basis of dietary selectivity in a specialist folivorous marsupial, the rainforest-dwelling green ringtail possum (*Pseudochirops archeri* Collett). Green ringtail possums are specialist folivores, with a diet primarily comprised of mature leaves from surprisingly few (4-10 %) of the available tree and vine species (Goudberg, 1990; Jones *et al.*, 2006; Procter-Gray, 1984). Early studies that considered diet at an interspecific level only, without considering intraspecific variation in preference, suggested that *P. archeri* selected high fibre, low nitrogen food species compared with sympatric folivorous possums, but avoided species with high concentrations of condensed tannin (Goudberg, 1990). We previously demonstrated both inter- and intraspecific preferences in *P. archeri*, and predicted that, like eucalypt folivores, diet selection in the green ringtail possum will be driven by avoidance of PSMs, or limitation of PSM diversity in the diet, and operate at an intraspecific level (Jones *et al.*, 2006). Furthermore, costs associated with procuring and digesting temporally unpredictable seasonal resources are minimised by focussing foraging effort on mature leaves from a few preferred species (Jones *et al.*, 2006). No previous study has been able to definitively demonstrate the chemical basis for dietary selectivity in rainforest marsupials. The aim of the present study was to determine the basis of dietary preference for *P. archeri*, both within and between available canopy species. As a specialist folivore we expect dietary preferences to minimise the diversity and/or the quantities of PSMs ingested.

Methods

Study site

This study was conducted at Nasser's Nature Refuge, a privately owned 33 ha fragment of complex notophyll vine forest, forest type 5b (Webb, 1959), located about 4 km south of Atherton on the Atherton Tablelands, Queensland (145°30¢E, 17°18¢S), elevation 750m (for more detail see Jones *et al.* 2006). The forest is relatively dry rainforest with the average annual rainfall about 1400 mm (Atherton: Bureau of Meteorology data), most of which falls during the wet season between December and May. This forest type is associated with fertile basaltic soils and is characterised by an uneven canopy (25–45m) with a large number of deciduous and semi-evergreen trees, a tendency for heavy leaf-fall during times of moisture stress and a dense shrub and scrambling vine layer. At this site there were approximately 94 species of trees and vines in the canopy (Jones *et al.*, 2006).

Field observations

Preferences by possums at the site were determined from field observations of radio-collared possums, as described by Jones *et al*. (2006).

Ten individual green ringtail possums, *P. archeri*, were fitted with radio-transmitters and relocated regularly during monthly five-day field trips between October 2004 and April 2005. The number of observations of possums within an individual tree was used as the measure of feeding preference in this study as outlined and discussed in Jones et al. (2006). The preferred tree species were; *Aleurites rockinghamensis, Ficus fraseri, Ficus copiosa* and *Arytera divaricata*. Two species were used in proportion to their abundance, *Dendrocnide photinophylla* and *Melia azedarach*, and all other tree species at the site were used less than would have been expected if tree use was random. Studies on other marsupial folivores have concluded that tree visitation is an acceptable and useful measure of foraging (Martin *et al.*, 1999; Moore *et al.*, 2005a; Scrivener *et al.*, 2004).

Approximately 200 leaf samples were collected during the dry (September) and wet (February) seasons from preferred and rarely used trees within the four food species preferred by *P. archeri*. Samples were divided into juvenile (not fully expanded), mature (fully expanded with final colouration) and mature-new (fully expanded but not as tough or dark in colour as mature) leaves, when they were present, which was mostly during the wet season for the younger age classes. Juvenile and mature-new leaves were regularly available only in the species *A. rockinghamensis* and *F. fraseri,* so analyses of the effect of leaf age were restricted to those species. Mature leaves (91% of food items directly observed to be consumed by *P. archeri* were mature leaves;

Jones *et al*. 2006) were also collected from seven non-food tree species that were common (>1.5% of individual trees) at the site (5-6 individuals per species from *Acronychia acidula*, *Castanospermum australe, Castanospora alphandii*, *Dendrocnide photinophylla*, *Ficus septica*, *Melia azedarach* and *Mallotus philippensis*) during the dry season. Half of each sample (35 to 50g) was immediately weighed into a paper bag, then dried for 24 hours at 100°C and reweighed to determine water content. The remaining half of each sample was immediately frozen in the field on solid CO_2 then stored in the laboratory at -20°C until preparation. These samples were lyophilised and ground through a 1000 µm screen (Cyclotec 1093 sample mill, Foss Pacific Pty Ltd, North Ryde, Australia), then stored in the dark, in sealed plastic containers surrounded by silica gel desiccant, until analysis.

Nutritional measures used in this study were; nitrogen concentration, nitrogen digestibility, digestible nitrogen concentration, dry matter digestibility, water content and the presence/absence of cyanogenic glycoside (cyanogenic glycoside concentration was quantified when present). Nitrogen concentration provides an index of protein content (Van Soest, 1982). Nitrogen digestibility and digestible nitrogen concentration represent the biological availability of nitrogen to the digestive processes of mammals, and incorporate the effects other factors such as tannins which may bind digestive enzymes and dietary proteins, reducing nitrogen digestibility (Robbins *et al.*, 1987; Zucker, 1983). When digestible nitrogen content was important in preference, the correlation between nitrogen content and digestible nitrogen content was compared with the correlation between nitrogen digestibility and digestible nitrogen content to help determine whether digestible nitrogen content was due primarily to the overall nitrogen content, or the digestibility of that nitrogen. Dry matter digestibility provides an overall *in vitro* measure of digestibility and is generally strongly related to fibre content (Choo *et al.*, 1981). Cyanogenic glycosides were tested as a possible herbivore deterrent in rainforest (Hruska, 1988; Miller, Simon & Woodrow, 2006b). Water content was included because it has been important in foliage selection by other marsupial folivores (Hume, Jazwinski & Flannery, 1993; Pahl *et al.*, 1990) and is likely to be important for a small folivore living in a dry rainforest (Andrew Krockenberger, unpublished data).

Nitrogen concentration and nitrogen digestibility

The nitrogen content of samples before and after *in vitro* digestion was determined using wet oxidation in sulphuric acid and hydrogen peroxide with a selenium catalyst. Lithium sulphate was added to raise the digestion temperature (Allen, 1974; Anderson *et al.*, 1989). Nitrogen was determined colorimetrically in the digest by the salicylate-hypochlorite method (Baethgen *et al.*, 1989). Nitrogen digestibility was calculated by subtracting the nitrogen content of each sample post in vitro digestion from the original nitrogen content of that sample. Digestible nitrogen concentration was calculated by multiplying the nitrogen digestibility by the original nitrogen concentration of the sample.

In vitro digestibility of dry matter and nitrogen

Dry matter and nitrogen digestibility was measured in duplicate using a pepsin/cellulase *in vitro* procedure (modified from Choo *et al*. 1981). 0.6000 ± 0.05 g of dry ground leaf material was weighed into an oven-dry pre-weighed filter bag (F57 Ankom Technology, Macedon, U.S.A.). The bags were heat-sealed and duplicates placed together in individual small beakers to which was added a pepsin solution (40 mL per bag/ 80ml per beaker) containing 2.0 g of Pepsin (1:10,000 from porcine stomach mucosa, MP Biomedicals Inc, Solon, U.S.A.) per litre of 0.1N HCl (pH 1.0). The beakers were then placed within an agitating incubator at 36ºC for 60 hours. Upon removal, the samples were washed five times in distilled water and then incubated (36ºC) for 75 h in a 0.05 M cellulase-acetate buffer solution (40 mL per bag/80 ml per beaker). The cellulase-acetate buffer solution contained 6.8 g of sodium acetate, 2.9 mL of glacial acetic acid (99%) and 6.25 g of cellulase (from *Aspergillus niger,* MP Biomedicals Inc, Solon, U.S.A.) per litre of solution (pH adjusted to 4.6). At the end of this incubation, the samples were washed as above, dried to constant mass at 60ºC, and then reweighed.

Cyanide content

The Fiegl-Anger paper method was used to determine cyanide presence/absence (Brinker *et al.*, 1989; Feigl & Anger, 1966) across the whole range of canopy species sampled. Approximately 3 grams of freeze-dried, ground leaf was weighed into a screw cap vial and a few drops of water added.

The indicator paper was suspended above the tissue and held in place by screwing the lid over the attached paper handle. The vials were incubated at 37 °C for 24 h. A strongly cyanogenic sample resulted in the indicator paper turning purple within minutes. A weakly cyanogenic sample turned the paper (or just the lower edge of the paper) purple over several hours. The papers were left for 24 h but no longer to avoid interference by bacterial cyanogenesis. *Prunus turneriana* was used as a positive control and *Alstonia scholaris* as a negative control (Miller *et al.*, 2006b).

Cyanogenic glycoside content was quantified in the only species that tested positive for presence of cyanogen, *Aleurites rockinghamensis*. The cyanogenic glycoside concentration (µg CN released/g dry tissue) was measured by hydrolysing the glycoside and trapping the resultant hydrogen cyanide in a NaOH well (Brinker *et al.*, 1989). Hydrolysis was achieved by adding 1 mL of 0.1 mM citrate buffer (pH 5.5) to a known amount of freeze-dried material (10-15 mg) in a sealed glass vial, and incubated for 20 h at 37 °C. Excess of a non-specific cyanogenic b-glucosidase enzyme (emulsin from *Prunus amygdalus* EC 3.2.1.21, Sigma-Aldrich, Castle Hill, Australia) was added so the assay quantified the full amount of cyanide in the tissue sample. Cyanide in the NaOH well was determined using a miniaturized cyanide assay, which was adapted from Brinker and Seigler (1989) for use with a photometric microplate reader (Labsystems Multiskan Ascent with incubator, Vantaa, Finland). The absorbance was measured at 590nm using NaCN as the standard (Goodger *et al.*, 2002).

Near Infrared Spectroscopy (NIRS)

All (118) samples collected were scanned using NIRS to produce predictive models of the nutritional parameters measured above. This analytical technique has been championed recently as a highly useful tool for ecologists investigating the basis of folivore dietary selectivity (Foley *et al.*, 1998). In particular, this method makes it possible to significantly increase data sets by greatly reducing time and resource costs per sample. Spectra were obtained with a Fourier Transform Near Infrared spectrometer - Multi Purpose Analyzer (MPA) (Bruker Optics Inc, Clayton, Australia), in a darkened room maintained at approximately 25°C and 55% RH. We used a 30-position sample wheel attached to the MPA to scan each sample of dried, ground leaf. This unit measured spectral reflectance at wavenumbers between 12000 and 4000 cm^{-1} (wavelengths 780 - 2780 nm). The spectral data were processed using the software package OPUS version 5.5. Calibration equations were developed using the statistical Quant software within the OPUS package comparing the spectra with known values of nutritional parameters measured above. This program produces approximately 340 possible calibration models using various combinations of pre-processing treatment and frequency region. It then ranks the models/ equations according to the lowest root mean standard error of the cross-validation (RMSECV) and the highest coefficient of determination (r^2). The model was further refined by manually removing any outlying spectra for which predictions were significantly different from the observed value. The model that offered the best predictive capability for each nutritional parameter was then used to predict them from the spectra of samples with unknown chemical profiles, for use in the determination of nutritional preferences, within the species included in the calibration models.

Statistical methods

The effect of leaf age on nutritional value was tested using paired t-tests. Discriminant function analysis was used to test whether any of the five nutritional variables could be used to discriminate between preferred and avoided tree species by *P. archeri* across all canopy species sampled. Within the four preferred tree species, the relationship between frequency of use and the measured nutritional parameters was determined using the randomisation procedure of Scrivener *et al.* (2004). The distributions of data were triangular (eg Figure 1), so traditional correlation or regression analyses were inappropriate. This type of distribution suggests that the nutritional characteristics measured did not fully determine the frequency of use, but rather imposed upper or lower constraints on frequency of use, similar to the pattern described by Scrivener et al. (2004). The randomisation procedure we used was designed to identify those limits and test for their statistical significance. The extreme highest (for cyanogenic glycoside content) or lowest (for all other parameters) nutritional values within each frequency of use class were used to determine the correlation between use and nutrition along the edge of the data, as described by the Pearson product-moment correlation coefficient. Data were log (natural) transformed prior to this analysis, to linearise the edge of the distribution. The correlation coefficient determined was compared directly with the distribution of such correlation coefficients calculated from 10,000 iterations where the distributions were generated by randomly assigning nutritional values from the dataset into frequency of use classes in the same proportion as that observed. The correlation of use with the extreme highest value of cyanogenic glycoside was tested because *a priori* we expected cyanogenic glycoside to be a deterrent (Gleadow & Woodrow, 2002) and conversely we tested the correlation of use with extreme lowest value of the other parameters because they were each expected to reflect positive nutritional value of the foliage (McKey *et al.*, 1981; Milton, 1979; Robbins *et al.*, 1987), so the possums were predicted to select the highest possible values of these parameters.

Results

Leaf age

Juvenile *A. rockinghamensis* foliage was significantly higher in water and cyanogenic glycoside content than mature leaves from the same tree (Table 5.1). Nitrogen concentration, nitrogen digestibility, digestible nitrogen concentration, dry matter digestibility did not differ between leaf age classes.

Within the species *F. fraseri*, mature foliage had higher nitrogen digestibility than juvenile foliage (Table 5.1). However juvenile foliage was higher in dry matter digestibility and water content than mature. There was no significant difference in nitrogen or digestible nitrogen content between juvenile and mature foliage.

Season

Only 7% of *A. rockinghamensis* samples collected during the dry season contained juvenile foliage compared with 50% in the wet season, for this reason seasonal analyses were restricted to mature foliage only. Water content and cyanogenic glycoside concentration were significantly higher in the wet season than in foliage from the same tree in the dry season (Table 5.2).

As in *A. rockinghamensis*, only 7% of samples collected in the dry season contained juvenile foliage compared with 50% in the wet season, so again the analyses were restricted to mature foliage. Foliage did not vary seasonally in nitrogen concentration, digestible nitrogen or dry matter digestibility, but wet season foliage was significantly higher in water content and lower in nitrogen digestibility than dry season foliage from the same tree (Table 5.2).

To avoid confounding the established effects of leaf age and season with possible intraspecific and interspecific variation in nutritional components, only mature foliage collected during the dry season was used in the following analyses.

Intraspecific variation in tree use

NIRS

Near infrared spectroscopy was successful in predicting nutritional value (nitrogen concentration, nitrogen digestibility, digestible nitrogen concentration and dry matter digestibility) from the scanned spectra of rainforest tree foliage. The predictive equations accounted for between 86% (digestible nitrogen concentration) and 95% (dry matter digestibility) of variability in nutritional measures (Table 5.3). Because the predictive models had high precision, they were used to generate values of nutritional quality for individual trees with known possum preference that had been spectrally scanned, but could not have the nutritional assays due to constraints of time and money. Sixty percent of the values in the following analyses for *A. rockinghamensis* were predicted using NIRS (except cyanogenic glycoside content where all individuals were chemically quantified because the NIRS predictions were less precise). Nutritional values of 15% of *Ficus fraseri* and *F. copiosa* samples and 55% of *A. divaricata* samples were predicted using NIRS.

Intraspecific preference

There was no significant relationship between use of *A. rockinghamensis* and nitrogen content, nitrogen digestibility, digestible nitrogen concentration, dry matter digestibility, or water content for mature foliage (Table 5.4). However, possums avoided trees with high concentrations of cyanogenic glycosides and only made frequent use of trees with low cyanogenic glycoside concentration in the foliage (Fig. 5.1, Table 5.5). In this case, P= 0.026, meaning that only 260 out of the 10,000 random distributions generated had a relationship along the edge that was as strong or better than that observed.

Both nitrogen digestibility and digestible nitrogen concentration of the foliage were important in determining use of individual trees from *F. fraseri* and *F. copiosa*. (Figs 5.2a and 5.2b, Table 5.5). No highly-used trees were low in nitrogen digestibility or digestible nitrogen content. However, there was no relationship between dry matter digestibility, nitrogen content, or water content and use of either *F. fraseri* or *F. copiosa* (Table 5.5). Within *Ficus* foliage the correlation between nitrogen digestibility and digestible nitrogen content (r = 0.728, P =0.001, N = 16), was slightly stronger than that between nitrogen content and digestible nitrogen content (r = 0.67, P = 0.005, N = 16).

As in the preferred *Ficus spp*. both nitrogen digestibility and digestible nitrogen concentration of the foliage were important in determining use of individual trees of *A. divaricata* (Figs 5.3a and 5.3b, Table 5.5). There was no significant relationship between use of *A. divaricata* and nitrogen content, dry matter digestibility, or water content (Table 5.5). There was a strong positive correlation between nitrogen digestibility and digestible nitrogen content of *Arytera* foliage ($r = 0.97$, $P < 0.001$, $N = 17$), but no correlation between nitrogen content and digestible nitrogen content ($r = 0.282$, $P = 0.272$, $N = 17$).

Interspecific variation in tree use

Despite the importance of the nutritional measures to intraspecific preferences, we could not predict species preference (preferred or not) based on the predictor variables measured in this study [nitrogen content (mg/g), nitrogen digestibility (% dw pre-post digestion), digestible nitrogen content (mg/g) dry matter digestibility (% dry weight - dw), water content (% wet weight - ww) (Table 5.4)], using two-group discriminant function analysis (Wilk’s Lambda = 0.942, df = 4, P = 0.300; Eigen value = 0.062, canonical correlation = 0.242).

Table 5.1: Nutritional composition of juvenile and mature foliage from two preferred food species of green ringtail possums, *Pseudochirops archeri*. (means ± SEM)

Species	Nutrient[1]	Leaf age[2]		N	P
		Juvenile	Mature		
Aleurites rockinghamensis	Nitrogen (mg/g)	20.9 ± 1.6	18.5 ± 0.9	5	0.265
	Nitrogen digestibility (%)	0.6 ± 8.5	-4.6 ± 1.0	5	0.539
	Digestible nitrogen (mg/g)	0.7 ± 1.7	-0.8 ± 0.2	5	0.398
	Dry matter digestibility (%)	46.3 ± 1.9	50.0 ± 3.0	5	0.457
	Water (%)	76.5 ± 1.2	68.8 ± 2.4	4	**0.014**
	Cyanogenic glycosides (mg/g)	45.3 ± 11.2	4.5 ± 0.9	6	**0.012**
Ficus fraseri	Nitrogen (mg/g)	25.8 ± 2.2	22.1 ± 1.5	7	0.299
	Nitrogen digestibility (%)	29.0 ± 2.3	36.1 ± 3.0	7	**0.020**
	Digestible nitrogen (mg/g)	7.7 ± 1.2	7.8 ± 0.6	7	0.913
	Dry matter digestibility (%)	76.4 ± 1.1	72.1 ± 0.7	7	**0.035**
	Water (%)	73.2 ± 1.7	65.3 ± 1.1	7	**0.010**

[1] All nutrient content is calculated on a dry matter basis except water content

[2] Leaf age was divided into three categories, juvenile foliage was not fully expanded, mature-new foliage was fully expanded but not mature colour and mature foliage was fully expanded and dark green in colour. The comparison is by paired t-test of juvenile and mature foliage only.

Table 5.2: The effect of season on nutritional composition of mature foliage of two species preferred by green ringtail possums, *Pseudochirops archeri* (means ± SEM).

Species	Nutrient[1]	Season[2]		N	P
		Wet	Dry		
Aleurites rockinghamensis	Nitrogen (mg/g)	20.1 ± 0.9	18.5 ± 0.4	7	0.135
	Nitrogen digestibility (%)	-2.6 ± 5.2	-8.7 ± 2.4	7	0.322
	Digestible nitrogen (mg/g)	-0.5 ± 1.1	-1.6 ± 0.4	7	0.387
	Dry matter digestibility (%)	45.17 ± 1.6	45.8 ± 2.4	7	0.851
	Water (%)	71.3 ± 0.9	67.7 ± 3.2	6	**0.008**
	Cyanogenic glycosides (µg/g)	10.4 ± 2.0	5.8 ± 0.7	33	**0.020**
Ficus fraseri	Nitrogen (mg/g)	23.8 ± 0.7	21.9 ± 1.2	9	0.112
	Nitrogen digestibility (%)	29.8 ± 2.8	39.0 ± 3.6	9	**0.050**
	Digestible nitrogen (mg/g)	7.1 ± 0.7	8.4 ± 2.2	9	0.201
	Dry matter digestibility (%)	74.4 ± 1.1	72.0 ± 0.9	9	0.202
	Water (%)	72.9 ± 0.6	64.9 ± 0.8	9	**<0.001**

[1] All nutrient content is calculated on a dry matter basis except water content

[2] Wet season includes samples from December to May and dry season includes samples from June to November

Table 5.3: Near infrared spectroscopy modelling of nutrient content of foliage from the eleven most common species[1] found in complex mesophyll vine forest at Nasser's nature reserve.

Nutrient[2]	N (outliers)	Pre-processing[3]	Frequency region[4] (nm)	Rank[5]	RMSECV[6]	R^2
Nitrogen (mg/g)	117 (2)	1st derivative	1333 – 1640 2173 – 2355	10	1.49	88.7
Nitrogen digestibility (%)	102 (17)	1st derivative	1333 – 1640 2173 – 2355	8	7.63	87.1
Digestible nitrogen concentration (mg/g)	100 (19)	1st derivative	1333 – 1640 2173 – 2355	9	1.76	86.7
Dry matter digestibility (%)	115 (4)	1st derivative	1333 – 1640 2173 – 2355	8	3.46	95.1
Water (% ww)	116 (3)	1st derivative	1333 – 2175	10	2.8	90.1
Cyanogenic glycosides (ug/g)	87 (0)	min-max normalization	1638 – 1836 2173 – 2355	8	8.55	63.2

[1] Species used in calibration are Acronychia acidula, Aleurites rockinghamensis, Arytera divaricata, Castanospermum australe, Castanospora alphandii, Dendrocnide photinophylla, Ficus copiosa, Ficus fraseri, Ficus septica, Mallotus philippensis and Melia azedarach.

[2] All nutrient content except for water are calculated on the basis of dry matter

[3] Pre-processing technique of spectral data applied before regression analysis as described in Woolnough and Foley (2002).

[4] Spectrum of wavelength used by each model, to nearest nm

[5] Number of factors of the matrix used to analyse the internal test samples

[6] Root-mean-square error of cross-validation

Table 5.4: Nutrient composition[1] of foliage from the 11 most common species in complex mesophyll vine forest at the Nasser's nature reserve study site (means ± SEM).

Species (n)	Proportion basal area at site (%)	Nitrogen (mg/g)	Nitrogen digestibility (%)	Digestible nitrogen (mg/g)	Dry matter digestibility (%)	Water content (%)
Acronychia acidula (6)	3	23.4 ± 1.0	45.4 ± 1.2	10.6 ± 0.4	61.8 ± 1.1	70.8 ± 0.3
Aleurites rockinghamensis (33)	22	20.1 ± 0.4	-4.3 ± 1.8	-0.7 ± 0.4	47.5 ± 1.0	72.0 ± 0.8
Arytera divaricata (8)	4	18.5 ± 0.3	3.7 ± 2.0	0.7 ± 0.4	32.6 ± 0.7	51.0 ± 1.5
Castanospermum australe (5)	6	22.8 ± 1.0	45.9 ± 2.9	10.4 ± 0.6	42.8 ± 1.6	61.7 ± 1.4
Castanospora alphandii (5)	8	17.6 ± 0.6	22.3 ± 3.7	4.0 ± 0.7	41.0 ± 0.7	54.4 ± 1.3
Dendrocnide photinophylla (5)	16	32.4 ± 0.9	-63.2 ± 6.8	-20.4 ± 2.1	74.7 ± 0.6	82.5 ± 1.3
Ficus copiosa (8)	3	14.9 ± 0.7	29.2 ± 2.6	4.4 ± 0.4	62.8 ± 0.9	70.3 ± 1.6
Ficus fraseri (32)	4	23.4 ± 0.7	32.7 ± 1.6	7.6 ± 0.4	74.0 ± 0.6	69.7 ± 0.8
Ficus septica (6)	1	19.7 ± 1.6	-36.4 ± 3.4	-7.0 ± 0.5	73.4 ± 1.2	74.7 ± 1.4
Mallotus philippensis (5)	1	17.5 ± 0.9	4.4 ± 1.3	0.8 ± 0.3	32.6 ± 0.5	48.3 ± 0.4
Melia azedarach (5)	18	25.6 ± 0.7	-24.0 ± 7.1	-6.2 ± 1.6	72.1 ± 1.1	71.9 ± 1.3

[1] All nutrient content except water were calculated on a dry matter basis

Table 5.5: Correlation between tree use by green ringtail possums, *Pseudochirops archeri* and minimum values of nutrients from mature foliage of the four preferred canopy species collected during the dry season.

Tree species	Nutrient[1]	N	r[2]	P[3]
Aleurites rockinghamensis	Nitrogen (mg/g)	35	0.519	0.707
	Nitrogen digestibility (%)	34	0.077	0.708
	Digestible nitrogen (mg/g)	34	0.204	0.578
	Dry matter digestibility (%)	35	0.850	0.291
	Water (%)	31	0.364	0.832
	Cyanogenic glycosides (mg/g)[4]	29	0.948	**0.026**
Ficus fraseri and *F. copiosa*	Nitrogen (mg/g)	18	0.055	0.269
	Nitrogen digestibility (%)	18	0.932	**0.017**
	Digestible nitrogen (mg/g)	16	0.742	**0.045**
	Dry matter digestibility (%)	20	0.015	0.358
	Water (%)	21	0.127	0.879
Arytera divaricata	Nitrogen (mg/g)	17	0.889	0.313
	Nitrogen digestibility (%)	18	0.963	**0.015**
	Digestible nitrogen (mg/g)	18	0.986	**0.028**
	Dry matter digestibility (%)	15	0.876	0.338
	Water (%)	25	0.689	0.680

[1] All nutrient content except water was calculated on a dry matter basis

[2] Pearson's product-moment correlation coefficient

[3] Probability of an equal or stronger correlation within 10 000 randomized distributions

[4] Cyanogenic glycosides are expected to be a deterrent, thus the correlation used maximum values of cyanogenic glycosides.

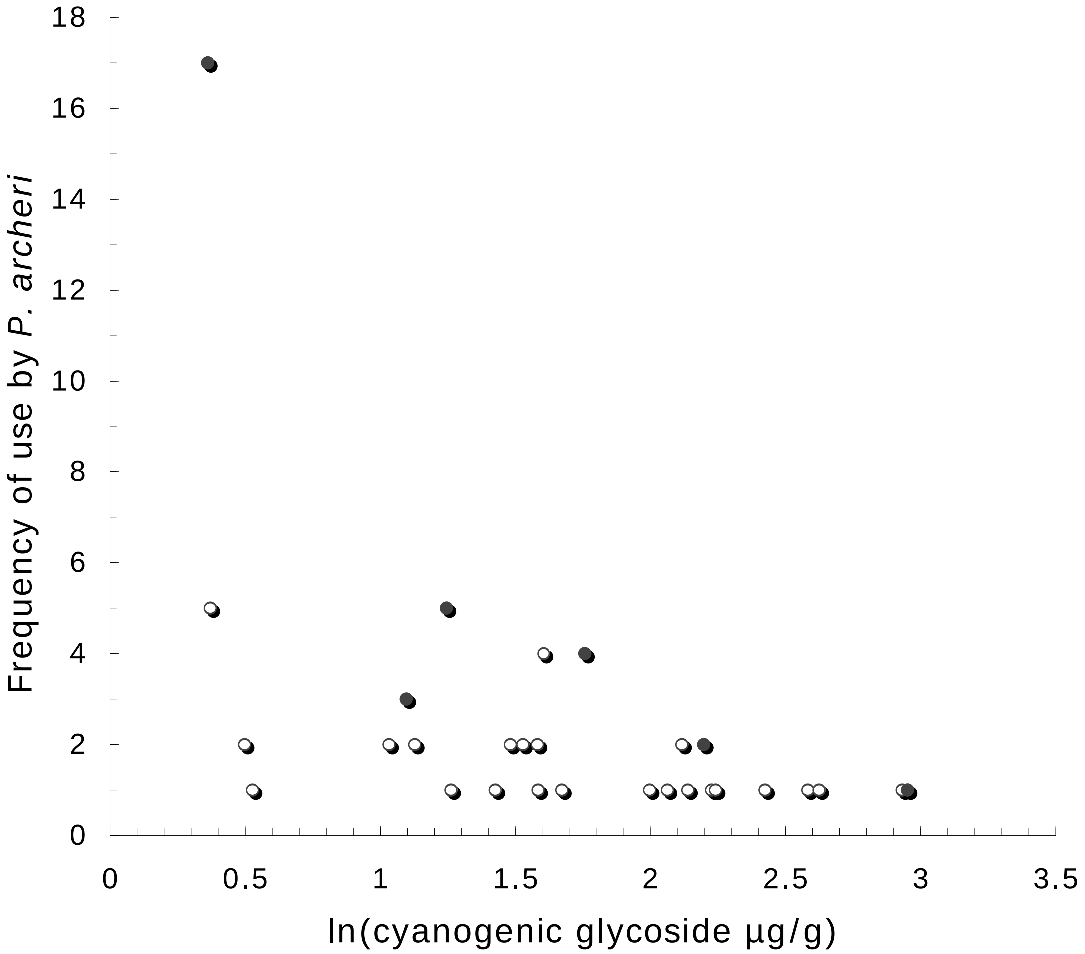

Figure 5.1: The relationship between cyanogenic glycoside concentration (ug cyanide produced/g dry matter) and use of individual *Aleurites rockinghamensis* by green ringtail possums, *Pseudochirops archeri*. The maximum values in each tree use category are shown as solid circles. (n = 29).

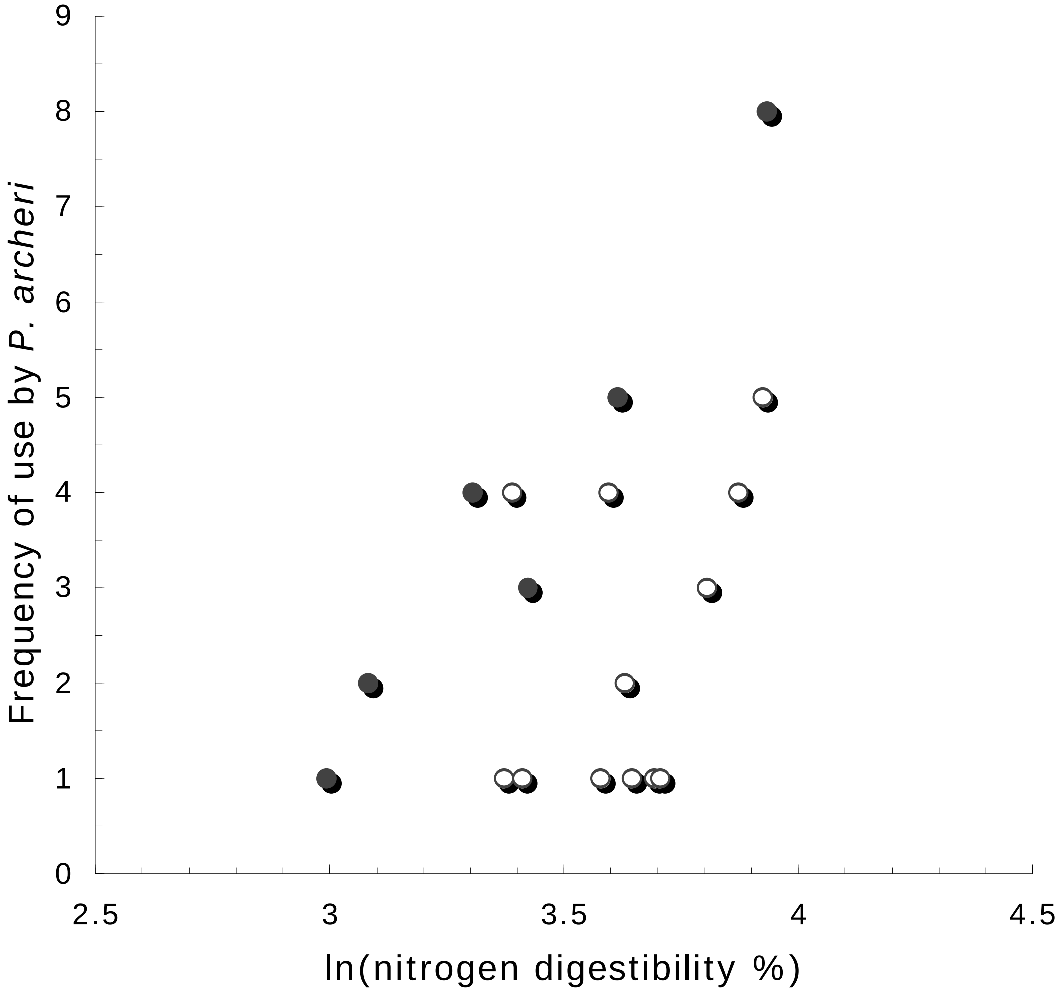

Figure 5.2a: The relationship between nitrogen digestibility (%) and use of *Ficus* (*Ficus fraseri* and *F. copiosa* combined) by green ringtail possums, *P. archeri*. The minimum values in each tree use category are shown as solid circles. (n = 17).

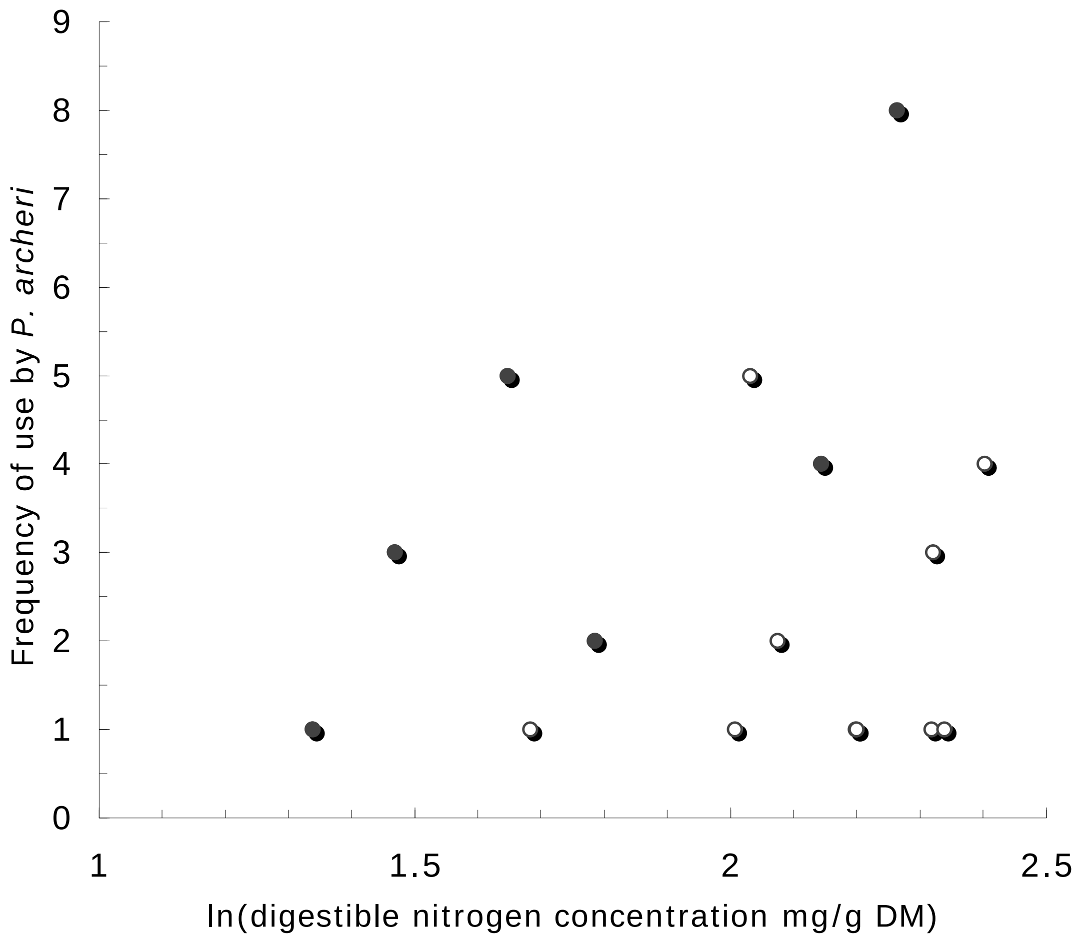

Figure 5.2b: The relationship between digestibile nitrogen concentration (mg/g dry weight) and use of *Ficus* (*Ficus fraseri* and *F. copiosa* combined) by green ringtail possums, *P. archeri*. The minimum values in each tree use category are shown as solid circles. (n = 16).

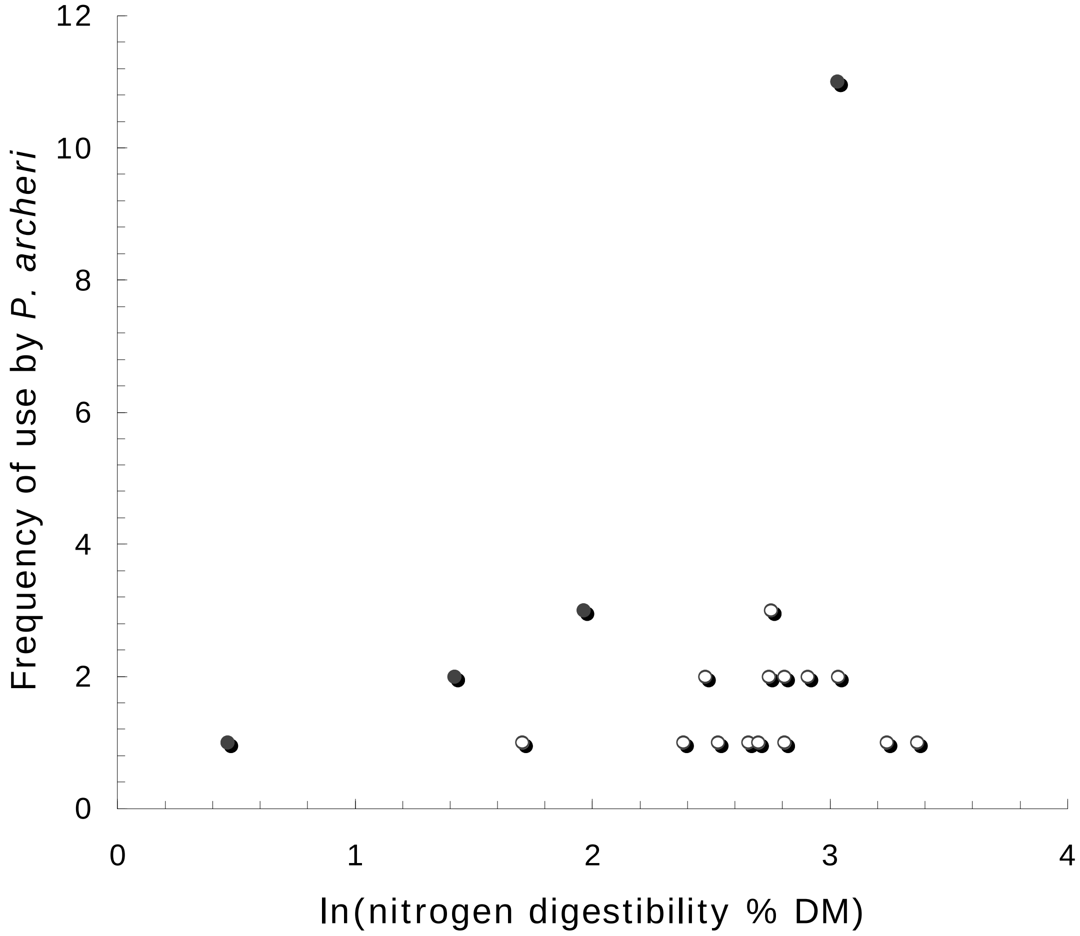

Figure 5.3a: The relationship between nitrogen digestibility (% dry weight) and use of *A. divaricata* by green ringtail possums, *P. archeri*. The minimum values in each tree use category are shown as solid circles. (n = 18).

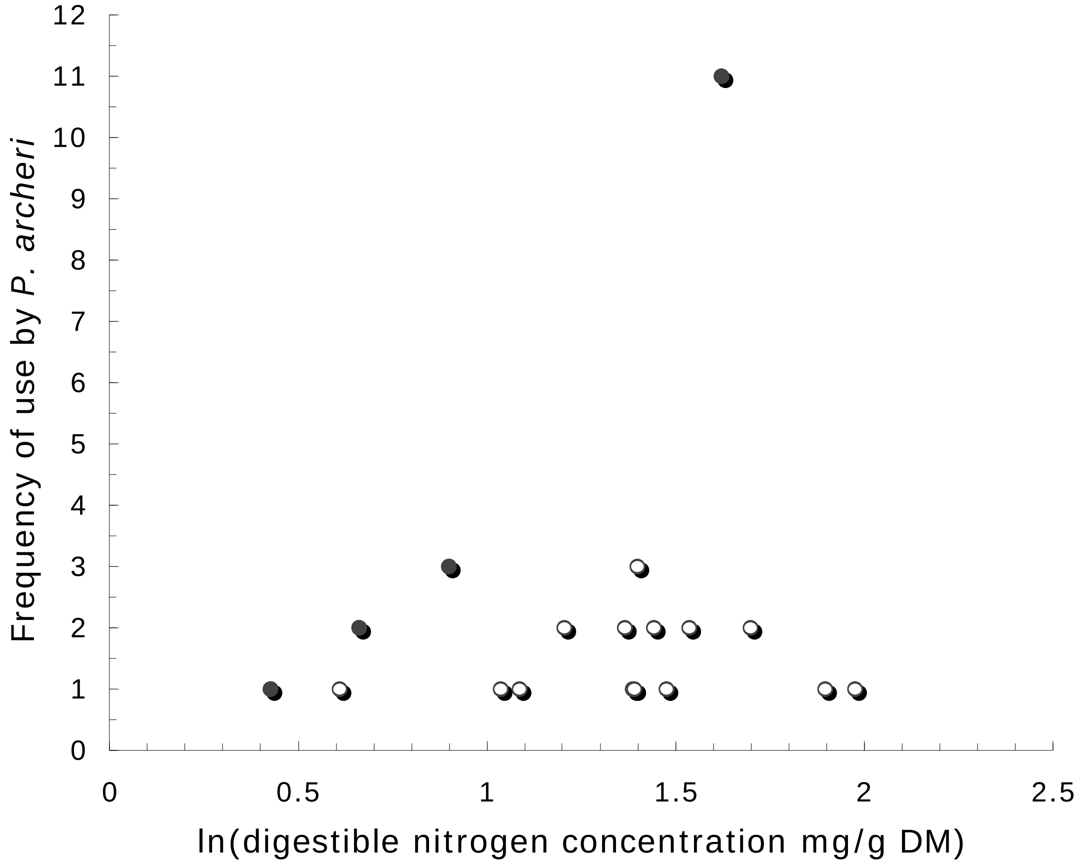

Figure 5.3b: The relationship between digestible nitrogen concentration (mg/g dry weight) and use of A. divaricata by green ringtail possums, P. archeri. The minimum values in each tree use category are shown as solid circles. (n = 18).

Discussion

The dietary preference of *Pseudochirops archeri* is related to nutritional characteristics of the available foliage. Even though interspecific selectivity by *P. archeri* was not well described by the nutritional characteristics measured in this study, intraspecific selection was related to nutritional quality of foliage, and more specifically, the activity of PSMs in that foliage. This was most clearly evident in choice within the preferred canopy species, candlenut, *Aleurites rockinghamensis*. Possums avoided trees with foliage high in cyanogenic glycosides, and only trees low in cyanogenic capacity were intensively used.

Cyanogenic glycosides

The candlenut, *A. rockinghamensis* (Euphorbiaceae) was the most common species at the site (22% of basal area; Table 4), and the species most preferred by green ringtails (38% of preferred trees were candlenut) (Jones *et al.*, 2006), but it was also the only species at the site that tested positive to cyanogenic glycosides. Cyanogenic taxa are relatively common in the family Euphorbiaceae (Thomsen & Brimer, 1997) but only represent around 4.5% of tree species in rainforests of the region (Miller *et al.*, 2006a). Cyanogenic glycosides are capable of releasing toxic hydrogen cyanide when consumed by herbivores and are thought to function primarily as defence against herbivores (Conn, 1979; Jones, 1998). Most generalist herbivores are strongly and rapidly affected by hydrogen cyanide and consequently tend to avoid plants containing high levels of this toxin (Gleadow *et al.*, 2002; Seigler, 1991). The more generalist marsupial folivore the common brushtail possum (*Trichosurus vulpecula*), develops conditioned food aversions (CFA) when subjected to sublethal doses of cyanide (O'Connor & Matthews, 1995), similar to the mechanism proposed for the deterrent effect of phenolic FPCs on the eucalypt specialist marsupial folivores (Lawler *et al.*, 1998a). Others suggest that the brushtail possums can directly detect cyanide and simply directly reject food items when cyanide is present (Warburton & Drew, 1994). Cyanide toxicity has previously been linked to deterrence of koalas feeding on *Eucalyptus viminalis* (Finnemore, Reichardt & Large, 1935). Specialisation on this cyanogenic taxon by *P.archeri* (Jones *et al.*, 2006) may reflect a consistently high abundance of candlenut foliage in their habitat (Freeland *et al.*, 1974), as the effectiveness of cyanogenic glycosides in defence can be variable and some specialist folivores select cyanogenic plants or plant parts. The golden bamboo lemur (*Hapalemur aurens*) eats the highly cyanogenic growing tips of the *Cephalostachym* cv. *viguieri* while two sympatric lemur species feed only on plant parts that do not contain cyanide (Glander *et al.*, 1989). Glander et al. (1989) attributed this to a competitive advantage accrued by *Hapalemur* through its ability to consume cyanogenic food that is unpalatable to other species. The mechanism by which specialist herbivores tolerate high cyanide remains unknown (Gleadow *et al.*, 2002), however there are four confounding factors in the efficacy of cyanogens to folivores (Gleadow *et al.*, 2002) that could each contribute to apparent immunity to the effects. The first two represent physiological adaptations of the folivore; the animal may be a specialist with mechanisms to cope with cyanide or the concentration of cyanide may be below threshold toxicity (possibly due to enhanced resistance of the folivore). The last two relate to behavioural solutions; the cyanogenic plant may not be toxic when part of a mixed diet, or if the feeding behaviour of the animal causes minimal damage to the leaf it will limit mixing of the cyanogenic glycoside with the degradative b-glucosidases, thus preventing release of HCN gas.

Leaf age

Mature foliage of temperate plants is often more highly defended by fibre and leaf toughness than young foliage (Coley & Barone, 1996a), although in some cases young foliage may have higher concentrations of total phenolics (Cork *et al.*, 1984). In general, this is reflected in the strong preference for young over mature foliage by temperate, eucalypt-specialist marsupial folivores (Kavanagh *et al.*, 1990; Moore *et al.*, 2000; Pahl, 1984). Within those temperate forests, foliage has less diversity of and lower overall levels of defence compared with that in rainforest (Coley *et al.*, 1991). In the rainforests, young leaves are generally better defended than mature (Coley *et al.*, 1996a). This may be a foliar response to higher herbivore pressure (mostly insect) in rainforests, and also may be a function of the much greater species and family level plant diversity in rainforests (Coley *et al.*, 1996b). Furthermore, high resource availability in the basalt soil of the site may promote production of nitrogen-containing PSMs such as cyanogens (Coley *et al.*, 1985). Jones et al (2006) suggested that the preference by *P. archeri* for mature foliage of preferred food plants (*A. rockinghamensis*, *F. fraseri, F. copiosa* and *Arytera* *divaricata*) might be due to its consistent availability in comparison with seasonal resources such as fruit, flowers and juvenile foliage. However, this study shows that mature leaves are also nutritionally superior, with higher concentrations of digestible nitrogen in the case of the fig species and *A. divaricata*. The cyanogenic glycoside content of juvenile foliage of *A. rockinghamensis* was an order of magnitude higher than that of mature foliage, and reached levels associated with deterrence or toxicity in other species (Glander *et al.*, 1989). We propose that *P. archeri* avoid eating juvenile foliage of *A. rockinghamensis* to evade especially high cyanogen concentrations. In a survey of over 400 Australian rainforest species, young foliage contained significantly higher concentrations of cyanogenic glycosides than old foliage, in all cyanogenic species where both young and mature foliage were tested (Miller *et al.*, 2006a). It is likely that juvenile leaves of *A. rockinghamensis* are highly defended by cyanogenic glycosides during their fragile growing phase, but when fully expanded, investment in chemical defence is relaxed (Coley *et al.*, 1996b), but not abandoned because even the lower concentrations of cyanogens seen in mature foliage are enough to drive intraspecific selectivity.

The seasonal variation in the cyanogenic glycoside content of *A. rockinghamensis* and nitrogen digestibility of *F. fraseri* mature foliage is probably explained by the confounded effects of season and leaf age. Mature leaves collected during the wet season were likely to have been younger due to the semi-deciduous nature of *A. rockinghamensis* (Jones *et al.*, 2006), where new leaves are flushed in the early wet season, causing mature leaves sampled in the wet to be younger than those sampled six months later before the major leaf fall of the dry season.

Nitrogen digestibility and tannins

We found no relationship between dietary preference by *P. archeri* and total nitrogen content, despite the fact that total protein has often been reported as important for defining nutritional quality of food plants and for interpreting their selection by wild herbivores (McKey *et al.*, 1981; Milton, 1979). Indeed, past studies have found relationships between nitrogen content of foliage and herbivore density on a landscape scale (Braithwaite *et al.*, 1983). However it does not necessarily follow that nitrogen determines herbivore preference at the scale of the individual tree. As discussed above, we now know that intraspecific variation in plant chemistry provides a more powerful predictor of herbivore preference, and particular PSMs show greater intra and inter specific variation than do amounts of nutrients (Moore *et al.*, 2000). Nitrogen digestibility of foliage is inhibited to various extents by tannins that bind dietary proteins and digestive enzymes (Mole *et al.*, 1987; Zucker, 1983). We suggest that nitrogen digestibility and digestible nitrogen concentration are superior measures to that of total nitrogen content when assessing the relationship between folivore food preference and dietary nitrogen, because they incorporate a more ecologically relevant measure of nitrogen availability.

Within the preferred species *F. fraseri*, *F. copiosa* and *A. divaricata*, visits by *P. archeri* to individual trees were positively related to the nitrogen digestibility and digestible nitrogen concentration of mature foliage. Furthermore, *P. archeri* were never observed eating the lower nitrogen digestibility juvenile foliage of *F. fraseri, F. copiosa* or *A. divaricata* despite their higher water content and dry matter digestibility. Nitrogen digestibility is likely to reflect the activity of tannins in reducing the digestibility of leaf proteins (Robbins *et al.*, 1991; Zucker, 1983). In the *in vitro* digestibility assay used in this study, negative nitrogen digestibilities (four of the eleven species tested, including *A. rockinghamensis*, had negative nitrogen digestibility and *A. divaricata* had very low digestibility) indicate that enzymes added to the samples were precipitated and caused the final nitrogen content of the sample to be greater than the initial. Such precipitation is likely to be the effect of tannins present in the foliage (Robbins *et al.*, 1991; Zucker, 1983). Furthermore, the actual nitrogen availability (digestible nitrogen) in foliage of *A. divaricata, F. fraseri* and *F. copiosa* was more strongly correlated with the nitrogen digestibility than with the gross nitrogen content, indicating that factors external to the nitrogen source in the leaf, such as the effect of tannins on protein digestibility (Mole *et al.*, 1987; Robbins *et al.*, 1991) were most important in determining the nutritional quality of the foliage. We interpret these results to mean that the importance of nitrogen digestibility and digestible nitrogen content to the preferences shown by *P. archeri* actually reflects an avoidance of high biological activity of tannins, within the preferred species. Chimpanzees in Guinea, Bossou predominately feed in species of Moraceae, which includes the genus *Ficus* (Takemoto, 2003), and also preferentially select foliage with low condensed tannin content. Similarly, colobus and rhesus monkey leaf preference is inversely correlated with tannin content (Marks *et al.*, 1988; Oates *et al.*, 1977). Digestibility of plant fibre can be reduced by tannins as well, although not as clearly as protein digestibility, possibly as a result of precipitation of digestive enzymes (Robbins *et al.*, 1991). This lesser effect, mediated by protein digestibility, may account for the lack of effect of dry matter digestibility on preference by *P. archeri*.

The actual effect of tannin on a folivore is subject to the chemical characteristics of the tannin and any physiological mechanisms the consumer employs to reduce the effects of the tannin (McArthur *et al.*, 1991a). Browsers often consume high tannin diets and may be better adapted to counteracting tannins than animals such as grazers living on a tannin-free diet (McArthur *et al.*, 1991b). Tannin-binding salivary proteins (TBSP) are produced by many browsers and are thought to prevent tannins interacting with other proteins, thus minimizing tannin absorption and reducing tannin toxicity (Shimada, 2006). It is currently unknown how important TBSPs are to tannin detoxification in marsupial folivores (Marsh *et al.*, 2003b). Tannins are highly diverse in structure and activity so it is difficult to chemically define tannin levels in foliage (Zucker, 1983). Leaves were analysed for nitrogen digestibility and digestible nitrogen concentration instead to provide a biologically relevant measure. The assay provides a relative measure of tannin activity and could not be expected to quantify the effect of the tannins in a specialist that may be adapted to minimise the tannin’s effect. Common ringtail possums (and possibly koalas and greater gliders) conserve nitrogen in the gut by absorbing and processing tannin using caecal microbes to dissociate protein complexes (McArthur *et al.*, 1991b), and the use of tree species with very low *in vitro* nitrogen digestibility may indicate similar capabilities in *P. archeri*. Recent experiments by Marsh *et al*. (2003a) and Marsh *et al*. (2003b) showed that common ringtail possums have a greater tolerance than common brushtail possums for tannins, but FPC phenolics limit their food intake. Ringtails fed foliage from trees of the FPC-free eucalypt subgenus *Monocalyptus* still showed a small (10%), but significant increase in feeding when the leaves were coated in tannin-binding poly-ethylene glycol (PEG) (Marsh *et al.*, 2003b), suggesting that tannins are also deterrent in common ringtail possums, though they may be often masked by the effect of FPCs (Marsh et al 2003a).

Interspecific variation in tree use

Despite the strong evidence for PSMs affecting intraspecific preferences, there was no relationship between possum preference for particular tree species and nutritional variables. It is likely that different PSMs affected the suitability of potential food plants within each species, producing a relationship too complex for the simple set of measures used. For example, use of *A. rockinghamensis* is only very weakly related to any of the measures except cyanogenic glycoside concentration, so if each plant group has a unique PSM, or group of PSMs, then no single parameter could explain interspecific selectivity. We suggest that green ringtail possums are dietary specialists either to minimise PSM intake of any sort and thus reduce the energy expenditure associated with PSM detoxification and elimination, or to limit the diversity of PSMs to which they are exposed. Interspecific choice may be based on the need to minimise the diversity of PSMs ingested, whereas intraspecific choice is based on the need to minimise the quantities of those few PSMs.

Accessibility to nitrogen in their preferred species caused *P. archeri* to choose some individual trees over others and mature foliage over juvenile. At the interspecific level *D. photinophylla* had very high nitrogen content but very low nitrogen digestibility (up to -81%) (Table 4). We suggest that this species is defended against herbivory through a nitrogen digestibility-reducing compound such as tannin. Possums were observed feeding on the petioles of *D. photinophylla* (Jones *et al.*, 2006) and it is possible that this behaviour allowed them to avoid tannins in the foliage. Our interpretation that *P. archeri* avoids high biological activity of tannins in intraspecific preferences broadly concurs with Goudberg's (1990) previous interspecific study. She found that condensed tannin content was significantly lower in 10 preferred food species of *P. archeri* than 10 randomly selected species that were never eaten and she concluded that tannin may play a major role in diet selection. Furthermore, preferred food had less than 0.2% condensed tannin content. However, the most preferred species in this study, *A. rockinghamensis* had negative nitrogen digestibility, indicating high biological activity of tannins in the foliage, and preferences were not affected by nitrogen digestibility, so it seems that cyanogenic capacity overides deterrent effects of tannins within this species. At an interspecific level dry matter digestibility did not seem to have any over-riding effect on food preference. Preferred species *A. rockinghamensis* and *A. divaricata* had significantly lower dry matter digestibility than avoided species *Ficus septica* and *Acronychia acidula*. Similarly, mature leaves of *F. fraseri* were preferred over young leaves, but young leaves had significantly higher dry matter digestibility. Furthermore, there was no relationship between tree use and dry matter digestibility within any *P. archeri* preferred food plants. Plant dry matter is composed of soluble, digestible cell contents and insoluble fractions made up of relatively indigestible cell polysaccharides and lignin. A high fibre diet reduces the efficiency of bacterial enzyme action in the gut of herbivorous mammals and slows the rate of digestion (Oates *et al.*, 1980). We measured dry matter digestibility to assess the possible effects of dietary fibre on selectivity in *P. archeri* (Choo *et al.*, 1981). We suggest that as a specialist folivore, *P. archeri* possesses physiological and behavioural adaptations that allow them to tolerate dietary items low in dry matter digestibility (high in fibre) in order to select food items based on avoidance of other PSMs such as nitrogen digestibility-reducing compounds and cyanogenic glycosides. This is consistent with previous studies showing that *P. archeri* has a high fibre diet compared with sympatric folivorous possums (Goudberg, 1990; Procter-Gray, 1984). This conclusion does not preclude avoidance of tree species with very low dry matter digestibility, such as *Mallotus philippensis*, that may be below a threshold level of digestibility.

Dietary selectivity by the specialist *P. archeri* may have been affected by the relative abundance of each tree species at the site. Frequency of tree use by koalas was primarily influenced by tree size, but also FPC and nitrogen concentration (Moore *et al.*, 2005a). Three of the four preferred species of *P. archeri*; *A. divaricata*, *F. fraseri* and *F. copiosa,* comprised only a small proportion of the plant biomass at the site (4, 4 and 3 % respectively, Table 4), but were used often suggesting that these species were extremely valuable food resources. The interesting exception is *A. rockinghamensis*, which comprised nearly a quarter of the total basal area at the site (Table 4). It is possible that the possums prefer this species, despite low nitrogen digestibility and cyanogens, because it is a common and therefore a predictable resource for a specialist folivore (Jones *et al.*, 2006) supporting the contention of Freeland and Janzen (1974) that specialisation is likely to arise when a defended plant is consistently and reliably available.

The findings of this study clearly show that PSMs determine intraspecific dietary preference by the rainforest marsupial folivore, *P. archeri*. Concentrations of deterrent compounds varied significantly between trees within species and leaf ages within trees. Within preferred tree species individuals were selected according to either cyanide concentration (*A.*

rockinghamensis) or nitrogen digestibility (tannin content) (*A. divaricata*, *F. copiosa*, *F. fraseri*). Similarly, within these species, the same factors determining tree preference also explained the preference for mature foliage over juvenile. This is the first study to isolate specific nutritional parameters responsible for intraspecific dietary preference in a rainforest marsupial folivore. We were not able to find a significant relationship between any of the nutritional parameters and overall interspecific selectivity by the possums. However, we suggest that each non-preferred species is defended by a unique PSM or set of PSMs, such as tannins in *D. photinophylla* and fibre in *M. philippensis* and this specialist folivore may limit PSM diversity in its diet by preferring a limited suite of food species, while minimising intake of deterrent PSMs at the intraspecific level.

CHAPTER 6.

Conclusions: The feeding strategy of *P. archeri*

Arboreal marsupial folivores face a range of feeding challenges due to their small size and a diet high in fibre and toxic plant defensive compounds. These animals are necessarily small due to branch strength and the energy required to move a large mass vertically (Grand, 1978), but small animals have higher mass specific metabolic requirements (Clutton-Brock *et al.*, 1977; Nagy, 1987). In addition gut size tends to scale linearly according to mass (Demment *et al.*, 1983; Parra, 1978), but because mass specific metabolic requirements increase the relative metabolic requirements per gut mass, it is harder for them to process a bulky diet high in indigestible fibre (Cork *et al.*, 1991). Dietary intake is further restricted by problems associated with processing and excreting large amounts of toxic PSMs ingested with a folivorous diet (Dearing *et al.*, 2005a). PSMs have been found to drive selectivity in herbivorous animals as diverse as birds (Guglielmo *et al.*, 1996), reptiles (Dearing & Schall, 1992; Schall & Ressel, 1991), primates (Ganzhorn & Wright, 1994; Marks *et al.*, 1988; Oates *et al.*, 1977), rodents (Sorensen *et al.*, 2005b) and marsupials (Lawler *et al.*, 1998a; Moore *et al.*, 2005a). In general, mammalian herbivores are able to subsist on a highly defended diet through behavioural and physiological adaptations (Cork *et al.*, 1991; McArthur *et al.*, 1991a).

Despite the challenges associated with a folivorous diet, I found that the small (1.0 – 1.5kg) arboreal, marsupial possum, *Pseudochirops archeri* was a strict folivore (Chapter 3). *P. archeri* used only four tree species, *Aleurites rockinghamensis*, *Ficus fraseri*, *Arytera divaricata* and *F. copiosa* significantly more frequently than would be expected if tree species were selected randomly in proportion to their relative abundance in the forest (Chapter 2). Conversely, 88 other tree species present were used less frequently than expected. Possums also favoured particular individual trees within some of the preferred tree species, behaviour that has been well established in *Eucalyptus*-specialised folivores and attributed to an intra-specific variation in deterrent chemicals (Lawler *et al.*, 1998a; Pass *et al.*, 1998). In 91% of feeding observations, possums consumed mature leaves only. The availability of young leaves, flowers and fruit varied throughout the year, with a peak in availability of these resources during the early wet season. I propose that *P. archeri* chose a strict, foliage-only diet due to the seasonality of the rainforest where most resources are not consistently available. By primarily selecting mature leaves, green ringtail possums reduced their dependence on seasonally variable resources, thus supporting Freeland and Janzen's (1974) hypothesis that herbivores will specialise on few of the available resources when these food items are easily and consistently available.

Within the four preferred tree species, specific nutritional parameters were responsible for variation in individual tree use. Within the predominant preferred species, *A. rockinghamensis,* cyanogenic glycosides acted as a deterrent (Chapter 5). Cyanide is a well-known plant defence against herbivores (Jones, 1998), and it seems that individual *A. rockinghamensis* trees are selected by *P. archeri* according to concentrations of this toxin. However, it is initially surprising that these animals would chose cyanogenic plants (no matter what the concentration) over so many other potential food species at the site. Gleadow and Woodrow (2002) reviewed the effect of cyanide on herbivores and concluded that cyanogenic glycosides are not effective against all herbivores, and some plants do not release enough cyanide to be considered toxic. In addition, specialist herbivores and folivores seem to be able to cope better with cyanogenic glycosides than generalists (Compton & Jones, 1985; Schappert & Shore, 2000). So it is possible that, as a specialist folivore, *P. archeri* has physiological adaptations to detoxify and excrete cyanogenic glycosides up to a threshold concentration. Madagascan golden bamboo lemurs (*Hapalemur aureus*) tolerate extremely high levels of cyanide in their diet (15mg CN/ 100g FW), consuming approximately 75mg per day (12 times the lethal dose) (Glander *et al.*, 1989). The concentration of cyanogenic glycosides in the mature leaves of *A. rockinghamensis* (21% of tree use) is much lower than this (0.18 mg CN/ 100g FW), so it is also possible that *P. archeri* simply don't consume enough cyanide for it to become chronic. Energy requirements of common ringtail possums are based on field metabolic rate (FMR) and were calculated as 2.5 times basal metabolic rate (BMR) (Munks & Green, 1995), which is a commonly used multiple in marsupials (Hume, 1999), therefore FMR = 2.5 X BMR. Krockenberger and Kanowski (in review) reported that average sized *P. archeri* have a BMR of 220 kJ/d, so FMR would be 550kJ/d. The energy content of foliage is generally around 20 kJ/d (Cork, Hume & Dawson, 1983a), digestibility of *A. rockinghamensis* is approx 45% (Jones *et al.*, in review), and if we look at urinary energy loss it is generally around 10% (Cork *et al.*, 1983a). This would give a total metabolic energy content of foliage of around 8.1 kJ/g dry matter (20 X 0.45 X 0.9 = 8.1). In comparison, total metabolic energy content of *Eucalyptus* foliage for koalas was similar at 8.975 kJ/g dry matter (Krockenberger, 2003). So if *P. archeri* need 550 kJ/d and the energy content of foliage is 8.1 (or 8.795) kJ/g, then they need to take in 550/8.1 = 67.9g/d or 550/8.795 = 62.5 g/d. If they ate foliage with around 4.5 ug/g (average CN content of mature *A. rockinghamensis* foliage) they would consume 281 ug/d CN to 305 ug/d. These levels are in the range that we might expect to see chronic effects. On the other hand if they ate the highest CN mature foliage 18 ug/g, which would be 1125 to 1222 ug/d, around the range that might be expected to be lethal to dogs (Von Breadow *et al.*, 2000), so they have good reason to avoid the high CN foliage. New leaves would result in levels around 2800 to 3000 ug/d, certainly enough to produce chronic neurological effects, if not be lethal.

Within the remaining preferred species, *F. fraseri* , *F. copiosa* and *A. divaricata,* preference corresponded with high nitrogen digestibility (low levels of deterrent tannins). Nitrogen digestibility of foliage is inhibited to various extents by tannins that bind dietary proteins and digestive enzymes (Mole *et al.*, 1987; Zucker, 1983), therefore it is not surprising that *P. archeri* avoided high concentrations of this PSM. Tannin is a well documented deterrent for arboreal herbivores such as the common ringtail and common brushtail possums (Cork, 1986; Marsh *et al.*, 2003a; Marsh *et al.*, 2003b) and primates (Marks *et al.*, 1988; Oates *et al.*, 1977; Takemoto, 2003). Goudberg (1990) tentatively suggested that the rainforest ringtail possums *P. archeri*, *P. herbertensis* and *H. lemuroides* also selected food items based on tannin concentration. However, Goudberg's study compared chemical parameters of preferred food species with species that were not eaten, she did not take into account variation within tree species. My research has highlighted the need for dietary studies to focus on the relationship between variation in nutritional and defensive characteristics and herbivore preference within preferred tree species, a phenomenon that until recently has largely been ignored (Moore & Foley 2000; Lawler *et al*. 2000). In addition, this study has confirmed the role that nitrogen digestibility-reducing compounds play in dietary selectivity by *P. archeri*.

The preference for mature leaves over juvenile was consistent with the factors determining tree preference, as juvenile leaves were either higher in cyanogenic potential or lower in nitrogen digestibility. In this way *P. archeri* minimise diversity of PSMs in the diet and also minimise concentration of those chosen PSMs. It is believed that PSMs are detoxified via separate metabolic pathways and so the specialist strategy is to have a few highly developed pathways that can detoxify the few chosen PSMs (Boyle *et al.*, 1999; Boyle *et al.*, 2001; Freeland *et al.*, 1974; Sorensen *et al.*, 2005a), rather than maintaining a diversity of metabolic pathways as is hypothesized for generalist herbivores (Wiggins *et al.*, 2006a; Wiggins *et al.*, 2006b). *P. archeri* achieve this behaviourally by selecting favourite tree species, individual trees and particular leaf ages to minimise the diversity and also concentration of the selected PSMs.

Dietary preference in green ringtail possums can be explained by nutritional characteristics I measured at the intraspecific level and within preferred trees, but not at an interspecific level. The relationship between tree species preference and nutritional parameters was not related to the same factors that determined preference within species or leaf age. It is likely that interspecific selectivity is driven by a complex interaction between nutrients and PSMs, including compounds that were not measured in this study, and each non-preferred tree species may be avoided due to a different undesirable combination of nutrients and PSMs, as well as other factors such as availability of that tree species at the site and morphological features of each species such as habit. I have previously suggested that intraspecific choice may be based on the need to minimise the quantities of the few PSMs ingested, it therefore follows that interspecific choice may be based on the need to minimise the diversity of PSMs. If *P. archeri* employ a specialist foraging strategy as suggested in this thesis, I would expect them to also respond poorly to novel PSMs. Specialist folivores are predicted to focus foraging effort on a few food types (and therefore PSM types) in order to reduce the diversity of metabolites that must be detoxified, and thus number of detoxification pathways that must be maintained (Freeland *et al.*, 1974). According to this theory, specialists such as *P. archeri* would have difficulty detoxifying and excreting novel PSMs because there would be no specialised detoxification pathways for these metabolites (Boyle *et al.*, 1999; Freeland *et al.*, 1974). So it is likely that *P. archeri* rely on a few food resources, which are defended by a few similar PSMs, for which they have suitable detoxification mechanisms, although this theory is yet to be tested.

Green ringtail possums have an altitudinal limit to distribution (Winter *et al.*, 1995). Kanowski (2004) suggested this might be due to thermal limitations because he tested and discarded alternatives (outlined in (Krockenberger *et al.*, in review)). Winter (1997) also suggested thermal limits due to the historical distribution of *P. archeri* in mountain-top refugia. Krockenberger and Kanowski (in review) proposed that thermal extremes specifically limit *P. archeri*, mediated by limitations on water intake imposed by PSMs. In this thesis I have shown that PSMs do limit food intake by *P. archeri* and it is possible that limits to dietary intake caused by PSMs may be exacerbated by climate change in this species (Krockenberger *et al.*, in review). Within preferred tree species, food intake by the specialist *P. archeri* appears to be limited by concentration of PSMs (Chapter 5). Because specialist animals choose food from such a small number of suitable resources they are less likely to be able to cope with rapid habitat changes (Renaud *et al.*, 2005; Travis, 2004; Warren *et al.*, 2001). It is possible that these possums are already water limited as moisture is mostly accessed in foliage, but this food is highly defended by PSMs Chapter 5, (Jones *et al.*, in review). Thermal limits to distribution and abundance may be driven by the interaction between food intake, foliar water and extreme temperatures (Kanowski, 2001). Thus, as a specialist folivore, *P. archeri* could be expected to be more at risk of the warmer temperatures and extreme drought events predicted as part of global climate change. An increase in daytime temperatures, or persistence of high temperatures for more than a few hours per day, could have significant detrimental effects on the dietary specialist, *P. archeri*. For example, if *P. archeri* had to increase intake of *Aleurites rockinghamensis* by 10% due to an increased need for moisture (Krockenberger *et al.*, in review), then their cyanogenic glycoside intake might increase by 10%, increasing problems associated with detoxification and excretion. Novel PSMs are likely to be highly toxic to *P. archeri* and the detoxification limitation hypothesis suggests that the few detoxification pathways used by this specialist may already be saturated, leaving little leeway for changes in dietary regime.

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Appendix 1. Nutritional parameters determined through wet chemistry (wet chem) or predicted using near infrared reflectance spectroscopy (NIRS). S – season (D – dry June to November, W – wet December to May), Visits – frequency of use by *P. archeri*, DM – dry matter digestibility (%DM), Water – water content (%WM), N – Nitrogen content (mg/g), N dig – nitrogen digestibility (%DM), Dig N – digestible nitrogen concentration (mg/g), Cyanide – cyanogenic glycoside concentration (µg /g DM) when present.

Tree #	Visits	Tree species	Leaf age	S	DM	Water	N	N dig	Dig N	Cyanide	Analysis
171	0	Acronychia acidula	mature	D	57.12	69.71	23.90	49.47	11.82	-ve	wet chem
565	0	Acronychia acidula	mature	D	60.76	71.10	22.25	47.42	10.55	-ve	wet chem
566	0	Acronychia acidula	mature	D	61.47	70.74	21.19	43.86	9.29	-ve	wet chem
568	0	Acronychia acidula	mature	D	64.36	71.10	26.99	43.24	11.67	-ve	wet chem
569	0	Acronychia acidula	mature	D	62.73	71.95	25.44	41.70	10.61	-ve	wet chem
570	0	Acronychia acidula	mature	D	64.63	70.18	20.85	46.72	9.74	-ve	wet chem
14	5	Aleurites rockinghamensis	juvenile	W	55.44	71.83	19.15	-8.07	-1.54	0.63	wet chem
18	2	Aleurites rockinghamensis	juvenile	W	59.86	78.10	25.94	-11.32	-2.94	78.57	wet chem
225	2	Aleurites rockinghamensis	juvenile	W	52.35	78.96	21.25	1.82	0.39	34.46	wet chem
250	1	Aleurites rockinghamensis	juvenile	W	48.94	78.52	25.76	22.22	5.72	65.17	wet chem
260	2	Aleurites rockinghamensis	juvenile	W	54.40	75.67	23.82	3.11	0.74	55.65	wet chem
296	1	Aleurites rockinghamensis	juvenile	W	54.42	74.06	21.72	-6.00	-1.30	37.42	wet chem
18	2	Aleurites rockinghamensis	mature	D	44.22	70.49	18.34	-16.55	-2.76	8.01	NIRS
49	1	Aleurites rockinghamensis	mature	D	42.39	52.14	16.64	3.79	2.26	6.37	NIRS
57	1	Aleurites rockinghamensis	mature	D	48.89	68.20	18.51	7.51	3.17	10.29	NIRS
78	1	Aleurites rockinghamensis	mature	D	51.02	68.52	19.86	6.90	2.28	0.69	NIRS
93	2	Aleurites rockinghamensis	mature	D	41.63	73.34	20.71	-2.46	-0.76	2.09	NIRS
130	1	Aleurites rockinghamensis	mature	D	43.76	68.38	15.63	-3.17	-0.85	8.41	NIRS
142	2	Aleurites rockinghamensis	mature	D	36.74	68.10	13.17			7.31	NIRS
165	1	Aleurites rockinghamensis	mature	D	72.85	73.87	33.51	0.47	1.32	2.53	NIRS
225	2	Aleurites rockinghamensis	mature	D	44.91	63.56	18.19	-1.54	-0.87	1.81	NIRS
237	1	Aleurites rockinghamensis	mature	D	49.26	68.17	21.96	6.84	0.22	17.78	NIRS
296	1	Aleurites rockinghamensis	mature	D	42.18	69.78	16.74	6.64	2.18	4.33	NIRS
413	1	Aleurites rockinghamensis	mature	D	42.46	67.30	17.68	7.01	2.77	6.86	NIRS
436	1	Aleurites rockinghamensis	mature	D	46.49	71.34	17.69	5.76	2.26	3.88	NIRS
438	1	Aleurites rockinghamensis	mature	D	42.04	68.53	19.79	-2.80	0.72	8.26	NIRS

Appendix 1. Nutritional parameters determined through wet chemistry (wet chem) or predicted using near infrared reflectance spectroscopy (NIRS). S – season (D – dry June to November, W – wet December to May), Visits – frequency of use by *P. archeri*, DM – dry matter digestibility (%DM), Water – water content (%WM), N – Nitrogen content (mg/g), N dig – nitrogen digestibility (%DM), Dig N – digestible nitrogen concentration (mg/g), Cyanide – cyanogenic glycoside concentration (µg /g DM) when present.

Tree #	Visits	Tree species	Leaf age	S	DM	Water	N	N dig	Dig N	Cyanide	Analysis
444	1	Aleurites rockinghamensis	mature	D	47.21	69.83	22.33	-4.54	-0.80	12.81	NIRS
460	1	Aleurites rockinghamensis	mature	D	42.40	67.95	16.63	-8.16	-2.47	12.25	NIRS
471	1	Aleurites rockinghamensis	mature	D	49.99	68.48	21.53	-0.07	-0.19	3.16	NIRS
478	2	Aleurites rockinghamensis	mature	D	45.98	66.32	19.70	-6.26	-1.56	3.40	NIRS
14	5	Aleurites rockinghamensis	mature	D	40.88	79.11	16.62	-27.36	-4.55	2.48	wet chem
128	2	Aleurites rockinghamensis	mature	D	38.32	65.12	17.76	-13.67	-2.43	0.64	wet chem
211	3	Aleurites rockinghamensis	mature	D	53.16	65.88	19.17	-2.68	-0.51	3.87	wet chem
238	3	Aleurites rockinghamensis	mature	D	43.63	68.29	17.24	-5.69	-0.98	1.99	wet chem
246	4	Aleurites rockinghamensis	mature	D	41.95	69.07	19.11	-3.78	-0.72	3.98	wet chem
250	1	Aleurites rockinghamensis	mature	D	55.65	65.90	17.96	-2.72	-0.49	7.50	wet chem
298	3	Aleurites rockinghamensis	mature	D	40.83	69.59	18.78	-16.26	-3.05	6.31	wet chem
18	2	Aleurites rockinghamensis	mature	W	59.41	76.27	26.17	10.12	2.77	6.56	NIRS
49	1	Aleurites rockinghamensis	mature	W	43.49	68.29	15.74	-29.68	-5.05	6.81	NIRS
78	1	Aleurites rockinghamensis	mature	W	48.27	76.60	19.38	1.09	0.08	0.81	NIRS
80	1	Aleurites rockinghamensis	mature	W	43.78	72.93	18.18	-6.24	-1.53	0.14	NIRS
130	1	Aleurites rockinghamensis	mature	W	45.72	71.49	16.73	-8.25	-1.48	6.07	NIRS
137	1	Aleurites rockinghamensis	mature	W	50.82	67.01	18.42	11.99	3.66	7.68	NIRS
166	2	Aleurites rockinghamensis	mature	W	43.30	73.91	16.15	-0.86	0.15	4.21	NIRS
205	1	Aleurites rockinghamensis	mature	W	54.10	66.56	19.86	23.97	6.75	0.35	NIRS
260	2	Aleurites rockinghamensis	mature	W	54.20	67.14	23.97	8.78	2.02	4.04	NIRS
268	1	Aleurites rockinghamensis	mature	W	45.36	71.47	17.92	-7.55	-0.47	5.53	NIRS
287	1	Aleurites rockinghamensis	mature	W	47.95	76.56	22.48	9.99	-0.13	18.14	NIRS
420	1	Aleurites rockinghamensis	mature	W	42.52	74.40	17.25	-11.15	-2.38	8.23	NIRS
438	1	Aleurites rockinghamensis	mature	W	42.76	69.94	14.48	5.46	0.65	7.98	NIRS
458	1	Aleurites rockinghamensis	mature	W	50.18	69.49	18.74	12.77	3.08	6.45	NIRS
528	1	Aleurites rockinghamensis	mature	W	49.74	77.88	20.75	4.61	2.15	6.29	NIRS

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Tree #	Visits	Tree species	Leaf age	S	DM	Water	N	N dig	Dig N	Cyanide	Analysis
549	2	Aleurites rockinghamensis	mature	W	48.64	70.92	19.82	-1.29	0.94	5.88	NIRS
558	1	Aleurites rockinghamensis	mature	W	43.08	67.96	20.27	-12.54	-2.71	4.44	NIRS
87	4	Aleurites rockinghamensis	mature	W	42.83	71.88	20.40	0.93	0.19	0.48	wet chem
96	5	Aleurites rockinghamensis	mature	W	49.58	75.44	21.02	-4.05	-0.85	0.45	wet chem
100	17	Aleurites rockinghamensis	mature	W	39.43	71.10	19.35	10.92	2.11	0.44	wet chem
252	1	Aleurites rockinghamensis	mature	W	49.24	74.19	21.65	12.80	2.77	1.26	wet chem
298	3	Aleurites rockinghamensis	mature	W	47.68	70.40	20.00	2.57	0.51	2.39	wet chem
77	2	Aleurites rockinghamensis	mature-new	D	40.88	72.17	14.66	-26.18	-4.75	3.61	NIRS
166	2	Aleurites rockinghamensis	mature-new	D	51.31	62.02	22.24	10.02	2.65	9.33	NIRS
445	1	Aleurites rockinghamensis	mature-new	D	51.27	67.34	20.08	-7.71	-1.31	4.55	NIRS
87	4	Aleurites rockinghamensis	mature-new	D	47.41	68.90	20.70	-2.62	-0.54	4.80	wet chem
96	5	Aleurites rockinghamensis	mature-new	D	41.51	72.62	18.74	-13.70	-2.57	9.29	wet chem
100	17	Aleurites rockinghamensis	mature-new	D	51.57	64.01	17.66	-11.37	-2.01	1.23	wet chem
252	1	Aleurites rockinghamensis	mature-new	D	44.99		17.48	-0.80	-0.14	10.60	wet chem
269	1	Aleurites rockinghamensis	mature-new	D	47.18	72.43	21.91	-6.63	-1.45	7.34	wet chem
270	1	Aleurites rockinghamensis	mature-new	D	46.26	70.28	19.55	-5.24	-1.02	1.89	wet chem
271	1	Aleurites rockinghamensis	mature-new	D	39.91	66.95	17.54	-13.14	-2.30	3.18	wet chem
142	2	Aleurites rockinghamensis	mature-new	W	39.28	68.76	13.27	-5.30	-2.88	18.43	NIRS
237	1	Aleurites rockinghamensis	mature-new	W	49.79	69.26	22.26	2.76	0.63	42.67	NIRS
413	1	Aleurites rockinghamensis	mature-new	W	46.68	72.31	23.15	20.02	3.99	26.59	NIRS
444	1	Aleurites rockinghamensis	mature-new	W	55.48	75.87	25.67	23.57	5.63	21.12	NIRS
445	1	Aleurites rockinghamensis	mature-new	W	49.40	72.26	20.29	23.21	5.33	12.95	NIRS
460	1	Aleurites rockinghamensis	mature-new	W	51.26	67.13	18.34	0.03	0.00	21.13	NIRS
471	1	Aleurites rockinghamensis	mature-new	W	47.87	73.78	21.31	-10.30	-1.92	10.18	NIRS
478	2	Aleurites rockinghamensis	mature-new	W	50.91	69.88	21.25	5.51	0.69	8.83	NIRS
77	2	Aleurites rockinghamensis	mature-new	W	53.15	76.84	23.32	3.71	1.19	3.71	NIRS

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Tree #	Visits	Tree species	Leaf age	S	DM	Water	N	N dig	Dig N	Cyanide	Analysis
93	2	Aleurites rockinghamensis	mature-new	W	54.73	74.10	25.63	20.30	3.84	10.21	NIRS
225	2	Aleurites rockinghamensis	mature-new	W	49.53	75.73	18.15	-4.21	-0.89	4.45	NIRS
296	1	Aleurites rockinghamensis	mature-new	W	46.13	74.06	18.79	2.16	0.15	2.38	NIRS
436	1	Aleurites rockinghamensis	mature-new	W	48.86	73.95	19.98	-4.18	-1.02	14.11	NIRS
512	2	Aleurites rockinghamensis	mature-new	W	49.78	77.92	15.74	1.48	1.00	2.64	NIRS
128	2	Aleurites rockinghamensis	mature-new	W	40.38	68.70	15.41	-18.08	-2.79	5.54	wet chem
250	1	Aleurites rockinghamensis	mature-new	W	47.14	70.07	23.22	-23.62	-5.48	15.30	wet chem
211	3	Aleurites rockinghamensis	mature-new	W	42.44		18.45	-7.63	-1.41	5.11	wet chem
238	3	Aleurites rockinghamensis	mature-new	W	49.81	74.04	20.50	4.67	0.96	12.14	wet chem
246	4	Aleurites rockinghamensis	mature-new	W	49.43	75.81	23.12	11.15	2.58	15.81	wet chem
269	1	Aleurites rockinghamensis	mature-new	W	51.71	77.28	21.51	3.85	0.83	2.02	wet chem
270	1	Aleurites rockinghamensis	mature-new	W	49.59	75.75	20.19	-3.41	-0.69	0.88	wet chem
271	1	Aleurites rockinghamensis	mature-new	W	50.55	73.32	20.95	-6.76	-1.42	2.41	wet chem
165	1	Aleurites rockinghamensis	mature-new	W						50.76	wet chem
515	1	Aleurites rockinghamensis	mature-old	W	47.91	66.05	18.50	9.54	2.42	2.33	NIRS
53	1	Arytera divaricata	mature	D	32.14	44.37	17.80	-10.34	-3.72	-ve	NIRS
56	1	Arytera divaricata	mature	D	34.51	47.19	19.62	5.27	0.10	-ve	NIRS
141	1	Arytera divaricata	mature	D	31.68	49.39	19.55	8.54	1.52	-ve	NIRS
162	1	Arytera divaricata	mature	D	35.91	53.08	23.22	-1.72	-1.10	-ve	NIRS
180	1	Arytera divaricata	mature	D		56.06		-2.35	-0.88	-ve	NIRS
208	1	Arytera divaricata	mature	D	35.25	50.90	22.80	4.07	0.15	-ve	NIRS
216	1	Arytera divaricata	mature	D	30.07	50.09	18.32	-9.36	-2.60	-ve	NIRS
272	0	Arytera divaricata	mature	D	35.25	52.83	20.98	11.04	1.26	-ve	NIRS
304	2	Arytera divaricata	mature	D	32.67	48.07	20.57	3.93	-0.14	-ve	NIRS
401	1	Arytera divaricata	mature	D		54.82				-ve	NIRS
480	1	Arytera divaricata	mature	D	41.26	53.17	18.11	9.66	1.62	-ve	NIRS

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Tree #	Visits	Tree species	Leaf age	S	DM	Water	N	N dig	Dig N	Cyanide	Analysis
482	1	Arytera divaricata	mature	D		50.58	16.63	-2.99	-2.36	-ve	NIRS
90	3	Arytera divaricata	mature	D	33.73	45.84	18.81	-2.89	-0.54	-ve	wet chem
98	2	Arytera divaricata	mature	D	32.59	47.50	18.12	-5.87	-1.06	-ve	wet chem
167	2	Arytera divaricata	mature	D	29.97	56.82	18.15	1.87	0.34	-ve	wet chem
232	11	Arytera divaricata	mature	D	35.15	47.42	19.27	10.69	2.06	-ve	wet chem
273	3	Arytera divaricata	mature	D	34.61	48.08	18.55	5.67	1.05	-ve	wet chem
306	2	Arytera divaricata	mature	D	30.15	52.41	16.60	5.54	0.92	-ve	wet chem
441	2	Arytera divaricata	mature	D	31.98	56.78	18.72	6.56	1.23	-ve	wet chem
457	2	Arytera divaricata	mature	D	32.61	51.87	19.95	8.27	1.65	-ve	wet chem
56	1	Arytera divaricata	mature	W		54.53		-5.37	-1.45	-ve	NIRS
141	1	Arytera divaricata	mature	W		58.31		24.51	4.96	-ve	NIRS
162	1	Arytera divaricata	mature	W	37.91	53.36	21.51	-2.43	-0.60	-ve	NIRS
167	2	Arytera divaricata	mature	W		53.69		-9.61	-2.73	-ve	NIRS
180	1	Arytera divaricata	mature	W	30.51	54.44	17.43	3.97	-0.69	-ve	NIRS
189	1	Arytera divaricata	mature	W	35.05	57.90	22.29	17.02	3.02	-ve	NIRS
208	1	Arytera divaricata	mature	W	36.07	55.26	21.44	8.29	1.03	-ve	NIRS
216	1	Arytera divaricata	mature	W		53.78	19.27	-13.26	-4.57	-ve	NIRS
232	11	Arytera divaricata	mature	W		57.80	16.99	2.58	0.50	-ve	NIRS
274	1	Arytera divaricata	mature	W	31.74	51.23	17.89	0.03	-0.76	-ve	NIRS
295	1	Arytera divaricata	mature	W		47.31	16.19	-9.77	-3.69	-ve	NIRS
304	2	Arytera divaricata	mature	W	35.97	51.62	21.99	2.30	-0.78	-ve	NIRS
306	2	Arytera divaricata	mature	W		51.49		16.19	1.91	-ve	NIRS
470	1	Arytera divaricata	mature	W	31.02	47.82	18.68	-11.81	-3.79	-ve	NIRS
480	1	Arytera divaricata	mature	W	36.08	62.38	22.15	15.71	3.06	-ve	NIRS
482	1	Arytera divaricata	mature	W	30.52	51.82	17.22	16.06	2.30	-ve	NIRS
526	2	Arytera divaricata	mature	W		60.65				-ve	NIRS

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Tree #	Visits	Tree species	Leaf age	S	DM	Water	N	N dig	Dig N	Cyanide	Analysis
533	2	Arytera divaricata	mature	W	35.02	53.29	20.58	6.68	0.02	-ve	NIRS
557	3	Arytera divaricata	mature	W	30.28	50.87	18.45	-2.75	-1.97	-ve	NIRS
53	1	Arytera divaricata	mature-new	W		66.49				-ve	NIRS
85	1	Arytera divaricata	mature-new	W		54.72		-5.80	-1.03	-ve	NIRS
90	3	Arytera divaricata	mature-new	W		59.25		34.07	7.62	-ve	NIRS
98	2	Arytera divaricata	mature-new	W		58.37	17.76	31.22	6.32	-ve	NIRS
272	0	Arytera divaricata	mature-new	W		59.30				-ve	NIRS
273	3	Arytera divaricata	mature-new	W		58.90		36.79	6.98	-ve	NIRS
401	1	Arytera divaricata	mature-new	W		58.52		26.38	4.71	-ve	NIRS
441	2	Arytera divaricata	mature-new	W		54.40	17.26	-10.77	-3.23	-ve	NIRS
493	1	Arytera divaricata	mature-new	W		59.70				-ve	NIRS
546	1	Arytera divaricata	mature-old	W	34.98	55.10	21.29	1.17	-0.37	-ve	NIRS
218	0	Castanospermum australe	mature	D	42.39	59.70	23.45	36.01	8.45	-ve	wet chem
577	0	Castanospermum australe	mature	D	41.66	61.32	21.17	49.24	10.42	-ve	wet chem
592	0	Castanospermum australe	mature	D	40.00	65.60	21.61	47.11	10.18	-ve	wet chem
594	0	Castanospermum australe	mature	D	41.11	63.85	21.37	53.20	11.37	-ve	wet chem
RX900	0	Castanospermum australe	mature	D	48.81	58.02	26.39	43.88	11.58	-ve	wet chem
263	0	Castanospora alphandii	mature	D	38.74	54.75	16.99	13.39	2.28	-ve	wet chem
564	0	Castanospora alphandii	mature	D	43.17	52.11	15.66	26.76	4.19	-ve	wet chem
580	0	Castanospora alphandii	mature	D	41.18	51.89	18.09	21.02	3.80	-ve	wet chem
591	0	Castanospora alphandii	mature	D	40.34	59.15	19.36	33.96	6.58	-ve	wet chem
593	0	Castanospora alphandii	mature	D	41.73	54.35	17.79	16.43	2.92	-ve	wet chem
155	0	Dendrocnide photinophylla	mature	D	74.53	77.54	30.64	-45.55	-13.96	-ve	wet chem
423	0	Dendrocnide photinophylla	mature	D	73.85	83.13	31.70	-59.93	-19.00	-ve	wet chem
424	0	Dendrocnide photinophylla	mature	D	76.58	82.82	36.05	-53.21	-19.18	-ve	wet chem
487	0	Dendrocnide photinophylla	mature	D	75.56	84.73	31.65	-80.99	-25.63	-ve	wet chem

Appendix 1. Nutritional parameters determined through wet chemistry (wet chem) or predicted using near infrared reflectance spectroscopy (NIRS). S – season (D – dry June to November, W – wet December to May), Visits – frequency of use by *P. archeri*, DM – dry matter digestibility (%DM), Water – water content (%WM), N – Nitrogen content (mg/g), N dig – nitrogen digestibility (%DM), Dig N – digestible nitrogen concentration (mg/g), Cyanide – cyanogenic glycoside concentration (µg /g DM) when present.

Tree #	Visits	Tree species	Leaf age	S	DM	Water	N	N dig	Dig N	Cyanide	Analysis
574	1	Dendrocnide photinophylla	mature	D	73.20	84.03	31.92	-76.52	-24.42	-ve	wet chem
447	1	Ficus copiosa	juvenile	W	67.05	71.17	18.28	47.75	12.19	-ve	NIRS
140	2	Ficus copiosa	mature	D	61.43	61.97	12.50	30.64	4.34	-ve	wet chem
286	5	Ficus copiosa	mature	D	58.63	69.35	13.97	37.14	5.19	-ve	wet chem
307	1	Ficus copiosa	mature	W	64.14	73.21	15.21	25.69	4.17	-ve	NIRS
416	0	Ficus copiosa	mature	W	62.55	75.43	20.19	28.43	5.12	-ve	NIRS
447	1	Ficus copiosa	mature	W	62.70	70.44	18.54	31.10	5.44	-ve	NIRS
473	1	Ficus copiosa	mature	W	66.62	67.49	16.59	40.83	8.75	-ve	NIRS
547	1	Ficus copiosa	mature	W	67.15	66.96	19.83	29.66	5.95	-ve	NIRS
140	2	Ficus copiosa	mature	W	61.67	69.73	12.50	15.37	1.92	-ve	wet chem
229	2	Ficus copiosa	mature	W	65.81	75.27	16.59	29.77	4.94	-ve	wet chem
286	5	Ficus copiosa	mature	W	61.42	73.04	13.97	37.34	5.11	-ve	wet chem
421	1	Ficus copiosa	mature	W	65.57	68.71	17.24	23.30	4.02	-ve	wet chem
532	2	Ficus copiosa	mature	W	63.16	68.35	15.27	27.79	4.24	-ve	wet chem
554	4	Ficus copiosa	mature	W	64.92	75.81	17.04	31.96	5.45	-ve	wet chem
410	1	Ficus copiosa	mature-new	W	65.66	75.28	15.71	27.73	6.19	-ve	NIRS
446	1	Ficus copiosa	mature-new	W	64.63	75.88	20.03	35.34	6.93	-ve	NIRS
489	0	Ficus fraseri	juvenile	W	79.83	75.37	23.86		8.01	-ve	NIRS
88	2	Ficus fraseri	mature	D	67.00	60.08	17.82	23.29	3.85	-ve	NIRS
175	2	Ficus fraseri	mature	D	74.41	67.45	20.02	24.20	7.41	-ve	NIRS
198	2	Ficus fraseri	mature	D	69.71	66.62				-ve	NIRS
239	1	Ficus fraseri	mature	D	70.67	64.07	20.08	16.00	1.69	-ve	NIRS
469	1	Ficus fraseri	mature	D	65.73	64.51				-ve	NIRS
489	0	Ficus fraseri	mature	D	67.84	63.09	20.05	23.50	2.25	-ve	NIRS
50	3	Ficus fraseri	mature	D	69.67	62.77	22.65	44.95	10.18	-ve	wet chem
92	5	Ficus fraseri	mature	D	69.33	61.72	15.05	50.62	7.62	-ve	wet chem

Appendix 1. Nutritional parameters determined through wet chemistry (wet chem) or predicted using near infrared reflectance spectroscopy (NIRS). S – season (D – dry June to November, W – wet December to May), Visits – frequency of use by *P. archeri*, DM – dry matter digestibility (%DM), Water – water content (%WM), N – Nitrogen content (mg/g), N dig – nitrogen digestibility (%DM), Dig N – digestible nitrogen concentration (mg/g), Cyanide – cyanogenic glycoside concentration (µg /g DM) when present.

Tree #	Visits	Tree species	Leaf age	S	DM	Water	N	N dig	Dig N	Cyanide	Analysis
95	8	Ficus fraseri	mature	D	69.27	60.94	18.83	51.05	9.61	-ve	wet chem
132	1	Ficus fraseri	mature	D	73.65	62.39	22.42	40.16	9.00	-ve	wet chem
145	4	Ficus fraseri	mature	D	76.60	65.57	23.00	48.04	11.05	-ve	wet chem
170	1	Ficus fraseri	mature	D	68.98	65.31	19.12	19.94	3.81	-ve	wet chem
173	1	Ficus fraseri	mature	D	75.52	68.05	25.20	35.81	9.03	-ve	wet chem
176	4	Ficus fraseri	mature	D	69.83	66.47	18.87	27.25	5.14	-ve	wet chem
195	4	Ficus fraseri	mature	D	73.20	65.98	23.45	29.60	6.94	-ve	wet chem
196	1	Ficus fraseri	mature	D	72.06	67.26	24.56	30.30	7.44	-ve	wet chem
212	1	Ficus fraseri	mature	D	73.01	66.62	24.97	40.67	10.16	-ve	wet chem
228	1	Ficus fraseri	mature	D	74.25	70.09	27.10	38.24	10.37	-ve	wet chem
290	4	Ficus fraseri	mature	D	71.98	63.08	23.42	36.39	8.53	-ve	wet chem
68	1	Ficus fraseri	mature	W	71.81	75.28	26.54	9.45	1.08	-ve	NIRS
88	2	Ficus fraseri	mature	W	76.54	71.68	23.53	8.94	1.92	-ve	NIRS
173	1	Ficus fraseri	mature	W	71.40	74.84	24.44	17.78	3.73	-ve	NIRS
175	2	Ficus fraseri	mature	W	76.63	69.97	22.74	12.27	3.09	-ve	NIRS
198	2	Ficus fraseri	mature	W	71.14	70.82	26.13	11.45	1.35	-ve	NIRS
255	1	Ficus fraseri	mature	W	72.11	68.26	22.17	9.03	1.13	-ve	NIRS
435	1	Ficus fraseri	mature	W	77.28	66.51	20.97	4.13	0.47	-ve	NIRS
452	3	Ficus fraseri	mature	W	75.43	72.31	24.68	14.10	3.81	-ve	NIRS
469	1	Ficus fraseri	mature	W	75.36	72.50	24.39	16.46	3.95	-ve	NIRS
519	4	Ficus fraseri	mature	W	78.17	72.52	22.34	14.06	1.67	-ve	NIRS
531	3	Ficus fraseri	mature	W	73.65	70.52	24.75	16.12	2.87	-ve	NIRS
538	1	Ficus fraseri	mature	W	75.58	66.31	20.89	7.68	1.18	-ve	NIRS
560	1	Ficus fraseri	mature	W	73.45	69.78	22.32	17.30	3.42	-ve	NIRS
50	3	Ficus fraseri	mature	W	80.00	75.54	24.66	47.05	11.60	-ve	wet chem
92	5	Ficus fraseri	mature	W	73.10	71.44	19.65	30.55	6.00	-ve	wet chem

Appendix 1. Nutritional parameters determined through wet chemistry (wet chem) or predicted using near infrared reflectance spectroscopy (NIRS). S – season (D – dry June to November, W – wet December to May), Visits – frequency of use by *P. archeri*, DM – dry matter digestibility (%DM), Water – water content (%WM), N – Nitrogen content (mg/g), N dig – nitrogen digestibility (%DM), Dig N – digestible nitrogen concentration (mg/g), Cyanide – cyanogenic glycoside concentration (µg /g DM) when present.

Tree #	Visits	Tree species	Leaf age	S	DM	Water	N	N dig	Dig N	Cyanide	Analysis
95	8	Ficus fraseri	mature	W	73.88	71.33	25.09	34.75	8.72	-ve	wet chem
145	4	Ficus fraseri	mature	W	71.27	73.97	22.42	21.77	4.88	-ve	wet chem
170	1	Ficus fraseri	mature	W	77.76	72.37	25.32	23.04	5.83	-ve	wet chem
173	1	Ficus fraseri	mature	W	73.24	74.84	25.50	23.30	5.94	-ve	wet chem
195	4	Ficus fraseri	mature	W	76.65	73.87	24.96	34.48	8.61	-ve	wet chem
196	1	Ficus fraseri	mature	W	74.61	72.60	21.76	31.41	6.83	-ve	wet chem
212	1	Ficus fraseri	mature	W	69.39	70.14	24.48	21.83	5.35	-ve	wet chem
290	4	Ficus fraseri	mature	W	74.62	68.56	27.14	37.86	10.28	-ve	wet chem
435	1	Ficus fraseri	mature-new	D	80.51	80.77	31.09	-6.69	-3.01	-ve	NIRS
239	1	Ficus fraseri	mature-new	W	72.60	78.64	23.54	14.69	2.03	-ve	NIRS
406	5	Ficus fraseri	mature-new	W	70.72	72.76	21.80	14.92	2.60	-ve	NIRS
489	0	Ficus fraseri	mature-new	W	72.75	73.02	23.75	19.07	3.95	-ve	NIRS
92	5	Ficus fraseri	juvenile	D	82.14	79.22	37.58	36.66	13.78	-ve	wet chem
132	1	Ficus fraseri	juvenile	W	74.02	72.20	21.31	28.47	6.07	-ve	wet chem
160	4	Ficus fraseri	juvenile	W	73.55	71.51	20.41	26.85	5.48	-ve	wet chem
176	4	Ficus fraseri	juvenile	W	74.20	73.25	21.71	21.56	4.68	-ve	wet chem
195	4	Ficus fraseri	juvenile	W	76.91	67.45	24.96	26.06	6.16	-ve	wet chem
196	1	Ficus fraseri	juvenile	W	77.77	78.93	21.76	27.38	6.25	-ve	wet chem
228	1	Ficus fraseri	juvenile	W	74.96	72.55	26.33	25.04	6.59	-ve	wet chem
406	5	Ficus fraseri	juvenile	W	74.73	72.76	20.13	29.43	5.92	-ve	wet chem
469	1	Ficus fraseri	juvenile	W	79.18	72.50	24.30	24.92	6.05	-ve	wet chem
241	0	Ficus septica	mature	D	72.51	72.87	18.13	-38.99	-7.07	-ve	wet chem
422	0	Ficus septica	mature	D	76.19	80.42	23.60	-33.94	-8.01	-ve	wet chem
550	0	Ficus septica	mature	D	73.76	75.14	18.83	-44.23	-8.33	-ve	wet chem
567	0	Ficus septica	mature	D	69.82	70.92	16.23	-31.95	-5.19	-ve	wet chem
571	0	Ficus septica	mature	D	71.26	72.85	16.30	-45.75	-7.46	-ve	wet chem

Appendix 1. Nutritional parameters determined through wet chemistry (wet chem) or predicted using near infrared reflectance spectroscopy (NIRS). S – season (D – dry June to November, W – wet December to May), Visits – frequency of use by *P. archeri*, DM – dry matter digestibility (%DM), Water – water content (%WM), N – Nitrogen content (mg/g), N dig – nitrogen digestibility (%DM), Dig N – digestible nitrogen concentration (mg/g), Cyanide – cyanogenic glycoside concentration (µg /g DM) when present.

Tree #	Visits	Tree species	Leaf age	S	DM	Water	N	N dig	Dig N	Cyanide	Analysis
573	0	Ficus septica	mature	D	77.14	75.87	25.27	-23.28	-5.88	-ve	wet chem
89	0	Mallotus philippensis	mature	D	33.96	48.93	18.99	1.35	0.26	-ve	wet chem
521	0	Mallotus philippensis	mature	D	31.88	49.18	16.85	2.03	0.34	-ve	wet chem
572	0	Mallotus philippensis	mature	D	33.75	48.06	19.80	7.27	1.44	-ve	wet chem
575	0	Mallotus philippensis	mature	D	32.12	47.19	16.88	7.92	1.34	-ve	wet chem
578	0	Mallotus philippensis	mature	D	31.49	48.34	15.02	3.56	0.53	-ve	wet chem
83	0	Melia azedarach	mature	D	70.22	67.94	27.78	-5.86	-1.63	-ve	wet chem
227	0	Melia azedarach	mature	D	69.61	74.41	24.62	-41.69	-10.26	-ve	wet chem
483	0	Melia azedarach	mature	D	73.14	74.27	23.74	-31.03	-7.37	-ve	wet chem
576	0	Melia azedarach	mature	D	72.14	72.73	26.03	-32.95	-8.58	-ve	wet chem
579	0	Melia azedarach	mature	D	75.49	70.00	25.61	-8.70	-3.13	-ve	wet chem

HOME RANGE OF A RAINFOREST MARSUPIAL FOLIVORE, THE GREEN RINGTAIL POSSUM, *PSEUDOCHIROPS ARCHERI.*

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Short running title: Home range of the green ringtail possum

ABSTRACT

The green ringtail possum, *Pseudochirops archeri* (Pseudocheiridae), is a small (1-1.5 kg), specialist marsupial folivore endemic to Australia's wet tropical rainforests. The bulk of its geographic range is extensively altered and fragmented by anthropogenic disturbance, so understanding spatial requirements of *P. archeri* forms an important part of conservation or management measures for the species. We used radiolocation to estimate home range of 13 individual *P. archeri* (9 females, 4 males) in remnant patches of complex mesophyll vine thicket (Webb and Tracey Type 5b) on the Atherton tablelands. We calculated home range using harmonic mean, minimum convex polygon, kernel and cluster techniques. At least 30 independent positions were required to adequately define individual home ranges. There were no differences in range size of male and females, but there was a positive relationship between possum mass and some measures of range size. The mean range size depended on the calculation technique, but was smaller than for other Pseudocheirids, varying from 0.188 ± 0.038 ha (95% Cluster) to 0.222 ± 0.043 ha (100% MCP). This was only 1/12th to 1/270th that predicted for a herbivorous mammal of the same size, and corresponds to an area of forest approximately 50m in diameter. *P. archeri* disproportionately depends on mature foliage low in plant defenses from a few individual trees of four tree species at the study sites. Because home range is determined by the interplay between resource requirements and the productivity and spatial heterogeneity of resources at the site, these very small ranges are dependent on productivity of a few individual trees low in defensive compounds. Further determination of the importance of plant defences on resource productivity and distribution and abundance of *P. archeri* would benefit from testing the effect of distribution of poorly defended chemotypes of preferred forage trees on home range.

Keywords: resource requirements, marsupial folivore, home range, plant secondary metabolites, Pseudocheiridae

INTRODUCTION

The home range of an organism is an important ecological measure of the habitat area required to meet the resource requirements needs of an individual, or group of individuals (Harris *et al.* 1990; Haskell *et al.* 2002) and is routinely used as a tool to determine areas required for viable populations in conservation planning (Belovsky 1987). Many factors can influence the size and shape of the home range, including population density, body size, diet, social organisation and food availability (Harestad & Bunnell 1979; Haskell *et al.* 2002; Lindstedt *et al.* 1986; McNab 1963; Taitt & Krebs 1981). Despite relatively little empirical support, there is a general expectation that home range should broadly reflect energetic requirements and animals should forage over as small a range as possible while meeting their resource requirements, though some authors suggest that this should merely define the lower limit to range size (Kelt & Van Vuren 2001). In general the determinants of home range size and variability are poorly understood (Reiss 1988). A number of authors have attempted to define allometric predictions of home range in mammals based on body mass and trophic level (Harestad & Bunnell 1979; Lindstedt *et al.* 1986; McNab 1963; Swihart & Slade 1989), however, log-linear allometric predictive schemes for home range are less successful than relating metabolic and life-history characteristics to mass (Kelt & Van Vuren 2001). This is largely because home range size must reflect not only the individual's resource requirements, but the productivity and spatial heterogeneity of those specific requirements from the habitat (Haskell *et al.* 2002; Mitchell & Powell 2004). In the case of specialist folivores, definition of suitable resources and habitat productivity can be deceptively difficult. For example, specialist folivores not only require foliage from particular species, but their intake is limited by plant secondary metabolites (PSMs) that vary within and between individual trees within those species (Lawler *et al.* 1998; Scrivener *et al.* 2004), so resource availability and productivity within a particular habitat can be very difficult to define.

The green ringtail possum, *Pseudochirops archeri (Marsupialia: Pseudocheiridae)*, is a small (1-1.5 kg) specialist folivore (Jones *et al.* 2006), one of four species of Pseudocheirids endemic to Australia's wet tropical rainforests (Winter & Goudberg 1995). It is restricted to altitudes above around 300m and declines in abundance above 900m (Winter & Goudberg 1995). The bulk of its geographic range lies on the Atherton and Evelyn tablelands, which are extensively altered and fragmented by anthropogenic disturbance (Laurance 1990). The rainforest Pseudocheirids are negatively impacted by fragmentation of forests. Some species, especially the lemuroid ringtail (*Hemibelideus lemuroides*), are absent from smaller fragments, although *P.*

archeri is the most robust of them, and can still be found in relatively small forest remnants (Laurance 1990). *P. archeri* is also one of a suite of vertebrates endemic to Australia's wet tropical rainforests that are predicted to be adversely impacted by the effects of climate change (Kanowski 2004; Krockenberger & Kanowski in review; Williams *et al.* 2003). Bioclimatic models predict a decline in the area of climatically suitable habitat, so understanding spatial requirements of *P. archeri* forms an important part of any conservation or management measures for the species. *P. archeri* does not use dens, so availability of suitable den sites is unlikely to affect their habitat requirements, as it can in other arboreal marsupials (Lindenmayer *et al.* 1997). However, as a specialist folivore, *P. archeri* requires sufficient foliage resources from suitable species and individual trees within its home range to support its nutritional requirements. Food preferences of *P. archeri* are strongly influenced by the concentration of PSMs, including cyanogenic glycosides and tannins, in the foliage of a few species of canopy trees (Jones & Krockenberger in review). Thus, productivity of their habitat is dependent on the productivity of relatively few preferred individual trees (Jones & Krockenberger in review; Jones *et al.* 2006), which is difficult to quantify at a broad scale.

This study quantifies the home range of the green ringtail possum, *P. archeri*, in comparison with expectations of the range size required by a herbivorous mammal of their mass, and considers the implications (that comparison has) for understanding the productivity of their habitat.

METHODS

Study sites

The study was conducted in two remnant fragments of complex notophyll vine forest (Type 5b, (Webb 1959); Regional ecosystem 7.8.3 (Sattler & Williams 1999)) at about 750 m altitude on the Atherton Tablelands of North-eastern Australia. These small (10 ha at 17°16′S, 145°34′E and 33 ha at 17°18′S, 145°30′E) irregularly shaped forest fragments are on a basalt parent material and surrounded by agricultural, grazing land. The forest is characterised by an uneven canopy (25–45m), a large number of deciduous and semi-evergreen trees, a tendency for heavy leaf-fall during times of moisture stress, and a dense shrub and scrambling vine layer (Webb 1959). Temperatures range from an average daily minimum of 9.9ºC in August to an average daily maximum of 29.7ºC in December and rainfall is strongly seasonal, with 85% of the average annual rainfall of 1413 mm falling in the 6 months between December and May (http://www.bom.gov.au/climate/averages/tables/cw_031002.shtml).

Animal Handling and Radio-tracking

Forty green ringtail possums were captured as a part of this study, although only thirteen adults (9 female, 4 male) were able to be located on enough occasions over a long enough period for calculation of home range (see below). Possums were initially located opportunistically by spotlighting along the forest edges, then captured using a tranquiliser dart-gun (Black Wolf CO_2

powered dart-gun and 0.5 ml Minidarts, Tranquil Arms, Victoria, containing 30 mg Zoletil; Zolazepam and Tiletamine, Virbac Australia Pty Ltd, NSW) and fitted with collar-mounted two-stage radio-transmitters (SirTrack Pty Ltd, Havelock North, New Zealand) as described in Jones *et al*. (2006). After a recovery period of 2 h they were released at the point of capture. Individuals were located over various lengths of time ranging from two to twenty-three months using a hand-held Yagi antenna and receiver (Regal 1000, Titley Electronics Pty Ltd, NSW). They were located no more than once daily during daylight hours (as they are nocturnal and roost during the day), and up to three times nightly. Because home range analysis relies on the assumption that successive location points are independent (Harris *et al.* 1990), most night-time locations were at least 2 hours apart, which is ample time for possums to traverse the full span (~40 m) of their home range (pers. obs). On the few occasions when possums were located more frequently the second point was discarded if the possum remained in the same tree.

When possums were located in an individual tree, by sight or by triangulation, the tree was permanently marked with a numbered metal tag, and its position was later determined by measuring the angle and distance from the centre of its trunk at breast height to one of a set of fixed reference points at the forest’s edge or to a nearby previously-marked tree if no reference point could be seen. The positions of reference points were determined using a Garmin 12 CX handheld GPS and GBR 21 differential radio beacon (Garmin International Inc., USA).

Analysis

To provide the maximum comparability of this study with others, we used four different measures of home range; Minimum Convex Polygons (MCP), Harmonic Mean Contours (HM; (Dixon & Chapman 1980)), Kernel Contours (Worton 1989), and Cluster Polygons (Kenward *et al.* 2001). Each measure was calculated using the software Ranges VI (Kenward *et al.* 2003). Even using the same measure of home range, varying parameters of the algorithm can produce substantial variation in results of the calculation. Unfortunately there are no clear guidelines or objective techniques for selecting specific parameters (Kenward *et al.* 2003), so we experimented with various combinations in each of the four methods and chose the ones that we felt most accurately represented the nature of our animals' home ranges. The final user-determined settings we selected for each method were: MCP – harmonic mean peel centre; HM – 100 x 100 matrix; Kernels – smoothing multiplier = 1, 40 matrix cells; and Clusters – convex polygons, single inclusive polygon. Tracking resolution was set to 3m and all other settings were left on Ranges VI default.

Unfortunately, there is little consistency in the level of home-range use reported in studies of wildlife, so we have chosen to report multiple levels of "core" area to maximise future comparability. We adopted Kenward's (2001) definition of home range as "an area repeatedly traversed by an animal". Thus, in order to exclude unwanted outliers, we used Ranges VI see whether there was a "core" of activity (for all methods other than MCP). We calculated total home range areas using all the location points for each animal at all 5% isopleth intervals between 20% and 100% (or 99% for harmonic mean and kernel contours). We then calculated the proportional increase in home range area with each successive 5% core increase and defined cores where there was little or no incremental increase in range area (Johnson 1991). Here we report these cores (55% and 85%), as well as the 70% isopleth (because it is commonly used as a measure of core range) and the 95% isopleth, which is commonly used to describe the total range size.

Another important consideration in home range analysis is ensuring that enough location points were obtained to accurately represent the home range of each individual. We estimated the minimum number of required points by plotting home range size versus number of locations, randomly selecting 10 replicates of *n* points from the total number of points available for each animal where n was every multiple of 5 up to the total number of locations, and seeking the value of n after which additional locations resulted in a minimal increase in the 85% core (because this minimises the effect of outliers) of each home range measure (Harris *et al.* 1990). Furthermore, because many of the animals' ranges were bordered on one or more sides by cleared areas, all range measures reported were adjusted to exclude cleared areas.

RESULTS

There were core areas of activity at 55% and 85% isopleths of home range, defined by little or no incremental increase in range size. Accurately defining home range in *P. archeri* required around 30 independent locations, depending on the algorithm used. The 85% isopleth of range size increased minimally after around 25 points using Kernel contours, 30 points using MCP and HM, and 45 points using Cluster polygons. Thus, only animals with 30 or more location points were included in further analysis of home range size.

Thirteen *P. archeri* (9 female, 4 male) each were located on 30 or more occasions, over an average of 11 months (range= 2 - 23). Their mass ranged from 960 g to 1530 g, with an average of 1150 ± 47 g (SEM, n = 13). Individuals had high site fidelity and there was no indication that their ranges shifted seasonally. A total of 666 independent locations of these 13 possums were obtained, in 58% of which the animal was sighted. The total home range of *P. archeri* ranges from 0.188 ± 0.038 ha (95% Cluster) to 0.222 ± 0.043 ha (100% MCP)(Table 1). This corresponds to a circular area of forest approximately 50 m in diameter. Around half of an individual's activity was concentrated within an area between 0.047 ± 0.009 ha (55% HM) and 0.090 ± 0.021 ha (55% Cluster) (Table 1), corresponding to a circle between 24 and 34 m in diameter. Individual ranges overlapped extensively, although we have not calculated and reported overlap because we did not have all, or even groups of contiguous individuals within an area radio-collared. Thus, any overlap we measured cannot be considered a true representation of the degree of overlap between contiguous individuals.

There tended to be a positive relationship between body mass and total range size, although this was only statistically significant for the 95% HM and Kernel measures (MCP $F_{1,11} = 3.808$, $P = 0.077$, $r^2 = 0.257$; 95% HM $F_{1,11} = 5.23$, $P = 0.043$, $r^2 = 0.322$; 95% KR $F_{1,11} = 5.231$, $P = 0.043$, $r^2 = 0.322$; 95%

CL $F_{1,11} = 4.538$, $P = 0.057$, $r^2 = 0.292$). There was no difference in home range size between males and females (ANCOVA with mass as a covariate for the measures of total range size and Independent samples t-tests for the cores) in any of the four measures at any core.

DISCUSSION

Green ringtail possums, *P. archeri*, had very small home ranges at the study sites. The total area used by individuals (95% KL: 0.189 ha) was equivalent to a circular area of forest approximately 50m in diameter, and 55% of their activity was concentrated within an area about 24-34m in diameter. This is 1/7th to 1/13th the size of comparable (95% KL) ranges reported for another Pseudocheirid, the similar-sized greater glider, *Petauroides volans*. Home range (95% KL) of *P. volans* is quite variable across their geographic range, from between 0.7 - 2.0 ha (females) and 1.7 - 2.5 ha (males) (Comport *et al.* 1996; Kavanagh & Wheeler 2004; Pope *et al.* 2004). *P. volans* males consistently range over larger areas than females (Comport *et al.* 1996; Kavanagh & Wheeler 2004; Pope *et al.* 2004), in contrast with *P. archeri* where there were no differences in male and female ranges. Perhaps it is not surprising that greater gliders, one of the most selective of the eucalypt specialist marsupial folivores (Hume 1999) range over larger areas than do green ringtail possums. As gliders, their ability to cover a large range is enhanced and their eucalypt forest habitat is less dense than the rainforest habitat of *P. archeri*, so they may have to cover greater areas to include enough suitable individual forage trees. The ranges of *P. archeri* in this study are also smaller than reported in the only other study measuring home range in rainforest Pseudocheirids. Wilson (2000) reported home range of a single *P. archeri* as between 0.6 ha (MCP and 95% Cluster) and 1.04 ha (95% Kernel). Her estimates of range in the similar sized lemuroid ringtail, *Hemibelideus lemuroides*, (0.46 ha, 95% Cluster to 1.13 ha, 95% Kernel; n = 8) were similar to that of the single *P. archeri* in her study and slightly smaller than the estimates from the single, slightly larger Herbert river ringtail, *Pseudochirulus herbertensis*, in her study (1.23 ha, 95% Cluster to 2.81 ha, 95% Kernel). It is difficult to make strong comparisons between the studies because Wilson (2000) used a different software package for her calculations and the specific form of the algorithm as well as default parameters used by a package can have large effects on the output (Gallerani Lawson & Rodgers 1997; Kenward *et al.* 2003). Methodological differences aside, the home range estimates she reports for *P. archeri* are all substantially larger than the means determined in this study (t-test comparison of mean with single value, $t_{12} = 9.02$, 100 % MCP; 16.55, 95% HM; 20.76, 95% Kernel; 10.84, 95% Cluster; all $P<<0.001$), so it is tempting to postulate that this difference may reflect differences in habitat quality between the studies. However, even though they are larger than estimates of range in this study, Wilson's (2000) estimates of home range in rainforest ringtail possums are also relatively small compared with that of the eucalypt forest greater glider, so the high diversity and density of trees in rainforest may contribute to a higher density of resources and consequent small range for marsupial folivores.

The home range of *P. archeri* is also small compared with the expected range for a mammalian herbivore of their size. Allometric relationships between home range and mass that have been derived for herbivorous mammals vary in their predictions by an order of magnitude (eg. (Harestad & Bunnell 1979; Haskell *et al.* 2002; McNab 1963; Swihart & Slade 1989)). For a 1kg herbivore, these relationships predict a range size between 2.6 and 60 ha, which is 12 to nearly 270 times larger than the simplest (and generally largest) MCP measure of home range of *P. archeri*. If the lower constraint to home range represents the smallest area of habitat which meets the resource requirements of the individual (Kelt & Van Vuren 2001), then a very small range such as described here indicates either very low requirements or high average productivity of suitable resources within the habitat. The energetic requirements of *P. archeri* are close (104%) to that predicted for a marsupial of their mass (Krockenberger & Kanowski in review). The energy requirements of a typical marsupial are about 30% lower than those of a typical placental mammal (Dawson & Hulbert 1970), however it is unlikely that this accounts for the very large difference between the predicted and observed home ranges. The alternative explanation is that this habitat is highly productive of suitable resources for this species, which is borne out by the occurrence, in one of the study fragments, of the highest measured density of *P. archeri* across the Atherton Tablelands (Kanowski 1999) and supported by observations of high densities of *P. archeri* in other small habitat fragments (Laurance 1990). Plant defense theory suggests that plants should defend foliage less when the resources they contain are abundant and easy to replace (Coley *et al.* 1985). This may be related to the positive effect of high-nutrient basalt substrate on densities of marsupial folivores across their range (Kanowski *et al.* 2001) and may partially explain the small ranges of *P. archeri* at these sites on basalt substrates. However, the higher estimate of range size made by Wilson (2000) was also at a site on basalt substrate, so it is likely that geological parent material does not explain all variation in range size. As a specialist folivore, *P. archeri* is dependent on the productivity of relatively few individual trees from four preferred species (4% of plant species at site)) (Jones *et al.* 2006) that express low levels of particular defensive plant secondary metabolites (PSMs) (Jones & Krockenberger in review). A combination of the spatial distribution of deterrent PSM chemotypes and size of individual trees influences the spatial patterns of foraging, distribution and abundance in koalas at the level of a site (Moore & Foley 2005). Similarly, the critical resources for *P. archeri* are individual trees low in specific deterrent PSMs, and we would predict that their home ranges within and between forest types should vary to reflect the spatial arrangement, density and productivity of those individual trees from preferred forage species that express low levels of PSMs, particularly cyanogenic glycosides and condensed tannins (Jones & Krockenberger in review).

There was a trend toward larger possums ranging over a larger area, although statistically significant only at the 95% harmonic mean and kernel estimates and not in any of the core measures or cluster or MCP estimates. Larger possums would have higher energy requirements (Krockenberger & Kanowski in review), so this trend toward larger range may indicate that larger possums required a larger area to support those requirements. However, if that were the case, we might reasonably expect those differences to be evident in all the measures of range. Even where statistically significant, variation associated with mass explained only a modest proportion (32%) of overall variation in range size, so we should interpret this result with some caution. Both kernel and harmonic mean measures of home range are susceptible to expanding home range predictions into unoccupied areas around foci of activity (Kenward *et al.* 2001), whereas MCP and cluster contours do not, so the relationship we found between harmonic mean and kernel contour estimates of total range could have been affected by changes in the distribution of activity across the range. The result could indicate that larger animals tended to visit trees around the periphery of their range slightly more often, although it should be noted that 55, 70 and 85% cores of activity were not increased. Alternatively, large possums require more resources and hence a greater home range than do smaller possums, but that this effect is less important than other factors influencing resource distribution within the forest, such as distribution of suitable forage trees.

One problem with comparing home ranges between studies is the lack of consistency in the home range measure used. Each technique has advantages and drawbacks. The MCP is the most commonly used technique, and is therefore most useful for comparability with previous studies (Harris *et al.* 1990; Kenward *et al.* 2001). However, although it is robust against lack of independence between successive location points, it can lead to inaccurate results because range shape and size are heavily influenced by outlying location points, and can expand to include large areas of unused habitat (Harris *et al.* 1990; Kenward *et al.* 2001). In addition, the MCP gives no indication of intensity of range use (Harris *et al.* 1990). In contrast, harmonic mean, kernel and cluster methods of home range analysis all allow intensity of range use to be examined, but are sensitive to autocorrelation in the data. Furthermore, different computer programs use different algorithms and can produce substantially different results from the same dataset (Gallerani Lawson & Rodgers 1997; Harris *et al.* 1990; Kenward *et al.* 2001). Even using the same program, HM and kernel contours can give significantly different results with only relatively small changes in particular parameters - smoothing parameters in the case of kernels, and tracking resolution relative to matrix size in HM (Kenward *et al.* 2001). Furthermore, when there is more than one location point at a particular set of co-ordinates (e.g. from repeated visits to a favourite tree), HM and kernel contours expand outwards as each additional point is added, potentially including areas that are not visited (Harris *et al.* 1990; Kenward *et al.* 2001). MCP and cluster analysis do not share this tendency (Kenward *et al.* 2001). Most studies neglect to mention which parameter settings they used (partly because the settings can be difficult to determine in some software), so it is difficult to ensure that results are comparable across studies.

Aside from methodological concerns, it is clear that green ringtail possums, *P. archeri*, have very small home ranges, despite their highly specialised dietary requirements. The small home range size of green ringtail possums means that even very small fragments of forest can provide suitable habitat, so conservation efforts for this species should not abandon small fragments. However, without further understanding of demographic and genetic processes within and between fragmented populations of *P. archeri,* it is unclear whether populations of in small fragments would be viable over the longer term. Because home range is determined by the interplay between resource requirements and the productivity and spatial heterogeneity of those specific resources at the site (Mitchell & Powell 2004), these very small ranges are dependent on the productivity of few individual trees low in defensive compounds. Further determination of the importance of plant defences on resource productivity and hence distribution and abundance of *P. archeri* would benefit from testing the effect of the distribution of poorly defended chemotypes of preferred forage trees on home range. The little comparative information we have (Wilson 2000) suggests that there is variation in home range size across the geographic range of *P. archeri*, although it is not clear whether that variation is linked in a systematic fashion with any aspect of habitat type. Estimates of the home range of *P. archeri*, as well as those of the other species of rainforest ringtails, across the range of forest types, parent material and altitudes these species inhabit will assist us to understand the influence of habitat characteristics in general, and more specifically plant defences, on habitat productivity, and folivore distribution and abundance across the landscape.

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Table 1: Home range size (ha) of green ringtail possums, *Pseudocheirops archeri*, in fragments of complex mesophyll vine forest on the Atherton tablelands of Australia’s Wet tropics region (means ± SEM).

Home range estimator	Isopleth	Male (n = 4)	Female (n = 9)	Mean (n = 13)
Minimum Convex Polygon	100	0.203 ± 0.050	0.231 ± 0.059	0.222 ± 0.043
Harmonic mean	55	0.032 ± 0.006	0.053 ± 0.012	0.047 ± 0.009
	70	0.056 ± 0.011	0.089 ± 0.022	0.078 ± 0.016
	85	0.110 ± 0.031	0.141 ± 0.036	0.132 ± 0.026
	95	0.184 ± 0.052	0.209 ± 0.051	0.201 ± 0.038
Kernel Contours	55	0.027 ± 0.013	0.067 ± 0.020	0.055 ± 0.015
	70	0.061 ± 0.028	0.103 ± 0.030	0.090 ± 0.022
	85	0.102 ± 0.039	0.167 ± 0.046	0.147 ± 0.034
	95	0.144 ± 0.049	0.209 ± 0.056	0.189 ± 0.041
Cluster Polygons	55	0.065 ± 0.019	0.100 ± 0.030	0.090 ± 0.021
	70	0.091 ± 0.031	0.126 ± 0.040	0.115 ± 0.029
	85	0.106 ± 0.030	0.162 ± 0.042	0.145 ± 0.031
	95	0.154 ± 0.039	0.203 ± 0.052	0.188 ± 0.038