

The effect of plant secondary metabolites on the interplay between the internal and external environments of marsupial folivores

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Abstract Most woody plants contain a diverse array of plant secondary metabolites (PSMs) that deter vertebrate herbivores. However, mammalian folivores have evolved a complex of physiological and behavioural strategies to counter these compounds, leading to the development of an “evolutionary arms race”. Marsupial folivores are ideal models to investigate the role of PSMs in the interaction between the external foraging environment and the digestive physiology of mammalian herbivores, as we have a very strong understanding of the diversity and modes of action of PSMs in *Eucalyptus*, as well as the mechanisms by which animals overcome the effects of these compounds. Studies of marsupial folivores have benefited from the facts that: these herbivores subsist on relatively poor quality diets; they include feeding types from specialist species such as the koala, to generalists; and life history factors such as maternal investment in reproduction can be measured more easily than in eutherians. Here, we describe patterns of spatial variation in the types and distributions of plant secondary metabolites in Australian forests and discuss how this variation influences foraging behaviour, habitat selection and life history strategies in arboreal, folivorous marsupials. We also provide a summary of our

understanding of the mechanisms by which marsupials detect and regulate their intake of toxic compounds. While our examples are drawn largely from studies of the interaction between marsupials and *Eucalyptus*, this knowledge is applicable to advancing our understanding of interactions in plant–mammal systems more broadly. We also identify and discuss key areas that should be the focus of future research.

Keywords Detoxification · *Eucalyptus* · Herbivory · Plant secondary metabolites

Introduction

Vertebrate herbivores are exposed to wide variation in concentrations and structural diversity of plant secondary metabolites (PSMs). Browsers in particular encounter secondary compounds in nearly all the foods that they eat (Dearing et al. 2005; Foley et al. 1999). Early studies of vertebrate browsers sought to correlate concentrations of secondary metabolites with diet choice, and although these led to significant improvements in analytical techniques (Hagerman et al. 1992; Mole and Waterman 1987), few clear trends emerged to enable diet selection to be linked to plant chemistry. Part of the difficulty was in understanding the diversity of actions of secondary metabolites and where to look to understand the effects of PSMs on the availability of primary nutrients (Foley and McArthur 1994).

Studies of leaf-eating marsupials have been important in testing many important questions concerning interactions between vertebrates and PSMs. These include the limitations to detoxification capacity (Freeland and Janzen 1974), the biological bases of varied diets (Levin 1976; Westoby 1978) and the effect of PSMs on the demography of

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free-ranging populations (Bryant and Kuropat 1980). This is partly because there is a continuum from specialist (e.g. the koala, *Phascolarctos cinereus*) to generalist (e.g. the common brushtail possum, *Trichosurus vulpecula*) species, including species that can be maintained readily in captivity as well as easily studied in the wild (Moore et al. 2004a; Shipley et al. 2009). Secondly, there is a strong understanding of the nutrition and digestive physiology of these species (Hume 1999). Finally, the marsupial life history, with the young being born after a short gestation and completing their development in the mother's pouch, allows reproductive success and maternal investment to be measured more easily than it could be in eutherian herbivores (Cork and Dove 1989; Krockenberger et al. 1998). In eutherian mammals the flow of nutrients from mother to young is mediated by the mother's placental tissue whereas the suckling of the young controls the flow of nutrients in marsupials. Thus, folivorous marsupials, with their narrow dietary niche, and poor quality diets, appear to be at the edge of their nutritional boundaries, and so provide an ideal model system to investigate the role of PSMs on the demography and population dynamics of mammalian herbivores.

In this paper, we focus on understanding the mechanisms by which marsupial folivores are able to regulate their intake of potentially toxic diets while still meeting their nutritional demands when faced with great variation in PSM distributions in the external environment. We also discuss the effects of PSMs on habitat selection and life history strategies in marsupial folivores. The marsupial species on which we concentrate are members of three families; the koala (5–14 kg) is the sole member of the family Phascolarctidae. In the Pseudocheiridae, the greater glider (*Petauroides volans*; 0.7–1.7 kg) eats *Eucalyptus* foliage exclusively whereas the common ringtail possum (*Pseudocheirus peregrinus*; 0.7–1.1 kg) eats a wider range of tree foliage. The common brushtail possum (1.5–4.5 kg), a member of the Phalangeridae, eats mainly tree foliage in some habitats, including up to 80–90% *Eucalyptus* (DeGabriel et al. 2009a; Scrivener et al. 2004), but may also include ground-layer vegetation, fruit and occasional insect material elsewhere. Other browsing members of the Pseudocheiridae and Phalangeridae occur in upland rainforests in northeast Australia where eucalypts are absent or are not known to include significant amounts of *Eucalyptus* foliage in their diets.

The natural diet provides some insight into the importance of PSMs in the ecology of these species. The koala and common brushtail possum feed primarily from the eucalypt subgenus *Symphyomyrtus* whereas the common ringtail possum and greater glider feed most often from the subgenus *Eucalyptus* (Moore et al. 2004a). The chemistry of these two subgenera differs in a number of important

respects. Although both contain similar mono- and sesquiterpenes, the symphyomyrtles also contain a diverse group of terpene–phloroglucinol adducts called formylated phloroglucinol compounds (FPCs; Eschler et al. 2000). These are potent feeding deterrents and koalas and brushtail possums have higher thresholds for these compounds compared to ringtail possums which are deterred by relatively low concentrations. In contrast, brushtail possums are strongly affected by tannins whereas ringtail possums are not (Marsh et al. 2003a). This opens the possibility that variable sensitivity to different classes of PSMs enables some degree of niche partitioning within the same forest and may potentially influence biodiversity at broader scales. Detailed studies of the greater glider, together with a better understanding of tannin chemistry in these large subgenera of eucalypts would help to evaluate this hypothesis.

The internal environment

How do animals avoid being poisoned?

Vertebrate herbivores ingest a very wide range of plant secondary metabolites (PSMs) from many different classes. Although most of these are not acutely toxic, they still represent a metabolic load that must be biotransformed and excreted (Foley et al. 1995). The time required for this process may be inconsistent with opportunities to ingest new food items and that in turn depends on how alternatives are distributed spatially in the foraging environment. Since almost all food items that are encountered by browsers contain PSMs, total avoidance is not a realistic option, but ingestion of PSMs brings consequences including toxicity, the loss of nutrients and other metabolites, and the opportunity costs of time that could have been spent foraging for other foods. These costs dictate that animals must evolve mechanisms that allow them to regulate their intake of secondary metabolites so as not to exceed some threshold intake. Such a regulatory mechanism must couple internal signals that reflect the current metabolic state with an ability to alter appetite and feeding behaviours. Early syntheses of plant–mammal interactions did not give sufficient attention to the mechanisms used to regulate intake, but doing so allows better connection with concepts of homeostasis and a wider framework for understanding the effects of PSMs on animal function (Foley et al. 1995). Several recent studies have demonstrated that at least in captivity, mammals respond to variable concentrations of PSMs in the diet by reducing meal size. Torregrossa and Dearing (2009) reviewed these studies and emphasised the uniform response between herbivorous marsupials, woodrats and laboratory rodents.

Studies in folivorous marsupials have highlighted three possible feedback mechanisms that serve to limit meal size. The first is nausea and emesis as part of a conditioned food or flavour aversion, the second is a non-conditioned aversion associated with bitter or “hot” compounds and the third is a limit to the rate of detoxification of ingested PSMs. Given our limited knowledge of these processes, we should be cautious in considering that these are discrete and non-overlapping mechanisms. For example, if ingested compounds are not detoxified rapidly enough, then it is possible that nausea could develop and a conditioned aversion might arise. In spite of these uncertainties, we can be certain that there is not a separate feedback mechanism for every compound or class of compounds. Animals must be able to rely on general feedback processes to recognise the effects of a huge range of secondary compound structures that are encountered and modify their feeding behaviour accordingly.

What are the characteristics of an ideal regulatory system? Most importantly, it needs to provide feedback rapidly and be able to be coupled with behavioural changes to modify feeding during a single meal. Although this does not preclude the possibility of aversions developing over a longer period of time, regulation at the time-scale of a single meal must be paramount. This suggests that the signal would most likely be transmitted via the nervous system, although blood-borne signals are also possible. Foley et al. (1995) suggested that blood pH could change following the creation of metabolic acid loads through the detoxification of PSMs. However, Edwards et al. (2009) showed that when the metabolic acid load from detoxification of orcinol was countered with exogenous bicarbonate, food intake was not restored to levels observed on a basal diet. This suggests that other toxin-related signals are controlling food intake even when significant acid loads are created through the process of detoxification.

Conditioned aversions

The importance of conditioned aversions in diet selection by herbivores has been a major research theme over the past three decades (Bryant et al. 1991). Studies in marsupials provide important ecological examples, together with detailed physiological studies to underpin the behavioural data. Conditioned aversions are well known in the behavioural sciences (Garcia 1989) and it has long been recognised that nausea and emesis are powerful conditioning agents influencing feeding. Thus, the nauseous malaise that might follow from the ingestion of secondary compounds could be associated with some cue related to the taste or smell (or even some other cue such as location), thereby allowing the consequences of

ingesting that food to be recognised in the future (Provenza 1996). Amongst *Eucalyptus* folivores, there is strong evidence that at least some secondary compounds initiate nausea, leading to reductions in food intake (Lawler et al. 1998; Stapley et al. 2000), and that these effects can be associated with volatile signals in the plants. Nausea is mediated by a complex network of neurotransmitters, which act ultimately in the brain and a variety of drugs have been developed to target different parts of this network (Andrews and Horn 2006).

Ondansetron is a specific 5HT₃ serotonin receptor antagonist that reduces vomiting in humans given therapeutic drugs such as cisplatin. Lawler et al. (1998) showed that when ondansetron was administered to common ringtail and brushtail possums, orally or by injection, both species consumed significantly more jensenone, an FPC (isolated from *Eucalyptus jensenii* but distributed more widely in eucalypts) that strongly deters feeding in marsupial folivores. However, Torregrossa (personal communication) cautioned that ondansetron could have other effects on gut motility, and indeed, 5HT₃ receptors are involved in gut motor activity (Sanger 2008). However, in a second series of experiments using a different drug that targeted an entirely separate pathway of the nausea and emetic response, we observed strikingly similar results (Fig. 1). Vofopitant is a tachykinin NK1 receptor antagonist and a member of a class of drugs that have been developed to better control nausea in humans (Andrews and Rudd 2004). Common brushtail possums fed jensenone in conjunction with vofopitant ate significantly more than animals fed the basal diet with jensenone whereas vofopitant itself had no significant effect on intake.

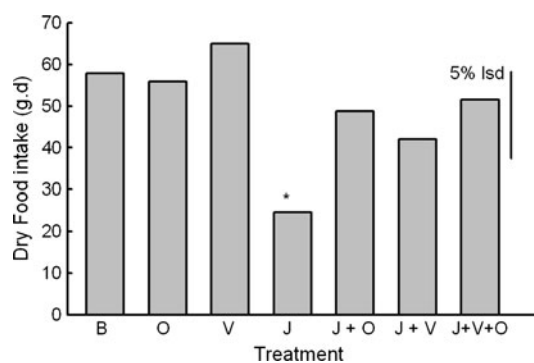


Fig. 1 The mean amount eaten by common brushtail possums of a basal diet and the basal diet with supplements in two Latin Square experiments. Treatment abbreviations as follows: B (basal diet), O [Ondansetron (0.007% dry matter)], V [basal + vofopitant (0.007% dry matter)], J [jensenone (0.4% dry matter)]. $n = 13$ for all diets except J + O + V where $n = 7$. The bar at the right indicates the 5% least significant difference between treatments. Treatments for which DMI is significantly different from that of the basal diet are marked with an asterisk

Although few drugs can be said to be completely selective, the similarity of responses of marsupials to these two drugs which are directed at separate mechanisms, suggests that inhibiting neuroreceptors associated with nausea and vomiting results in marsupial folivores eating greater amounts of PSMs. Lawler et al. (1998) hypothesised that ingestion of jensenone leads to the release of serotonin from enterochromaffin cells in the stomach and small intestine and that this binds to serotonin receptors in the vagus nerve. Ondansetron blocks the binding of serotonin to 5HT₃ receptors and so the resulting nausea is reduced and animals eat more. Most animals do not vomit and of course we can never know if an animal feels nausea the same way that we do. Nonetheless, the similarity of the response to two different drugs that attenuate nausea and vomiting in humans and other animal models strongly suggests a similar response in the marsupials.

Provenza and Villalba (Provenza et al. 1994, 2000; Villalba and Provenza 2000a, b) have shown that aversions can be conditioned by pairing the emetic substance LiCl with a wide range of taste and odour stimuli, such as onion and cinnamon flavours, but a better example of the ecological potential of conditioned aversions can be seen in *Eucalyptus*. There is a strong positive phenotypic correlation between foliar concentrations of the major monoterpenes (e.g. 1,8-cineole) and FPCs in eucalypts (Moore et al. 2004b). Thus, the smell and taste of monoterpenes can provide cues to marsupials of the concentration of non-volatile FPC compounds that cause nausea. The terpenes themselves can condition the aversion and Lawler et al. (1999) showed that animals could learn this association over a period of several days. Interestingly, genetic linkage analysis has identified a number of places where the QTL for monoterpenes and FPC compounds are indistinguishable on linkage groups of *Eucalyptus nitens* even though there is no apparent biosynthetic link between the compounds (Henery et al. 2007). Irrespective of the cause of this linkage (e.g. a shared promoter or transcription factor), it provides the means for conditioned aversions to be an important part of diet selection in mammals relying on *Eucalyptus* foliage. In summary, the body of evidence suggests that marsupials are able to monitor internal signals of nausea that arise during a single meal and modify the amount of food that they eat in response to these signals. Importantly, they can learn the association between the smell and taste of food and the underlying concentration of these nausea-inducing compounds. This allows a much more precise response to variations in the concentrations of toxins in their diets and allows avoidance of individual plants that present a risk of intoxication which will result in lower rates of feeding and a greater risk of death.

Non-conditioned aversions

Animals can be deterred from eating foods by many factors. For example, strongly bitter foods and those that contain compounds that are perceived as hot can limit feeding, but importantly these do not appear to cause long-term conditioned aversions. The best characterised ecological example involves capsaicin (Tewksbury and Nabhan 2001), as differences in the capsaicin receptor explain the relatively higher tolerance of birds for plants such as peppers (*Capsicum* spp.) compared to mammals (Jordt and Julius 2002), a fact which in turn has profound consequences for the ecology of these plants (Tewksbury et al. 2008). Amongst marsupials, common brushtail possums limit their consumption of bitter compounds such as salicin in response to effects in the oral cavity rather than via any post-ingestional effects such as nausea (Pass and Foley 2000). Thus, the consumption of a single meal is limited but the aversion is neither long-lasting nor classically conditioned by the taste of the food.

Limitations to detoxification

Detoxification is assumed to be a costly process for herbivores, involving energy and protein for inducing and maintaining enzymes and specific conjugates for excretion (Foley et al. 1999). The detoxification limitation hypothesis also suggests that animals eating diverse diets should choose a diet that avoids saturating particular detoxification pathways (Marsh et al. 2006a). This notion has been highly influential in early hypotheses of plant–herbivore relationships (Freeland and Janzen 1974), but in spite of its pivotal place in the history of the discipline, it has been difficult to test because of the lack of knowledge of the fundamental parameters of pharmacokinetics of most secondary metabolites. Studies in marsupials initiated a pharmacological perspective that has since provided a fertile area for new syntheses in both terrestrial and marine systems (Forbey and Foley 2009; Forbey et al. 2009; Sotka et al. 2009). A pharmacological perspective explicitly addresses details of absorption, distribution, metabolism and excretion (ADME) of PSMs (Sorensen et al. 2006) and doing so requires an ability to sample blood and urine over a prolonged period.

Recent studies in marsupials and woodrats (*Neotoma* spp.) have shown that limitations to detoxification can in fact act as signals to stop feeding. For example, McLean et al. (2007) characterised the pharmacokinetics of 1,8-cineole in common brushtail possums and found that they ate until the cineole consumed saturated the pre-systemic metabolism, which led to a rapid rise in the concentration of cineole in the blood and a cessation of the meal. Marsh et al. (2005) showed that when additional glycine was

provided orally to common brushtail possums fed benzoic acid, they were able to increase the rate of conjugation and consequential excretion of urinary benzoyl glycine (hippuric acid) and so eat more than control animals. However, we still do not know if accumulation of the parent compound and/or metabolites has other effects via nausea, or whether animals can monitor the disposal of these compounds via other means.

If the rate of detoxification is limited, then animals could diversify their diets to include items with different types of secondary metabolites. This has been one of the major predictions of the detoxification limitation hypothesis (Marsh et al. 2006a). Marsh et al. (2006b) tested this idea with common brushtail possums fed a range of different PSMs mixed into a basal diet of fruits and cereals. Consistent with predictions, when animals were offered two diets containing PSMs that were excreted via non-competing pathways, they ate more than when the PSMs in the two diets were excreted via the same pathways. Although the diets eaten by wild herbivores are more complex and the choices may be less stark than in these experiments conducted with simple diets, they are the only direct evidence that implicates detoxification as a cause of diet mixing.

The evidence above suggests that there are multiple physiological mechanisms that animals can use to detect the consequences of ingested PSMs and modify their feeding behaviour. These mechanisms should allow animals to forage efficiently because toxicosis, which may lead to a reduction in the rate of feeding, or the need to seek alternative foods, is reduced. We cannot overemphasise the importance of learning in mediating these behaviours. The alternative feeding options are set by the external environment and in particular, the frequency and spatial arrangement of foods and other factors such as competitors and predators. The challenge is to describe the external environment in a way that is relevant to animal perceptions and can link to variations in reproductive success. Below we highlight recent progress in doing this, again concentrating on studies with marsupial herbivores.

The external environment

High levels of intra-specific variety in plant nutrition and chemical defence commonly occur in the external environment encountered by marsupial folivores. PSMs play a fundamental role in interactions between marsupials and the plants they eat, by restricting the transfer of energy and nutrients between trophic levels from net producers (plants) to net consumers (animals) in the ecosystem. Thus, PSMs have the potential to strongly influence community structure and biodiversity of plants and animals, both at

fine scales and across landscapes (Ganzhorn 1992; Iason et al. 2005). Some PSMs in eucalypts, such as FPCs and terpenes, act as toxins or anti-feedants which reduce the number of trees that are available for animals to eat, whereas others, such as tannins, reduce the availability of nutrients (Foley et al. 1999; Iason 2005). Thus, there is a strong theoretical framework to envisage a role for PSMs in the “bottom-up” regulation of marsupial populations (Agrawal 2004; Wheat et al. 2007).

Studies with captive animals have demonstrated clear effects of PSMs on diet selection and digestion (Foley et al. 1999); however, we lack examples that demonstrate these processes amongst wild marsupials. Many have recognised the potential for PSMs to influence the distribution and abundance of vertebrate herbivores; however, most have attempted to correlate broad-scale measures of soil fertility, plant nutrients (particularly N) and PSMs to the biomass of animals (Cork 1992; Ganzhorn 1992; Johnson et al. 2005; McKey et al. 1978; Oates et al. 1990). Braithwaite (1996) showed that the patchy distributions of arboreal marsupials in south-eastern Australian forests tend to be concentrated on better quality soils, and food quality is often cited as the most important factor predicting the occurrence of marsupial folivores (Braithwaite et al. 1984; Cork and Catling 1996; Jones et al. 1994; Pausas et al. 1995; Turner and Kelly 1981). Cork and Catling (1996) proposed that food quality sets a threshold to suitability of habitat to arboreal marsupial folivores, but above that threshold, environmental factors, including forest structure, influence the abundance and persistence of populations. Nevertheless, while there are clearly links between soil fertility, foliar quality and populations of folivores, a common element of previous studies is that they have focused simply on herbivore abundance, without considering how PSMs influence the demographic mechanisms that regulate density, such as the reproductive rate. In order to achieve this, a necessary step is to understand how the types and distributions of PSMs vary in the external environment.

Spatial variation in food quality

Although they are usually dominated by trees, and indeed often by trees of a single genus or species, spatial and temporal heterogeneity are universal features of the external environment of most folivorous marsupials. Because folivores move through their environment in both space and time, the extent and structure of this heterogeneity determines how and when they encounter different foods and it may also limit or enhance their scope for avoiding intoxication by PSMs and assembling a balanced, nutritious diet.

The strongest influence on environmental heterogeneity is variation in plant assemblages, because even closely

related tree species can differ greatly in their suitability as food. For example, two co-occurring and closely related species, *Eucalyptus ovata* and *Eucalyptus strzeleckii* differ in the type and amount of FPCs they contain, and as a consequence, captive koalas willingly eat *E. ovata* but not *E. strzeleckii* (Moore et al. 2005). Species composition matters in very diverse plant assemblages, too; for example green ringtail possums (*Pseudocheirops archerii*) inhabiting tropical rainforest in North Queensland direct the majority of their feeding towards a very small minority of available tree species (4 out of ≥ 94 species), suggesting that this species' interaction with its external environment may be shaped by the distribution of these few species (Jones et al. 2006). Many factors can structure plant assemblages, from climate, local microclimate and soil conditions, to fire and browsing history (Williams and Woinarski 1997), but in many cases, particularly for generalist herbivores, plant diversity may be equally as important as plant species identity. Wiggins et al. (2006) demonstrated that captive brushtail possums ate mixed diets and fed more efficiently in mixed patches of *E. globulus* and *E. tenuiramis* than in pure patches, and that efficiency improved most when the scale of species heterogeneity was small.

The nutritional environment can be heterogenous even in monospecific stands of forest, with heterogeneity occurring amongst trees within a stand, or at scales from hundreds of metres to thousands of kilometres. The scale at which heterogeneity in foliar quality occurs is dependent upon the degree of spatial structure, or autocorrelation, amongst tree attributes and also upon the processes responsible for such structure. Spatial autocorrelation in plant defence traits can be generated by endogenous processes such as isolation by distance (Wright 1943), whereby limited gene dispersal (either limited seed or pollen dispersal or both) causes genetically similar plants to occur in close spatial proximity to one another. In plant species with heritable plant defences, 'patches' of chemically similar plants will result. This process underlies the spatial dependence that occurs in terpene and FPC concentrations in natural populations of *Eucalyptus*. At a site in southeastern Australia, Andrew et al. (2007) detected spatial autocorrelation at distances of up to 60 m and also detected strong local spatial autocorrelation for a large proportion of trees in a population of *E. melliodora*. Genetic differences and resulting chemical phenotypic differences also occur between plant populations, as a result both of genetic isolation and differing selective pressures.

Such larger scale genetic differences combined with exogenous processes, such as edaphic, hydrological or climatic shifts, can produce large differences in the average level of defence and nutritional quality of foliage from

different populations of the same plant species. In *Eucalyptus microcorys*, mean concentrations of the FPC sideroxylonal ranged from 14–31 mg g⁻¹ across 42 sites in eastern Australia and concentrations of the monoterpene, cineole, total nitrogen and tannins also varied greatly between sites. Differences in levels of plant defence were associated with differences in the degree of cold that sites were subjected to and to the availability of resources for plant growth (Moore et al. 2004c). Similarly, populations of *E. globulus* in Tasmania differ in their level of FPC defences and in their palatability to brushtail possums (O'Reilly-Wapstra et al. 2004).

Consequences of spatial variability in the external environment for marsupial folivores

Herbivores move across a number of spatial scales. When foraging, marsupial folivores might restrict their movement to the canopy of a single tree while feeding, or they might move between trees within their home range, or make larger scale dispersal movements. All of this variation interacts with the spatial distribution of food resources, be they trees of different species or trees of a single species with favourable foliar quality, to influence the rates at which folivores encounter suitable feeding trees, and consequently, the suitability of a patch as habitat. In environments in which food resources are rare and dispersed (Fig. 2), less mobile folivores may not easily be able to include a sufficient number of food trees in their home range. For example, greater gliders can readily glide distances of up to 100 m between trees (McKay 1983) and access distant food sources with relatively little energy expenditure. The similarly sized ringtail possum would generally need to make the same journey through the forest canopy, assuming it was continuous. In habitats with less continuous canopy cover, brushtail possums would generally make the journey along the ground; however, such movements incur the cost of a greatly increased risk of predation. DeGabriel (2008) found very high rates of predation by carpet pythons (*Morelia spilota*) on common brushtail possums in open ironbark woodland in northern Australia. The incidence of predation was significantly biased towards males, across all age groups, which they concluded was the result of the larger home ranges of males compared to females, presumably in response to increased movement patterns of males seeking mating opportunities. Consequently, males invested earlier in reproduction than did populations in southern Australia (Clinchy et al. 2004). Thus, the spatial distribution of trees has the potential to exert strong effects on the social structure and mating system of folivore populations. Similarly, Martin and Martin (2007) demonstrated that the spatial arrangement of silver wattle trees (*Acacia*

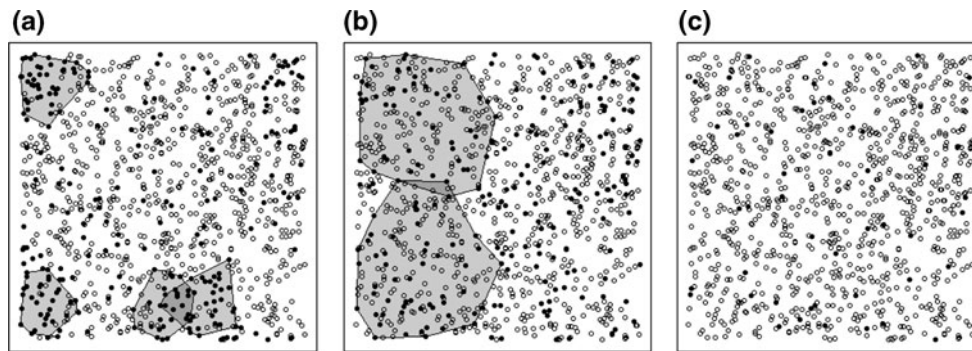


Fig. 2 Illustration of the effects of patchiness and food tree density on individual animal distributions. *Open circles* represent trees of an unsuitable species or with foliar chemistry unsuitable for folivores; *closed circles* represent suitable food trees. In **a**, feeding trees are spatially clumped, allowing small folivore home ranges (*shaded*

polygons). In **b**, the number and proportion of feeding trees here is identical to **(a)**, but the trees are more spatially dispersed, forcing folivores to use larger home ranges. **c** Potential feeding trees are too sparse and too dispersed for folivores with limited mobility to include a sufficient feeding resource in a single home range

dealbata), an important diet component of the bobuck (*Trichosurus cunninghamii*), relative to den sites maintained a monogamous mating system in a forest population of these possums.

Spatial dependence in foliar characteristics, or patchiness, can increase the density of food trees locally and increase the suitability of patches as habitat for folivores. However, a further complication of the external environment is that marsupials do not choose trees solely on the basis of their foliar chemistry and nutrient status. At various times, tree choice might be driven by the presence of tree hollows for denning, shade or shelter from the sun, weather or predators, distance from other resources and proximity to or distance from other marsupials.

From a physiological perspective, variation in the spatial distributions of PSMs across broad geographic scales may result in the evolution of within-species differences in the responses of herbivores to these compounds. For example, populations may become more specialised on local plants, as in the case of woodrats (McEachern et al. 2006), or they may be better able to cope with higher concentrations of PSMs in their diets compared to their conspecifics (Mangione et al. 2000). Amongst the marsupials, DeGabriel et al. (2009b) demonstrated differences in the ability of spatially and genetically separated populations of brushtail possums to tolerate PSMs, with possums from southern Australia showing a more marked decrease in food intake from diets containing terpenes, tannins, a bitter compounds (salicin) and FPCs, than were possums from northern Australia. In contrast, common brushtail possums from eucalypt woodland in northern Australia and the rainforest specialist coppery brushtail possums showed very similar responses to PSMs, despite originating from habitats with very different plant assemblages and PSM profiles. This appears to be the result of local adaptation, which may be mediated by a combination of genetic and

behavioural mechanisms, and should prompt caution in generalising about the effects of PSMs on wild populations.

Availability of nutrients

A prominent nutritional hypothesis is that the availability of protein is limiting for populations of mammalian browsers (Robbins 1993), because animals require adequate protein for maintenance, growth, reproduction and lactation (Robbins 1993). However, previous attempts to correlate the abundance of folivores with mean concentrations of foliar N (Braithwaite et al. 1984; Chapman et al. 2004; Pettoirelli et al. 2001; White 1993) have failed to take into account the negative effects of tannins, which are ubiquitous in browse, on the amount of N that can be digested (Hagerman et al. 1992; Robbins et al. 1987). *Eucalyptus* leaves typically contain very low concentrations of N (0.8–2% dry matter; Moore et al. 2004a), while also containing up to 12% condensed tannin (Fox and Macauley 1977). Marsh et al. (2003a) demonstrated that tannins in *E. melliodora* foliage reduce the digestion of N by 26–41% and cause decreases in food intake by common brushtail possums. Similarly, Cork et al. (1983) found that koalas only digested 45% of the N in *E. punctata* foliage, largely because of tannin activity. In contrast, the caecotrophic common ringtail possum appears to be relatively unaffected by dietary tannins (Marsh et al. 2003a).

Recently, DeGabriel et al. (2008) developed an integrative in vitro approach to quantify the combined negative effects of tannins and fibre on the digestibility of N in *Eucalyptus* foliage, and determine how much is actually available for marsupials to use (termed “digestible N”). They estimated the effects of tannins by measuring the affinity of foliage for polyethylene glycol (PEG), a measure that has been successfully used to demonstrate the effects of tannins on food intake by captive possums (Foley and

Hume 1987; Marsh et al. 2003b). The use of PEG provides a more meaningful method to quantify the effects of tannins on animal nutrition than do traditional colorimetric assays, as it can be directly related to reductions in food intake and effects on digestion in vivo. This is the most common approach used in agricultural studies (Landau et al. 2004; Silanikove et al. 1996), but it has not been widely applied in ecology. A comparison of 11 ironbarked *Eucalyptus* woodlands across northern Australia demonstrated significant variation in concentrations of foliar N, but even more marked variation in concentrations of digestible N, with little or no correlation between the two measures (DeGabriel et al. 2008). Similarly, in a single stand of *E. drepanophylla*, tannins reduced the availability of N by 7–76% (DeGabriel et al. 2009a). Thus, the logical progression is to apply this assay to understand how N availability influences the presence and density of marsupial populations.

Effects of the external environment on marsupial life histories

The availability of food resources is assumed to be a key factor shaping the life histories of individual animals and consequently the dynamics of herbivore populations. Studies in agricultural systems and with captive animals have demonstrated links between concentrations of nutrients and PSMs and reproductive performance (Min et al. 2003), but there remain relatively few studies linking particular aspects of nutrition to the life history strategies of wild mammalian herbivores.

Many researchers have speculated that cues from the external environment determine both the seasonality of breeding and the potential reproductive success of marsupial folivores. For example, Wayne et al. (2005a), found that the timing and frequency of births of the western ringtail possum (*Pseudocheirus occidentalis*) in Jarrah woodlands were correlated with flushes of new, presumably more nutritious leaves, a theory that has also been posited for common ringtail possums (Munks 1995). Similarly, Wayne et al. (2005b) suggested that inter-population differences in growth rates of pouch young of western brushtail possums (*T. v. hypoleucus*) were due to nutritional variation, but this hypothesis was not explicitly tested.

DeGabriel et al. (2009a) specifically tested similar nutritional hypotheses and demonstrated that the negative effects of tannins on the availability of protein had strong effects on the reproductive success of free-living female common brushtail possums (*Trichosurus vulpecula*). They found a fivefold difference in breeding success between females with access to the best quality home ranges and those with the poorest quality ranges. While most females

were able to produce an offspring in the peak breeding season each year, those with higher levels of available protein were more likely to breed twice in a year than were those with the poorest quality ranges. Furthermore, they demonstrated that the growth rates of offspring were positively correlated with the average concentrations of in vitro digestible N in their mother's home ranges. Recently, McArt et al. (2009) demonstrated similar effects of availability of digestible protein on the reproductive performance of moose in Alaska, confirming that measuring available protein may be the key to understanding reproductive success and ultimately, population distributions, in wild mammalian herbivores. In both DeGabriel et al.'s (2009a) (Fig. 3) and McArt et al.'s (2009) studies most of the difference between total N and digestible N could be directly attributed to the impact of tannins on protein availability, but there will always be some part of the dietary protein which remains undigested because it is bound in the cell-wall matrix. Nonetheless, these two studies give new impetus to connecting variations in PSMs directly with the demography of wild animals.

There is, however, evidence for the evolution of flexible life history strategies amongst marsupial folivores in response to the constraints imposed by the external environment. Eucalypt foliage is generally low in energy and protein compared to many other leaf diets, thereby limiting the amount of nutrients that mothers can transfer to offspring. Munks and Green (1997) showed that the rate of supply of milk by common ringtail possums was low compared to other marsupial species of a similar size. Consequently, these possums have an extended lactation, to compensate for the lower growth rates of young resulting from restricted energy transfer. Krockenberger and

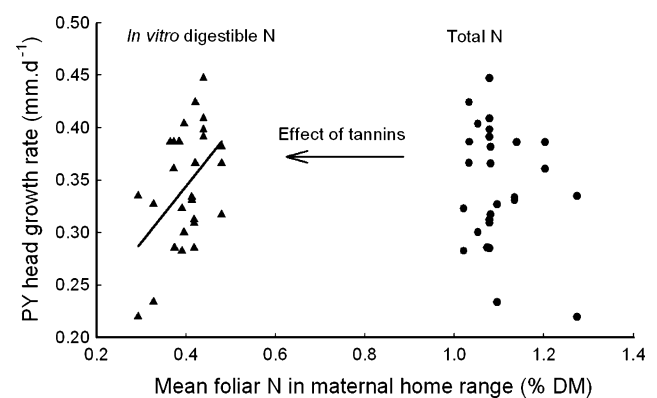


Fig. 3 Relationship between head growth rates of pouch young (PY) of 13 female common brushtail possums and mean in vitro digestible nitrogen (N) concentrations or mean total N concentration of foliage from the home range of each female ($P = 0.014$, $n = 27$ pouch young). The difference between the total N and in vitro digestible N concentrations is attributed to the effect of tannins although a small proportion of the difference results from N bound in the cell-wall matrix. Re-calculated from DeGabriel et al. (2009a)

Hume (2007) found that koalas employ a flexible digestive strategy to cope with the increased nutritional demands of lactation. Lactating female koalas ingested up to 35% more leaf per day compared to non-lactating animals, which they coped with by increasing the solute digesta pool size, as well as the passage rate of large particles or the efficiency of the particle separation mechanism. It is clear that the interaction between nutrients and chemical defences in the external environment of marsupials imposes stringent limitations on the reproductive potential of folivore species and thus, is undoubtedly a key factor underlying the presence and persistence of marsupial populations in Australian forests. However, this relationship is highly sensitive to external shocks, and a critical area of immediate concern is the effects of climate change on marsupial populations.

Effects of environmental change on leaf-eating marsupials

Understanding the potential effects of PSMs on populations of marsupial folivores and their strategies for coping with these compounds is increasingly important in the face of global environmental change. Studies in a number of plant–animal systems have demonstrated that increased temperatures and elevated concentrations of atmospheric CO₂ can have complex impacts on concentrations of nutrients and PSMs in leaves and consequent levels of herbivory (Close et al. 2005; Huttunen et al. 2008; Kanowski 2001; Lawler et al. 1997; Mattson et al. 2004; Veteli et al. 2007). For example, Lawler et al. (1997) found that *E. tereticornis* seedlings grown under high CO₂ treatments, had lower concentrations of total N and higher concentrations of total phenolics and were therefore less palatable to Chrysomelid beetles. Similarly, Kanowski (2001) reported reduced foliar N and increased tannin concentrations in seedlings of rainforest plant species eaten by tropical marsupial folivores, when they were grown under elevated CO₂. The body of evidence suggests that foliar N will decline as atmospheric CO₂ rises. However, the effects of predicted temperature increases on the nutritional quality of foliage are less clear. Foliar concentrations of phenolic compounds, including tannins, have been shown to be higher in eucalypts growing in cold climates (Moore et al. 2004c), which is hypothesised to be an adaptive response to protect from cold-induced photo inhibition (Close and McArthur 2002). Studies in other systems have also shown that concentrations of phenolics are negatively related to temperature (Mattson et al. 2004). Nevertheless, given the effects of the interaction of foliar nitrogen and tannin concentrations on marsupial reproductive success, predicted climate change could have cascading impacts on the population ecology of marsupial folivores and may ultimately limit their persistence in particular habitats.

Conclusions

By integrating detailed studies of the chemical ecology of food plants with an understanding of the physiology of the vertebrate browsers, we have highlighted how plant secondary compounds influence diet selection. However, field studies have shown that the spatial arrangement of resources together with the availability of alternative foods and the presence of predators provides a complex fabric on which foraging decisions must be made. Understanding how these factors interact in current conditions will enable us to better predict how these plant–herbivore interactions will be affected by climate change.

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References

- Agrawal AA (2004) Plant defense and density dependence in the population growth of herbivores. *Am Nat* 164:113–120
- Andrew RL, Peakall R, Wallis IR, Foley WJ (2007) Spatial distribution of defense chemicals and markers and the maintenance of chemical variation. *Ecology* 88:716–728
- Andrews PL, Horn CC (2006) Signals for nausea and emesis: Implications for models of upper gastrointestinal diseases. *Auton Neurosci* 125:100–115
- Andrews PLR, Rudd JA (2004) The role of tachykinins and the tachykinin NK1 receptor in nausea and emesis. *Handb Exp Pharmacol* 164:359–440
- Braithwaite LW (1996) Conservation of arboreal herbivores: The Australian scene. *Aust J Ecol* 21:21–30
- Braithwaite LW, Turner J, Kelly J (1984) Studies on the arboreal marsupial fauna of eucalypt forests being harvested for wood-pulp at Eden N.S.W. III. Relationships between faunal densities, eucalypt occurrence and foliage nutrients, and soil parent materials. *Aust Wildl Res* 11:41–48
- Bryant JP, Kuropat PJ (1980) Selection of winter forage by sub-arctic browsing vertebrates—the role of plant chemistry. *Annu Rev Ecol Syst* 11:261–285
- Bryant JP, Provenza FD, Pastor J, Reichardt PB, Clausen TP, Dutoit JT (1991) Interactions between woody-plants and browsing mammals mediated by secondary metabolites. *Annu Rev Ecol Syst* 22:431–446
- Chapman CA, Chapman LJ, Naughton-Treves L, Lawes MJ, McDowell LR (2004) Predicting folivorous primate abundance: Validation of a nutritional model. *Am J Primatol* 62:55–69
- Clinchy M, Taylor AC, Zanette LY, Krebs CJ, Jarman PJ (2004) Body size, age and paternity in common brushtail possums (*Trichosurus vulpecula*). *Mol Ecol* 13:195–202
- Close DC, McArthur C (2002) Rethinking the role of many plant phenolics—protection from photodamage not herbivores? *Oikos* 99:166–172
- Close DC, McArthur C, Hagerman AE, Fitzgerald H (2005) Differential distribution of leaf chemistry in eucalypt seedlings

- due to variation in whole-plant nutrient availability. *Phytochemistry* 66:215–221
- Cork SJ (1992) Polyphenols and the distribution of arboreal, folivorous marsupials in *Eucalyptus* forests of Australia. In: Hemingway RW, Laks PE (eds) *Plant polyphenols: synthesis, properties, significance*. Plenum Press, New York, pp 653–663
- Cork SJ, Catling PC (1996) Modelling distributions of arboreal and ground-dwelling mammals in relation to climate, nutrients, plant chemical defences and vegetation structure in the eucalypt forests of southeastern Australia. *For Ecol Manage* 85:163–175
- Cork SJ, Dove H (1989) Lactation in the tammar wallaby (*Macropus eugenii*). 2. Intake of milk components and maternal allocation of energy. *J Zool* 219:399–409
- Cork SJ, Hume ID, Dawson TJ (1983) Digestion and metabolism of a natural foliar diet (*Eucalyptus punctata*) by an arboreal marsupial, the koala (*Phascolarctos cinereus*). *J Comp Physiol B Biochem Syst Environ Physiol* 153:181–190
- Dearing MD, Foley WJ, McLean SR (2005) The influence of plant secondary metabolites on the nutritional ecology of terrestrial herbivorous vertebrates. *Annu Rev Ecol Evol Syst* 36:169–189
- DeGabriel JL (2008) Demographic constraints imposed by plant nutrients and secondary chemistry on the common brushtail possum (*Trichosurus vulpecula* Kerr). Ph.D. thesis, The Australian National University
- DeGabriel JL, Wallis IR, Moore BD, Foley WJ (2008) A simple, integrative assay to quantify nutritional quality of browses for herbivores. *Oecologia* 156:107–116
- DeGabriel JL, Moore BD, Foley WJ, Johnson CN (2009a) The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. *Ecology* 90:711–719
- DeGabriel JL, Moore BD, Shipley LA, Krockenberger AK, Wallis IR, Johnson CN, Foley WJ (2009b) Inter-population differences in the tolerance of a marsupial folivore to plant secondary metabolites. *Oecologia* 161:539–548
- Edwards MJ, Wallis IR, Foley WJ (2009) Acid loads induced by the detoxification of plant secondary metabolites do not limit feeding by the common brushtail possum (*Trichosurus vulpecula*). *J Comp Physiol B Biochem Syst Environ Physiol*. doi: [10.1007/s00360-009-0404-y](https://doi.org/10.1007/s00360-009-0404-y)
- Eschler BM, Pass DM, Willis R, Foley WJ (2000) Distribution of foliar formylated phloroglucinol derivatives amongst *Eucalyptus* species. *Biochem Syst Ecol* 28:813–824
- Foley WJ, Hume ID (1987) Digestion and metabolism of high-tannin *Eucalyptus* foliage by the brushtail possum (*Trichosurus vulpecula*) (Marsupialia: Phalangeridae). *J Comp Physiol B Biochem Syst Environ Physiol* 157:67–76
- Foley WJ, McArthur C (1994) The effects and costs of allelochemicals for mammalian herbivores: an ecological perspective. In: Chivers DJ, Langer P (eds) *The Digestive System in Mammals: Food, Form and Function*. Cambridge University Press, Cambridge
- Foley WJ, McLean S, Cork SJ (1995) Consequences of biotransformation of plant secondary metabolites on acid-base metabolism in mammals—a final common pathway. *J Chem Ecol* 21:721–743
- Foley WJ, Iason GR, McArthur C (1999) Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: How far have we come in 25 years? In: Jung H-JG, Fahey GCJ (eds) *Nutritional ecology of herbivores: proceedings of the Vth International Symposium on the Nutrition of Herbivores*. American Society of Animal Science, Savoy, Illinois, pp 130–209
- Forbey JS, Foley WJ (2009) PharmEcology: A pharmacological approach to understanding plant–herbivore interactions: an introduction to the symposium. *Integr Comp Biol* 49:267–273
- Forbey JS, Harvey AL, Huffman MA, Provenza FD, Sullivan R, Tasdemir D (2009) Exploitation of secondary metabolites by animals: A response to homeostatic challenges. *Integr Comp Biol* 49:314–328
- Fox LR, Macauley BJ (1977) Insect grazing on *Eucalyptus* in response to variation in leaf tannins and nitrogen. *Oecologia* 29:145–162
- Freeland WJ, Janzen DH (1974) Strategies in herbivory by mammals: the role of plant secondary compounds. *Am Nat* 108:269–287
- Ganzhorn JU (1992) Leaf chemistry and the biomass of folivorous primates in tropical forests. Test of a hypothesis. *Oecologia* 91:540–547
- Garcia J (1989) Food for Tolman: Cognition and cathexis in concert. In: Archer T, Nilsson L (eds) *Aversion, avoidance and anxiety*. Erlbaum, Hillsdale, N.J., pp 45–85
- Hagerman AE, Robbins CT, Weerasuriya Y, Wilson TC, McArthur C (1992) Tannin chemistry in relation to digestion. *J Range Manage* 45:57–62
- Henery ML, Moran GF, Wallis IR, Foley WJ (2007) Identification of quantitative trait loci influencing foliar concentrations of terpenes and formylated phloroglucinol compounds in *Eucalyptus nitens*. *New Phytol* 176:82–95
- Hume ID (1999) *Marsupial nutrition*. Cambridge University Press, Cambridge, U.K.
- Huttunen L, Niemela P, Julkunen-Tiitto R, Heiska S, Tegelberg R, Rousi M, Kellomaki S (2008) Does defoliation induce chemical and morphological defenses in the leaves of silver birch seedlings under changing climate? *Chemoecology* 18:85–98
- Iason G (2005) The role of plant secondary metabolites in mammalian herbivory: ecological perspectives. *Proc Nutr Soc* 64:123–131
- Iason GR, Lennon JJ, Pakeman RJ, Thoss V, Beaton JK, Sim DA, Elston DA (2005) Does chemical composition of individual Scots pine trees determine the biodiversity of their associated ground vegetation? *Ecol Lett* 8:364–369
- Johnson CN, Vernes K, Payne A (2005) Demography in relation to two herbivorous marsupials: testing for source-sink dynamics versus independent regulation of population size. *Oecologia* 143:70–76
- Jones BA, How RA, Kitchener DJ (1994) A field study of *Pseudocheirus occidentalis* (Marsupialia, Petauridae). 1. Distribution and habitat. *Wildl Res* 21:175–187
- Jones KMW, Maclagan SJ, Krockenberger AK (2006) Diet selection in the green ringtail possum (*Pseudochirops archeri*): A specialist folivore in a diverse forest. *Austral Ecol* 31:799–807
- Jordt SE, Julius D (2002) Molecular basis for species-specific sensitivity to “hot” chili peppers. *Cell* 108:421–430
- Kanowski J (2001) Effects of elevated CO₂ on the foliar chemistry of seedlings of two rainforest trees from north-east Australia: Implications for folivorous marsupials. *Austral Ecol* 26:165–172
- Krockenberger AK, Hume ID (2007) A flexible digestive strategy accommodates the nutritional demands of reproduction in a free-living folivore, the koala (*Phascolarctos cinereus*). *Funct Ecol* 21:748–756
- Krockenberger AK, Hume ID, Cork SJ (1998) Production of milk and nutrition of the dependent young of free-ranging koalas (*Phascolarctos cinereus*). *Physiol Zool* 71:45–56
- Landau S, Dvash L, Decandia M, Cabiddu A, Shapiro F, Molle G, Silanikove N (2004) Determination of poly(ethylene glycol)-binding to browse foliage, as an assay of tannin, by near-infrared reflectance spectroscopy. *J Agric Food Chem* 52:638–642
- Lawler IR, Foley WJ, Woodrow IE, Cork SJ (1997) The effects of elevated CO₂ atmospheres on the nutritional quality of *Eucalyptus* foliage and its interaction with soil nutrient and light availability. *Oecologia* 109:59–68
- Lawler IR, Foley WJ, Pass GJ, Eschler BM (1998) Administration of a 5HT₃ receptor antagonist increases the intake of diets containing *Eucalyptus* secondary metabolites by marsupials. *J Comp Physiol B Biochem Syst Environ Physiol* 168:611–618

- Lawler IR, Stapley J, Foley WJ, Eschler BM (1999) Ecological example of conditioned flavor aversion in plant–herbivore interactions: Effect of terpenes of *Eucalyptus* leaves on feeding by common ringtail and brushtail possums. *J Chem Ecol* 25:401–415
- Levin DA (1976) Chemical defenses of plants to pathogens and herbivores. *Annu Rev Ecol Syst* 7:121–159
- Mangione AM, Dearing MD, Karasov WH (2000) Interpopulation differences in tolerance to creosote bush resin in desert woodrats (*Neotoma lepida*). *Ecology* 81:2067–2076
- Marsh KJ, Foley WJ, Cowling A, Wallis IR (2003a) Differential susceptibility to *Eucalyptus* secondary compounds explains feeding by the common ringtail (*Pseudocheirus peregrinus*) and common brushtail possum (*Trichosurus vulpecula*). *J Comp Physiol B Biochem Syst Environ Physiol* 173:69–78
- Marsh KJ, Wallis IR, Foley WJ (2003b) The effect of inactivating tannins on the intake of *Eucalyptus* foliage by a specialist *Eucalyptus* folivore (*Pseudocheirus peregrinus*) and a generalist herbivore (*Trichosurus vulpecula*). *Aust J Zool* 51:31–42
- Marsh KJ, Wallis IR, Foley WJ (2005) Detoxification rates constrain feeding in common brushtail possums (*Trichosurus vulpecula*). *Ecology* 86:2946–2954
- Marsh KJ, Wallis IR, Andrew RL, Foley WJ (2006a) The detoxification limitation hypothesis: Where did it come from and where is it going? *J Chem Ecol* 32:1247–1266
- Marsh KJ, Wallis IR, McLean S, Sorensen JS, Foley WJ (2006b) Conflicting demands on detoxification pathways influence how common brushtail possums choose their diets. *Ecology* 87:2103–2112
- Martin JK, Martin AA (2007) Resource distribution influences mating system in the bobuck (*Trichosurus cunninghami*: Marsupialia). *Oecologia* 154:227–236
- Mattson WJ, Kuokkanen K, Niemela P, Julkunen-Tiitto R, Kellomaki S, Tahvanainen J (2004) Elevated CO₂ alters birch resistance to *Lagomorpha* herbivores. *Glob Chang Biol* 10:1402–1413
- McArt SH, Spalinger DE, Collins WB, Schoen ER, Stevenson T, Bucho M (2009) Summer dietary nitrogen availability as a potential bottom-up constraint on moose in south-central Alaska. *Ecology* 90:1400–1411
- McEachern MB, Eagles-Smith CA, Efferson CM, Van Vuren DH (2006) Evidence for local specialization in a generalist mammalian herbivore, *Neotoma fuscipes*. *Oikos* 113:440–448
- McKay GM (1983) Greater glider (*Petauroides volans*). In: Strahan R (ed) *The Australian Museum complete book of Australian mammals*. Angus and Robertson, Sydney
- McKey D, Waterman PG, Mbi CN, Gartlan JS, Struhsaker TT (1978) Phenolic content of vegetation in two African rain forests: ecological implications. *Science* 202:61–63
- McLean S, Boyle RR, Brandon S, Davies NW, Sorensen JS (2007) Pharmacokinetics of 1, 8-cineole, a dietary toxin, in the brushtail possum (*Trichosurus vulpecula*): Significance for feeding. *Xenobiotica* 37:903–922
- Min BR, Barry TN, Attwood GT, McNabb WC (2003) The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Anim Feed Sci Technol* 106:3–19
- Mole S, Waterman PG (1987) A critical analysis of techniques for measuring tannins in ecological studies. I. Techniques for chemically defining tannins. *Oecologia* 72:137–147
- Moore BD, Wallis IR, Marsh KJ, Foley WJ (2004a) The role of nutrition in the conservation of the marsupial folivores of eucalypt forests. In: Lunney D (ed) *Conservation of Australia's forest fauna*, 2nd edition edn. Royal Zoological Society of New South Wales, Mosman, NSW, pp 549–575
- Moore BD, Wallis IR, Pala-Paul J, Brophy JJ, Willis RH, Foley WJ (2004b) Antiherbivore chemistry of *Eucalyptus*—Cues and deterrents for marsupial folivores. *J Chem Ecol* 30:1743–1769
- Moore BD, Wallis IR, Wood J, Foley WJ (2004c) Foliar nutrition, site quality and temperature affect foliar chemistry of tallwood (*Eucalyptus microcorys*). *Ecol Monogr* 74:553–568
- Moore BD, Foley WJ, Wallis IR, Cowling A, Handasyde KA (2005) A simple understanding of complex chemistry explains feeding preferences of koalas. *Biol Lett* 1:64–67
- Munks S (1995) The breeding biology of *Pseudocheirus peregrinus viverrinus* on Flinders Island, Bass Strait. *Wildl Res* 22:521–534
- Munks SA, Green B (1997) Milk consumption and growth in a marsupial arboreal folivore, the common ringtail possum, *Pseudocheirus peregrinus*. *Physiol Zool* 70:691–700
- O'Reilly-Wapstra JM, McArthur C, Potts BM (2004) Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species. *Funct Ecol* 18:677–684
- Oates JF, Whitesides GH, Davies AG, Waterman PG, Green SM, Dasilva GL, Mole S (1990) Determinants of variation in tropical forest primate biomass: new evidence from West Africa. *Ecology* 71:328–343
- Pass GJ, Foley WJ (2000) Plant secondary metabolites as mammalian feeding deterrents: separating the effects of the taste of salicin from its post-ingestive consequences in the common brushtail possum (*Trichosurus vulpecula*). *J Comp Physiol B Biochem Syst Environ Physiol* 170:185–192
- Pausas JG, Braithwaite LW, Austin MP (1995) Modelling habitat quality for arboreal marsupials in the south coastal forests of New South Wales, Australia. *For Ecol Manage* 78:39–49
- Pettorelli N, Gaillard JM, Duncan P, Ouellet JP, Van Laere G (2001) Population density and small-scale variation in habitat quality affect phenotypic quality in roe deer. *Oecologia* 128:400–405
- Provenza FD (1996) Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. *J Anim Sci* 74:2010–2020
- Provenza FD, Lynch JJ, Burritt EA, Scott CB (1994) How goats learn to distinguish between novel foods that differ in post-ingestive consequences. *J Chem Ecol* 20:609–624
- Provenza FD, Kimball BA, Villalba JJ (2000) Roles of odor, taste, and toxicity in the food preferences of lambs: implications for mimicry in plants. *Oikos* 88:424–432
- Robbins CT (1993) *Wildlife feeding and nutrition*. Academic Press Inc., Orlando
- Robbins CT, Hanley TA, Hagerman AE, Hjeljord O, Baker DL, Schwartz CC, Mautz WW (1987) Role of tannins in defending plants against ruminants: reduction in protein availability. *Ecology* 68:98–107
- Sanger GJ (2008) 5-Hydroxytryptamine and the gastrointestinal tract: where next? *Trends Pharmacol Sci* 29:465–471
- Scrivener NJ, Johnson CN, Wallis IR, Takasaki M, Foley WJ, Krockenberger AK (2004) Which trees do wild common brushtail possums (*Trichosurus vulpecula*) prefer? Problems and solutions in scaling laboratory findings to diet selection in the field. *Evol Ecol Res* 6:77–87
- Shiple LA, Sorensen JS, Moore BD (2009) Revisiting the dietary niche: When is a mammalian herbivore a specialist? *Integr Comp Biol* 49:274–290
- Silanikove N, Shinder D, Gilboa N, Eyal M, Nitsan Z (1996) Binding of poly(ethylene glycol) to samples of forage plants as an assay of tannins and their negative effects on ruminal degradation. *J Agric Food Chem* 44:3230–3234
- Sorensen JS, Skopec MM, Dearing MD (2006) Application of pharmacological approaches to plant–mammal interactions. *J Chem Ecol* 32:1229–1246
- Sotka EE, Forbey J, Horn M, Poore AGB, Raubenheimer D, Whalen KE (2009) The emerging role of pharmacology in understanding consumer–prey interactions in marine and freshwater systems. *Integr Comp Biol* 49:291–313
- Stapley J, Foley WJ, Cunningham R, Eschler B (2000) How well can common brushtail possums regulate their intake of *Eucalyptus*

- toxins? *J Comp Physiol B Biochem Syst Environ Physiol* 170:211–218
- Tewksbury JJ, Nabhan GP (2001) Seed dispersal—directed deterrence by capsaicin in chillies. *Nature* 412:403–404
- Tewksbury JJ, Reagan KM, Machnicki NJ, Carlo TA, Haak DC, Penalzoa ALC, Levey DJ (2008) Evolutionary ecology of pungency in wild chillies. *Proc Natl Acad Sci USA* 105:11808–11811
- Torregrossa AM, Dearing MD (2009) Nutritional toxicology of mammals: regulated intake of plant secondary compounds. *Funct Ecol* 23:48–56
- Turner J, Kelly J (1981) Relationships between soil nutrients and vegetation in a north coast forest, New South Wales. *Aust For Res* 11:201–208
- Veteli TO, Mattson WJ, Niemela P, Julkunen-Tiitto R, Kellomaki S, Kuokkanen K, Lavola A (2007) Do elevated temperature and CO₂ generally have counteracting effects on phenolic phytochemistry of boreal trees? *J Chem Ecol* 33:287–296
- Villalba JJ, Provenza FD (2000a) Discriminating among novel foods: effects of energy provision on preferences of lambs for poor-quality foods. *Appl Anim Behav Sci* 66:87–106
- Villalba JJ, Provenza FD (2000b) Roles of novelty, generalization, and postingestive feedback in the recognition of foods by lambs. *J Anim Sci* 78:3060–3069
- Wayne AF, Rooney JF, Ward CG, Vellios CV, Lindenmayer DB (2005a) The life history of *Pseudocheirus occidentalis* (Pseudocheiridae) in the jarrah forest of south-western Australia. *Aust J Zool* 53:325–337
- Wayne AF, Ward CG, Rooney JF, Vellios CV, Lindenmayer DB (2005b) The life history of *Trichosurus vulpecula hypoleucus* (Phalangeridae) in the jarrah forest of south-western Australia. *Aust J Zool* 53:265–278
- Westoby M (1978) What are the biological bases of varied diets? *Am Nat* 112:627–631
- Wheat CW, Vogel H, Wittstock U, Braby MF, Underwood D, Mitchell-Olds T (2007) The genetic basis of a plant-insect coevolutionary key innovation. *Proc Natl Acad Sci USA* 104:20427–20431
- White TCR (1993) *The inadequate environment: nitrogen and the abundance of animals*. Springer, Berlin
- Wiggins NL, McArthur C, Davies NW, McLean S (2006) Spatial scale of the patchiness of plant poisons: A critical influence on foraging efficiency. *Ecology* 87:2236–2243
- Williams JE, Woinarski J (1997) *Eucalypt ecology: Individuals to ecosystems*. Cambridge University Press, Cambridge
- Wright S (1943) Isolation by distance. *Genetics* 28:114–138