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The effect of inactivating tannins on the intake of *Eucalyptus* foliage by a specialist *Eucalyptus* folivore (*Pseudocheirus peregrinus*) and a generalist herbivore (*Trichosurus vulpecula*)

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Abstract

The paucity of evidence on eucalypt browsing by common brushtail and common ringtail possums suggests that ringtails preferentially eat foliage from trees within the subgenus *Monocalyptus*. In contrast, brushtails eat less eucalypt foliage than do ringtails and prefer trees from the subgenus *Symphyomyrtus*. Trees from these subgenera differ in their defensive chemicals. Both contain tannins but it appears that only the symphyomyrts synthesise formylated phloroglucinol compounds (FPCs). We fed possums foliage from several individual *Eucalyptus rossii* and *E. consideniana*, both monocalypts, to avoid the confounding factor of FPCs, and examined the effects of blocking tannins by dipping foliage in polyethylene glycol (PEG). Ringtails and brushtails differed in their abilities to eat foliage from these eucalypts. The ringtails ate much more than did the brushtails and showed a small (about 10%) but significant increase in feeding in response to PEG. The brushtails were reluctant to eat foliage from either eucalypt species but doubled their intake when leaves were coated with PEG. Even so, they still did not eat enough to meet maintenance requirements for energy and nitrogen. Neither ringtails nor brushtails preferred foliage from any individual *E. rossii* tree, suggesting that all trees were equally defended. However, brushtails preferred foliage from some *E. consideniana* to others. Monocalypt tannins are clearly important barriers to feeding in brushtail possums, but further research with higher doses of PEG will confirm whether they are the only deterrent chemicals in monocalypt foliage.

Introduction

The profiles of plant secondary metabolites (PSMs) may vary widely within a genus and even between individual plants of a species (Lawler *et al.* 2000; Wallis *et al.* 2002). Variation of this nature is thought to contribute to dietary specialisation amongst species that consume similar diets (Glander 1978). It is widely assumed that specialist feeders are better adapted than generalists to ingest large quantities of toxic compounds, presumably because they can metabolise these chemicals. Among the four folivores that eat *Eucalyptus*, koalas (*Phascolarctos cinereus*) (Moore and Foley 2000) and greater gliders (*Petauroides volans*) (Kavanagh and Lambert 1990) are specialists that feed almost entirely from the genus, whereas the diet of the common brushtail possum (*Trichosurus vulpecula*) is more general, with animals eating a wide range of foods (Kerle 1984). The fourth species, the common ringtail possum (*Pseudocheirus peregrinus*), eats a diet that lies somewhere in between, although it is predominantly a folivore (Cork and Pahl 1984).

It was once thought that the high tannin and terpene content of eucalypt foliage limited feeding, but recently Lawler *et al.* (1998, 2000) showed that feeding decisions depend largely on the foliar concentration of formylated phloroglucinol compounds (FPCs). In particular, koalas appear to have a much higher threshold for FPCs and thus can feed from a wider range of trees than can ringtail possums (Lawler *et al.* 1998), again suggesting the specialist–generalist paradigm.

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However, even within *Eucalyptus*, major differences in the profiles of PSMs occur that correspond to several distinct informal taxa, the most important of which are the informal subgenera, *Symphyomyrtus* and *Monocalyptus*. Eschler *et al.* (2000) showed that FPCs were absent from *Monocalyptus* yet widely distributed within *Symphyomyrtus*, while both groups contain high concentrations of tannins (Fox and MacCauley 1977). This diversity in chemical defences provides the basis for diversity among herbivores and past research suggests that the marsupial folivores of *Eucalyptus* show at least two levels of specialisation. The first concerns the amount of foliage, particularly eucalypt, in the diet. Thus, ringtail possums appear more specialised than are brushtail possums. The second level of specialisation concerns the capacity of each possum species to metabolise particular eucalypt PSMs. In captivity, ringtails have often been maintained exclusively on monocalypts, e.g. *E. radiata* (Foley 1992); *E. andrewsii* (Chilcott and Hume 1984*a*, 1984*b*); *E. haemostoma* (Hume *et al.* 1996); *E. rossii* (Lawler *et al.* 2000). In contrast, captive brushtail possums tend to be fed foliage from symphyomyrts, e.g. *E. crebra* and *E. tereticornis* (Freeland and Winter 1975); *E. melliodora* (Foley and Hume 1987; Wallis *et al.* 2002). There is surprisingly little information on the eucalypts eaten by wild brushtail and ringtail possums but evidence (Cork and Pahl 1984; Kerle 1984) confirms these broad patterns. Thus, brushtail possums may be able to metabolise large amounts of FPCs but ringtail possums seem adept at dealing with eucalypt tannins (Marsh *et al.* 2003).

We predicted that high concentrations of tannins explain why brushtail possums avoid some monocalypt species that ringtails readily eat. Consequently, blocking these tannins would cause an increase in feeding. As the natural diet of ringtail possums usually contains high concentrations and presumably a wide range of tannins, we would predict that tannin inactivation would have a smaller impact on feeding by ringtails than by brushtails. If ringtail or brushtail possums do benefit from a diet containing less tannin, we would expect them, given the choice, to select foliage coated with polyethylene glycol (PEG) 4000, a tannin-blocking agent.

Previous studies of eucalypt tannin–possum interactions have used only symphyomyrt species (Foley and Hume 1987; McArthur and Sanson 1991; Marsh *et al.* 2003) and two of these were published before the discovery of the effects of FPCs on herbivores (Pass *et al.* 1998). Foley and Hume (1987) fed foliage from a single *E. melliodora* and showed that brushtail possums ate more when their drinking water contained PEG. In contrast, McArthur and Sanson (1991) found that ringtail possums did not increase their intake of *E. ovata* when the foliage was coated with PEG. Our experience suggests that the *E. melliodora* foliage fed by Foley and Hume (1987) almost certainly contained low concentrations of FPCs. In contrast, McArthur and Sanson (1991) fed foliage from several trees and subsequent analysis by Lawler *et al.* (1998) found variable concentrations of the FPC, macrocarpal G, which may have disguised any influence of the tannins. After showing that PEG does not remove the effects of FPCs (Marsh *et al.* 2003), we decided to repeat the studies of Foley and Hume (1987) and McArthur and Sanson (1991) but feeding monocalypts to remove the confounding effect of FPCs.

The experiments described in this paper compare how much foliage ringtail and brushtail possums are willing to eat from two monocalypt species, *E. rossii* and *E. consideniana*, in the presence and absence of PEG. We chose *E. rossii* because, in Canberra, ringtail possums eat it extensively, whereas brushtail possums do not. In contrast, *E. consideniana* is regarded as an indicator of the poorest-quality eucalypt forests in southern New South Wales (Braithwaite *et al.* 1983), as indicated by the low densities of marsupial folivores in forests where the species is common. In addition, the tannins of

E. consideniana have been partially characterised by modern analytical techniques (Santos and Waterman 2001).

Methods

The capture and maintenance of the possums, as well as leaf collection, storage and feeding resembled the procedures of Wallis *et al.* (2002). Briefly, cages were held in temperature-controlled rooms (20 ± 2°C) under a 12 : 12 light : dark cycle. Bunches of foliage were weighed at 1000 hours each morning and dipped in either a 20% w/v solution of PEG 4000 or water. All bunches were left to stand with their stems in water until 1700 hours, when the PEG had dried on the leaf. After reweighing, the bunches were placed in the possum cages with their stems in water. A similar bunch of control leaf was placed outside the cages to monitor changes in water content. PEG precipitates tannins through hydrogen bonding with the hydroxyl group of the tannin side-chain (Jones and Mangan 1977), as shown diagrammatically in Marsh *et al.* (2003). This reduces the amount of tannin available to bind protein or cause toxicity to the consumer.

Each morning all uneaten leaf was removed from the cages and weighed. The amount eaten was determined by drying the spilled leaf and samples of the control leaf at 60°C to constant weight. Food intake was corrected for the mass of PEG and for the dry matter content.

Experiment 1. The effect of PEG on the intake of E. rossii *and* E. consideniana *foliage by common ringtail and common brushtail possums*

One female and five male ringtail possums (mean body mass of 0.79 kg) received foliage from one of six *E. rossii* trees each night. The foliage was fed either untreated or coated with PEG, and each possum received every treatment in two 6 × 6 Latin squares (12 nights) (Ratkowsky *et al.* 1993). Dry matter intake (DMI) was measured each night. The experiment with *E. consideniana* followed the same protocol.

The experiments with six male brushtail possums (mean body mass of 1.96 kg) followed the protocol used for the ringtail possums, except that a rest night was provided between each experimental night (therefore each experiment was 24 nights). This was to ensure that possums maintained body weight, as they ate very little on experimental nights. On the rest night brushtail possums were fed *E. melliodora* foliage from a tree they favoured.

Experiment 2. Do common ringtail possums prefer E. rossii *foliage with the tannins inactivated?*

Part A

Six male common ringtail possums (mean body mass of 0.73 kg) were placed in individual metabolism cages (135 × 85 × 60 cm), at each end of which was a metal water container and a polythene tube (30 × 10 cm) filled with water in which to stand bunches of foliage. A wooden perch provided equal access to both ends of the cage. Ringtail possums received a choice between untreated foliage and foliage coated with PEG from the same branch of a single tree, with the treatments assigned randomly to a particular end of the cage. We measured DMI over six nights when the ringtails were fed foliage from six different *E. rossii* trees in a 6 × 6 Latin square design (Ratkowsky *et al.* 1993).

Ringtail possums dropped some foliage but there was only occasional mixing of treated and untreated foliage and it was easy to visually separate the PEG-coated leaves from the untreated foliage. In this part of the experiment, treatment nights were consecutive (i.e. there were no rest nights).

Part B

During Part A there was a statistically significant decline in the proportion of PEG-coated foliage eaten, but no change in total food intake (see Results). This suggested that ringtails learn that they benefit by eating at least some PEG each night. The procedure was similar to Part A except that we included a rest night when possums could choose between two bunches of untreated foliage. In the analysis we compared food intake on ‘experimental’ and ‘rest’ days and compared how much the ringtails ate of PEG-coated foliage and untreated foliage on ‘experimental’ days.

Statistical analysis

Experiments were designed as digram-balanced Latin squares so that every possum received all treatments and every treatment preceded and followed all other treatments once. These designs are appropriate for studies with few animals and where animals vary widely. Carryover effects were never significant and so were dropped from statistical models and are not reported.

The different experimental protocols used for ringtail and brushtail possums made a statistical comparison tenuous so we analysed the data for each species separately but present the combined data graphically. One aim of the research was to examine differences in food intake between ringtail and brushtail possums fed the same foliage. We adjusted for the 2–3-fold differences in body mass by arbitrarily expressing all food-intake data as g per $kg^{0.75}$ per day. It was not sensible to use an exponent derived from the data because brushtails reacted aversely to the *Monocalyptus* foliage and often ate less than did the smaller ringtails. Expressing food intake per unit of metabolic body mass makes it possible to compare our data with those previously reported. However, we also provide some data on absolute food intake. Results are cited throughout the text as a mean and standard deviation.

Most experiments were analysed using the Graeco-Latin Square algorithm in Genstat 5 (4th Edn). In Experiments 1 and 2A, 'PEG' and 'Tree' were treatments and 'Possum'*'Experimental Day' the blocking effect. We analysed Experiment 2B using the residual maximum likelihood algorithm (REML) in the same version of Genstat.

In all cases, full models tested all possible combinations of effects, and non-significant effects were sequentially dropped from models leaving a reduced model containing only the statistically significant effects. Any non-significant terms reported in the Results were obtained from the full model, while significant terms are reported using values from the reduced model. In the REML analyses this significance was measured using a submodel, whereby the term of interest is dropped from the full model enabling the change in deviance to be measured between the full model and the submodel. This change in deviance is attributable to the term of interest and its significance is calculated using a Chi-square test.

Results

Experiment 1, Part A: The effect of PEG on the intake of E. rossii *foliage by common ringtail and common brushtail possums*

Ringtail possums ate a similar amount of untreated foliage (mean = 41, s.d. = 5.0 g DM per $kg^{0.75}$ per day) from each of the six *E. rossii* trees, indicating that they considered them all equally palatable ($F_{1,49} = 1.14$, $P = 0.350$). They ate significantly more *E. rossii* when the foliage was coated with PEG (mean = 44, sd = 4.7 g DM per $kg^{0.75}$ per day) ($F_{1,49} = 22.08$, $P < 0.001$) (Fig. 1). The absolute intakes averaged over the six trees, for each ringtail, were

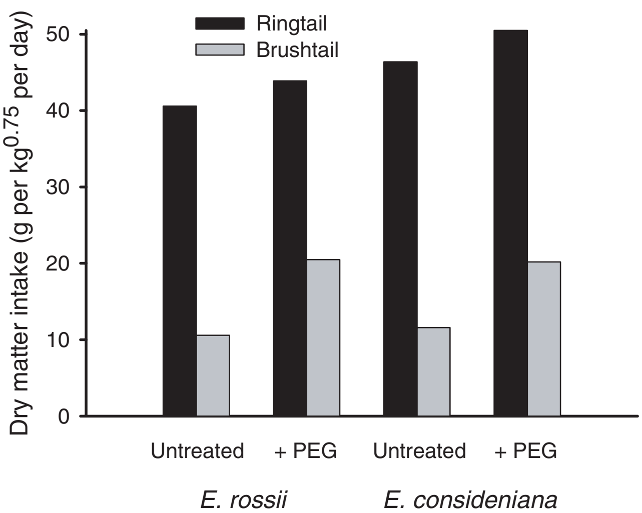

Fig. 1. The amount of untreated and PEG-coated foliage eaten by brushtail and ringtail possums in a no-choice experiment. $N = 36$ for all treatments. The 5% least significant difference values, which are too small to depict graphically, were 0.7, 1.7, 2.1 and 2.3 g for ringtail and brushtail possums fed *E. rossii* and *E. consideniana*, respectively.

24–41 g DM per day (mean = 33, s.d. = 5.1 g) for the untreated foliage and 28–44 g DM per day (mean = 36, s.d. = 4.7 g) for the foliage treated with PEG.

Brushtail possums considered foliage from all six *E. rossii* to be similarly unpalatable, eating just 11 g (s.d. = 4.2) DM per $kg^{0.75}$ per day ($F_{5,49}$ = 0.85, P = 0.52). The absolute intakes of untreated foliage were 5–32 g per day (mean = 17.5, s.d. = 6.5 g). The brushtail possums responded dramatically to the PEG by almost doubling their intake to 21 g (s.d. = 7.5) DM per $kg^{0.75}$ per day ($F_{1,49}$ = 133.3, P < 0.001) (Fig. 1). This corresponded to an absolute intake of 34 g DM per day (range = 16–55, s.d. = 9.9).

Experiment 1, Part B: The effect of PEG on the intake of E. consideniana *foliage by common ringtail and common brushtail possums*

The feeding responses of both ringtail and brushtail possums to *E. consideniana* mirrored those described for *E. rossii*. Ringtail possums ate similar amounts of foliage from individual *E. consideniana* ($F_{5,49}$ = 1.55, P = 0.192). Again, they ate more foliage coated with PEG (mean = 51, s.d. = 6.4 g DM per $kg^{0.75}$ per day) than they did untreated foliage (mean = 46, s.d. = 6.4 g DM per $kg^{0.75}$ per day) ($F_{1,49}$ = 14.93, P < 0.001) (Fig. 1). The absolute intakes for each ringtail possum averaged over the six trees were 30–50 g DM per day (mean = 39, s.d. = 4.9 g) for the untreated foliage and 35–52 g DM per day (mean = 43, s.d. = 3.7 g) for the foliage treated with PEG.

Brushtail possums ate little foliage from *E. consideniana* (mean = 12, s.d. = 7.5 g DM per $kg^{0.75}$ per day) but preferred some individual trees to others ($F_{5,48}$ = 4.22, P = 0.003). As with *E. rossii*, brushtail possums ate about twice as much foliage when it was coated with PEG (mean = 21, s.d. = 9.2 g DM per $kg^{0.75}$ per day) ($F_{1,48}$ = 58.03, P < 0.001) (Fig. 1). In addition, there was a trend towards a PEG × tree interaction, with the brushtails responding differently to PEG depending on the tree ($F_{5,44}$ = 2.08, P = 0.075). The absolute amount of foliage eaten was 0–40 g DM per day for the untreated foliage (mean = 19, s.d. = 8.5 g) and 6.5–63 g DM per day for the foliage treated with PEG (mean = 33, s.d. = 12.1 g).

Experiment 2: Do common ringtail possums prefer E. rossii *foliage with the tannins inactivated?*

Part A

We observed that ringtail possums usually moved to the same end of the cage after leaving their nest-boxes, but statistical analysis showed that the placement of foliage within the cage had no effect on DMI ($F_{1,18}$ = 0.007, P = 0.932). Ringtail possums chose to eat more of the *E. rossii* foliage that had been coated in PEG than of the untreated foliage ($F_{1,35}$ = 15.67, P < 0.001) (Fig. 2) with a trend suggesting that this depended somewhat on the individual tree (PEG × tree interaction, $F_{5,20}$ = 2.35, P = 0.078). The preference for PEG-coated foliage declined through Days 1–6 ($F_{5,20}$ = 2.62, P = 0.044) (Fig. 3) even though there was no change in the total amount eaten over this time ($F_{5,18}$ = 0.33, P = 0.885).

Part B

We repeated the choice experiment to determine whether ringtail possums ate more on nights that PEG was available than they did on ‘rest nights’, when the choice was limited to two bunches of untreated leaf. Ringtail possums tended to choose the PEG-coated foliage over the untreated foliage (χ^2 = 3.1, P = 0.078). However, their total intake was significantly higher on nights when PEG was available than on rest nights (χ^2 = 54.5, P < 0.001) (Fig. 4).

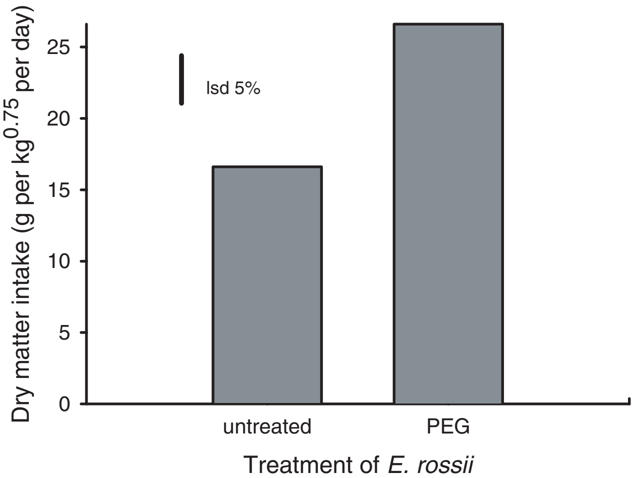

Fig. 2. The amount eaten by ringtail possums given a choice between untreated or PEG-coated *E. rossii* foliage.

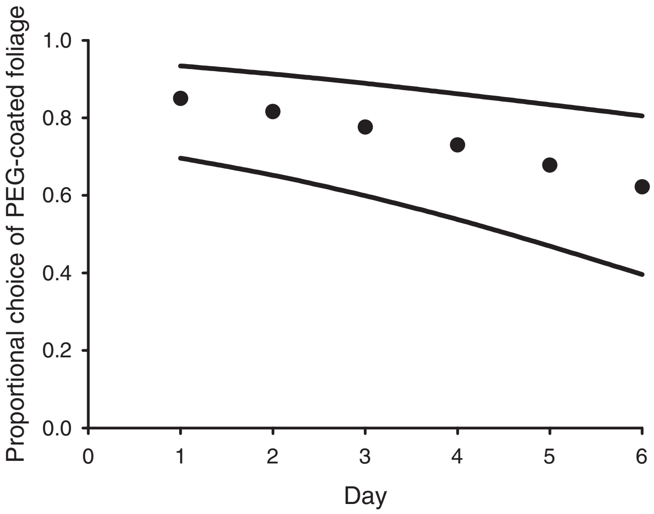

Fig. 3. The declining preference for PEG-coated *E. rossii* foliage with time shown by ringtail possums. $N = 6$ for each day. The lines indicate a 95% confidence interval.

Discussion

Scientists have long been baffled over why the various marsupial folivores prefer food from certain species of eucalypt. Indeed, the preferences of ringtail and brushtail possums appear even broader than first realised, with ringtails apparently favouring foliage from species within the informal subgenus *Monocalyptus*. In contrast, the eucalypt foliage eaten by brushtails tends to come from symphyomyrt species and, if anything, they show a distinct dislike for monocalypt foliage (see Table 1). Nevertheless, animals exhibit preferences even within these informal taxa. For example, arboreal marsupials were not recorded (greater

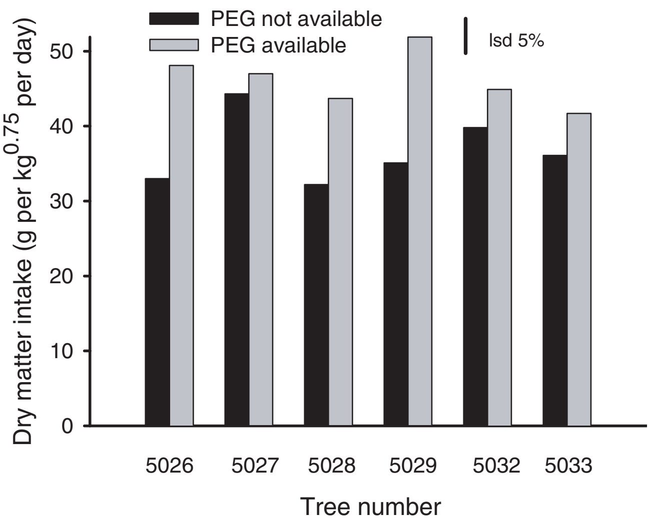

Fig. 4. The intake of *E. rossii* foliage by ringtail possums on 'rest nights' when leaf was offered untreated (black bars) and on 'experimental nights' (grey bars) when they had the choice of eating either untreated or PEG-coated foliage.

gliders, brushtail possums, ringtail possums, eastern pygmy possums, squirrel gliders) or were recorded in low densities (feathertail gliders, sugar gliders, yellow-bellied gliders) where *E. consideniana* occurred in the Eden district of New South Wales (Braithwaite *et al.* 1988). It was the purpose of the current study to shed some light on these issues.

In this study, ringtail possums ate similar amounts of *E. rossii* and *E. consideniana* foliage as did ringtail possums fed a variety of eucalypt foliage in other studies (Table 1). Moreover, ringtail possums ate enough *E. consideniana* foliage to maintain bodyweight and actually gained weight when first introduced to the foliage. Thus, it is unclear nutritionally why there are, at most, low densities of ringtail possums in natural stands of *E. consideniana*. One explanation is that *E. consideniana* offers a lower plane of nutrition than do other species, as suggested by its low concentration of nitrogen. Braithwaite *et al.* (1983) reported that the foliage of *E. consideniana* contained 0.81 ± 0.12% N, while the six trees used in this study contained 0.9–1.1% N. In contrast, the six *E. rossii* trees we studied contained 1.1–1.3% N. Although we did not directly compare the DMI of either ringtail or brushtail possums fed foliage from the two eucalypt species, the ringtail possums ate approximately 10% more of the *E. consideniana* than they did of the *E. rossii*. These animals were non-lactating individuals housed in metabolism cages at 20°C and clearly have much lower metabolic demands than do free-living animals, especially those in terminal lactation. It may be that ringtails cannot eat enough foliage from *E. consideniana* when nutrient demands are high.

We expected that the generalist feeding behaviour of wild brushtail possums would make them more susceptible than ringtail possums to the effects of tannins. This proved to be the case. Brushtail possums always ate less of the untreated *E. rossii* and *E. consideniana* foliage than did ringtail possums. More importantly, brushtail possums ate less *E. rossii* and *E. consideniana* foliage than they do of some symphyomyrt species (e.g. *E. melliodora*: Table 1). Thus, their intakes of monocalypt foliage were not only low relative to those of

Table 1. The mean dry matter intakes of common ringtail and brushtail possums fed eucalypt foliage
Intake (amount eaten) is given as g per $kg^{0.75}$ per day

Eucalyptus species	Possum species	Amount eaten	Reference
Monocalyptus			
E. andrewsii	Common ringtail	42	Chilcott and Hume 1984*a*
E. consideniana	Common ringtail	46	This study
E. consideniana + PEG	Common ringtail	51	This study
E. consideniana	Common brushtail	12	This study
E. consideniana + PEG	Common brushtail	20	This study
E. dives	Common ringtail	45	Cork and Foley, unpublished
E. haemostoma (summer)	Common ringtail	45	Hume *et al.* 1996
E. haemostoma (winter)	Common ringtail	56	Hume *et al.* 1996
E. radiata	Common ringtail	39	Foley 1992
E. radiata	Common ringtail	36	Cork and Foley, unpublished
E. rossii	Common ringtail	40	This study
E. rossii + PEG	Common ringtail	44	This study
E. rossii	Common brushtail	11	This study
E. rossii + PEG	Common brushtail	21	This study
Symphyomyrtus			
E. melliodora	Common brushtail	43	Foley and Hume 1987
E. melliodora + PEG	Common brushtail	52	Foley and Hume 1987
E. melliodora	Common brushtail	22	Marsh *et al.* 2003
E. melliodora + PEG	Common brushtail	31	Marsh *et al.* 2003
E. ovata	Common ringtail	34	McArthur and Sanson 1991
E. ovata + PEG	Common ringtail	33	McArthur and Sanson 1991
E. ovata	Common ringtail	15#50	Lawler *et al.* 1998
E. polyanthemos	Common ringtail	3–44	Lawler *et al.* 2000
E. punctata (summer)	Common ringtail	54	Hume *et al.* 1996
E. punctata (winter)	Common ringtail	42	Hume *et al.* 1996
E. sideroxylon	Common brushtail	10–27	Watson, unpublished
E. viminalis	Common ringtail	0–8	Lawler *et al.* 1998
Corymbia			
C. maculata	Common ringtail	36	Cork and Foley, unpublished

ringtail possums, but they were also low compared with their known consumption of other eucalypt foliage.

Treating foliage with PEG allowed the brushtail possums to almost double their intake of foliage, suggesting that tannins offer eucalypts some protection against browsing by brushtail possums. The phenolic fraction of *E. consideniana* is composed of hydrolysable tannins (galloyl esters and ellagitannins) and flavonol glycosides (Santos and Waterman 2001). The structures of these compounds are known so in future it may be possible to demonstrate unequivocally that these substances deter feeding and to describe the mechanism involved.

It is probable that monocalypts contain undiscovered chemical compounds that deter feeding by brushtail possums but not by ringtail possums. Although supplementary PEG enabled brushtails to eat much more, they still did not eat enough to meet predicted maintenance requirements. It may be that the brushtails did not ingest enough PEG to bind all of the tannins. An *in vitro* PEG-binding assay, based on that of Silanikove *et al.* (1996), showed that the amount of PEG coated on the leaves was sufficient to bind 20–25% and 25–35% of the tannins in the foliage from *E. rossii* and from *E. consideniana*, respectively.

If the brushtail possums had ingested enough PEG to bind all of the tannins, then we could deduce whether tannins were the only components deterring them from eating *E. rossii* and *E. consideniana*.

We tried various methods, such as dipping the leaves twice, to increase the amount of PEG on foliage. None of these techniques succeeded so alternative methods, like self-medication, are necessary to increase the animals' consumption of PEG. There is some evidence that animals will not only self-medicate with PEG but will also adjust the dose to counter secondary plant chemicals. For instance, Provenza *et al.* (2000) and Villalba and Provenza (2001) showed that lambs will eat more PEG as the tannin content of their diet increases.

We found a small but significant increase in intake (about 7% for *E. rossii* and 10% for *E. consideniana*) when ringtail possums were fed foliage coated with PEG to inactivate tannins. These findings contrast with those of McArthur and Sanson (1991) in which ringtail possums ate the same amount of *E. ovata* foliage whether or not it was treated with PEG. However, as mentioned in the Introduction, it is likely that the negative effects of FPCs on feeding in ringtail possums confounded their results.

Ringtail possums are tannin specialists (McArthur and Sanson 1991), so a little PEG may enable them to counter increasing amounts or different types of tannins, whereas additional PEG provides no further benefit.

Ringtail possums initially preferred PEG-coated foliage to untreated foliage, but this preference gradually declined. It is unlikely that PEG was the direct cause of this decline because the same possums maintained a constant plane of feeding during a ten-day digestibility experiment on other PEG-coated eucalypt foliage (Marsh *et al.* 2003). Also, regardless of the degree of preference for PEG-coated foliage, the presence of PEG induced ringtail possums to eat more. In other words, they ate significantly more on nights when they at least had the option of ingesting PEG.

We attributed the decline in preference for PEG-coated foliage to the general action of PEG and the ability of the ringtails to learn self-medication. PEG does not bind only the tannins in the leaf on which it is coated but may also benefit the animal by binding tannins in the gut arising from untreated foliage or from previous meals. Initially, it appears that these ringtail possums, which were all familiar with PEG, associated the taste of PEG with a positive effect of blocking tannins. However, with time they learnt to ingest their requirement of PEG to bind any excess tannins that were not otherwise detoxified.

Regardless of the nature of the difference, the current research showed that ringtail and brushtail possums differ markedly in their abilities to eat the foliage of two monocalypt species and that part of the difference lies in their contrasting abilities to counter monocalypt tannins. Variation in how tannins affect eutherian mammals is often attributed to tannin-binding salivary proteins (TBSPs). For example, quebracho tannins have little effect on mule deer (*Odocoileus hemionus*), which have TBSPs, but greatly affect feeding by sheep, which have little TBSP (Robbins *et al.* 1991). TBSPs are rich in proline and have a high affinity for tannins thus preventing tannins from binding dietary proteins.

McArthur *et al.* (1995) found that the saliva of brushtail possums was rich in protein but suggested that the low rate of secretion may prevent the effective binding of tannins. The only study that we know of examining TBSPs in ringtail possums used whole salivary glands from killed animals (Mole *et al.* 1990). The proline-rich proteins isolated had little affinity for sorghum tannins. However, salivary proteins might only attain their high affinity for tannins through conformational changes or glycosylation after they leave the salivary gland (Hagerman and Robbins 1993). Additionally, Hagerman and Robbins (1993) showed that the TBSPs of different herbivore species specifically bind the tannins naturally encountered by the animal. Thus, the salivary proteins of ringtail possums may have a higher affinity for *Eucalyptus* tannins than they do for sorghum tannins. Related to this is the recent discovery of the histatin salivary proteins, which have a similar function to the proline-rich salivary proteins (Bennick 2002). It is therefore difficult to conclude how great a contribution saliva makes to countering eucalypt tannins.

Finally, animals can counter the deleterious effects of tannins in several other ways. Intestinal bacteria, capable of dissociating tannin–protein complexes, have been isolated from both ringtail and brushtail possums (Osawa and Sly 1992). The typical numbers of these bacteria in each possum species are not known, but the bacteria in ringtail possums may be more effective than those in brushtail possums simply because, through caecotrophy, digesta in ringtail possums is exposed to the microbial population for longer (Foley *et al.* 1999). In summary, ringtails are well adapted to deactivate tannins but the precise mechanism is still unknown.

Conclusions

The results in this paper support the hypothesis that the specialised herbivore, the common ringtail possum, is less susceptible to the detrimental effects of monocalypt tannins than is the generalist herbivore, the common brushtail possum. This is likely related to physiological differences between the two species. While monocalypt tannins are clearly important barriers to feeding in brushtail possums, we do not know whether they are the only deterrent compounds in the foliage. Likewise, the findings invite questions about how monocalypt tannins differ from those in other plants that brushtail possums eat. This paper shows that when the confounding effect of FPCs is removed, ringtail possums can eat approximately 10% more eucalypt foliage if the tannins are inactivated. It remains to be seen how brushtail possums, in particular, detect and regulate their intake of tannins and how the inclusion of other groups of secondary compounds, such as the formylated phoroglucinol compounds, alters their response to PEG. Unfortunately, there is no way known to block FPCs and thus repeat these experiments in ringtail and brushtail possums fed the same foliage.

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References

Bennick, A. (2002). Interaction of plant polyphenols with salivary proteins. *Critical Reviews in Oral Biology and Medicine* **13**, 184–196.

Braithwaite, L. W., Dudzinski, M. L., and Turner, J. (1983). Studies on the arboreal marsupial fauna of eucalypt forests being harvested for woodpulp at Eden, N.S.W. II. Relationship between the fauna density, richness and diversity, and measured variables of the habitat. *Australian Wildlife Research* **10**, 231–247.

Braithwaite, L. W., Binns, D. L., and Nowlan, R. D. (1988). The distribution of arboreal marsupials in relation to eucalypt forest types in the Eden (N.S.W.) woodchip concession area. *Australian Wildlife Research* **15**, 363–373.

Chilcott, M. J., and Hume, I. D. (1984*a*). Digestion of *Eucalyptus andrewsii* foliage by the common ringtail possum, *Pseudocheirus peregrinus*. *Australian Journal of Zoology* **32**, 605–613.
Chilcott, M. J., and Hume, I. D. (1984*b*). Nitrogen and urea metabolism and nitrogen requirements of the common ringtail possum, *Pseudocheirus peregrinus*, fed *Eucalyptus andrewsii* foliage. *Australian Journal of Zoology* **32**, 615–622.
Cork, S. J., and Pahl, L. (1984). The possible influence of nutritional factors on diet and habitat selection by the ringtail possum (*Pseudocheirus peregrinus*). In 'Possums and Gliders'. (Eds A. P. Smith and I. D. Hume.) pp. 269–276. (Surrey Beatty: Sydney.)
Eschler, B. M., Pass, D. M., Willis, R., and Foley, W. J. (2000). Distribution of foliar formylated phloroglucinol derivatives amongst *Eucalyptus* species. *Biochemical Systematics and Ecology* **28**, 813–824.
Foley, W. J. (1992). Nitrogen and energy retention and acid–base status in the common ringtail possum (*Pseudocheirus peregrinus*) – evidence of the effects of absorbed allelochemicals. *Physiological Zoology* **65**, 403–421.
Foley, W. J., and Hume, I. D. (1987). Digestion and metabolism of high-tannin *Eucalyptus* foliage by the brushtail possum (*Trichosurus vulpecula*) (Marsupialia: Phalangeridae). *Journal of Comparative Physiology B* **157**, 67–76.
Foley, W. J., Iason, G. R., and McArthur, C. (1999). Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? In 'Nutritional Ecology of Herbivores: Proceedings of the Vth International Symposium on the Nutrition of Herbivores'. (Eds H.-J. G. Jung and G. C. J. Fahey.) pp. 130–209. (American Society of Animal Science: Savoy.)
Fox, L. R., and MacCauley, B. J. (1977). Insect grazing on *Eucalyptus* in response to leaf tannins and nitrogen. *Oecologia* **29**, 145–162.
Freeland, W. J., and Winter, J. W. (1975). Evolutionary consequences of eating: *Trichosurus vulpecula* (Marsupialia) and the genus *Eucalyptus*. *Journal of Chemical Ecology* **1**, 439–455.
Glander, K. E. (1978). Howler monkey feeding behaviour and plant secondary compounds: a study of strategies. In 'Ecology of Arboreal Folivores'. (Eds G. G. Montgomery.) pp. 561–574. (Smithsonian Institution Press: Washington DC.)
Hagerman, A. E., and Robbins, C. T. (1993). Specificity of tannin-binding salivary proteins relative to diet selection by mammals. *Canadian Journal of Zoology* **71**, 628–633.
Hume, I. D., Bladon, R. V., and Soran, N. (1996). Seasonal changes in digestive performance of common ringtail possums (*Pseudocheirus peregrinus*) fed *Eucalyptus* foliage. *Australian Journal of Zoology* **44**, 327–336.
Jones, W. T., and Mangan, J. L. (1977). Complexes of the condensed tannins of sainfoin (*Onobrychis vicifolia* Scop.) with fraction 1 leaf protein and with submaxillay mucoprotein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture* **28**, 126–136.
Kavanagh, R. P., and Lambert, M. J. (1990). Food selection by the greater glider, *Petauroides volans* – is foliar nitrogen a determinant of habitat quality. *Australian Wildlife Research* **17**, 285–299.
Kerle, J. A. (1984). Variation in the ecology of *Trichosurus*: its adaptive significance. In 'Possums and Gliders'. (Eds A. P. Smith and I. D. Hume.) pp. 115–128. (Surrey Beatty: Sydney.)
Lawler, I. R., Foley, W. J., Eschler, B. M., Pass, D. M., and Handasyde, K. (1998). Intraspecific variation in *Eucalyptus* secondary metabolites determines food intake by folivorous marsupials. *Oecologia* **116**, 160–169.
Lawler, I. R., Foley, W. J., and Eschler, B. M. (2000). Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology* **81**, 1327–1338.
Marsh, K. J., Foley, W. J., Cowling, A., and Wallis, I. R. (2003). Differential susceptibility to secondary compounds in *Eucalyptus* explains feeding by the common ringtail possums (*Pseudocheirus peregrinus*) and the common brushtail possums (*Trichosurus vulpecula*). *Journal of Comparative Physiology B* **173**, 69–78.
McArthur, C., and Sanson, G. D. (1991). Effects of tannins on digestion in the common ringtail possum (*Pseudocheirus peregrinus*), a specialized marsupial folivore. *Journal of Zoology* **225**, 233–251.
McArthur, C., Sanson, G. D., and Beal, A. M. (1995). Salivary proline-rich proteins in mammals – roles in oral homeostasis and counteracting dietary tannin. *Journal of Chemical Ecology* **21**, 663–691.
Mole, S., Butler, L. G., and Iason, G. (1990). Defense against dietary tannin in herbivores – a survey for proline rich salivary proteins in mammals. *Biochemical Systematics and Ecology* **18**, 287–293.
Moore, B. D., and Foley, W. J. (2000). A review of feeding and diet selection in koalas (*Phascolarctos cinereus*). *Australian Journal of Zoology* **48**, 317–333.

Osawa, R., and Sly, L. I. (1992). Occurrence of tannin–protein complex degrading *Streptococcus* sp. in feces of various animals. *Systematic and Applied Microbiology* **15**, 144–147.
Pass, D. M., Foley, W. J., and Bowden, B. (1998). Vertebrate herbivory on *Eucalyptus* – identification of specific feeding deterrents for common ringtail possums (*Pseudocheirus peregrinus*) by bioassay-guided fractionation of *Eucalyptus ovata* foliage. *Journal of Chemical Ecology* **24**, 1513–1527.
Provenza, F. D., Burritt, E. A., Perevolotsky, A., and Silanikove, N. (2000). Self-regulation of intake of polyethylene glycol by sheep fed diets varying in tannin concentrations. *Journal of Animal Science* **78**, 1206–1212.
Ratkowsky, D. A., Evans, M. A., and Alldredge, J. R. (1993). 'Cross-over Experiments – Design, Analysis, and Application.' (Marcel Dekker, Inc.: New York.)
Robbins, C. T., Hagerman, A. E., Austin, P. J., McArthur, C., and Hanley, T. A. (1991). Variation in mammalian physiological responses to a condensed tannin and its ecological implications. *Journal of Mammalogy* **72**, 480–486.
Santos, S. C., and Waterman, P. G. (2001). Polyphenols from *Eucalyptus consideniana* and *Eucalyptus viminalis*. *Fitoterapia* **72**, 95–97.
Silanikove, N., Shinder, D., Gilboa, N., Eyal, M., and 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. *Journal of Agricultural and Food Chemistry* **44**, 3230–3234.
Villalba, J. J., and Provenza, F. D. (2001). Preference for polyethylene glycol by sheep fed a quebracho tannin diet. *Journal of Animal Science* **79**, 2066–2074.
Wallis, I. R., Watson, M. L., and Foley, W. J. (2002). Secondary metabolites in *Eucalyptus melliodora*: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. *Australian Journal of Zoology* **50**, 1–13.

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