Which trees do wild common brushtail possums (*Trichosurus vulpecula*) prefer? Problems and solutions in scaling laboratory findings to diet selection in the field

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ABSTRACT

In this study, we examined whether a group of plant secondary metabolites – the formylated phloroglucinol compounds (FPCs), which are known to deter feeding on eucalypt foliage by captive marsupial folivores – influence feeding by wild common brushtail possums. There was at least a six-fold range of FPC concentrations in the foliage of individual trees within a single eucalypt species. Possums largely avoided trees with high FPC concentrations. While some trees with low FPCs were used heavily, others were not; thus the relationship between FPC concentration and tree use was polygonal rather than linear. We used a randomization procedure to demonstrate a relationship between the upper limits of FPC concentration and tree use. In contrast, this procedure showed that neither the nitrogen content nor the in vitro digestibility of the leaves influenced the use of trees by possums. Our results show that plant secondary metabolites, with a demonstrable mode of action, affect the selection of individual eucalypt trees by wild folivores. Thus, FPC concentrations may influence the distribution and abundance of marsupial folivores at landscape scales.

**Keywords:** diet selection, *Eucalyptus*, marsupial folivores, plant secondary metabolites.

INTRODUCTION

Few species of mammals eat eucalypt foliage, despite its abundance in Australia (Landsberg and Cork, 1997). One explanation for this is that eucalypt leaves contain few nutrients but high concentrations of plant secondary metabolites that reduce digestibility or intoxicate...
herbivores (Cork and Foley, 1991; Foley, 1992). In the past 20 years, there have been many studies of diet selection by *Eucalyptus*-feeding marsupial folivores, in both the laboratory and the field (Cork and Pahl, 1984; Hindell and Lee, 1987; Hume and Esson, 1995; Cork and Catling, 1996). Most studies have found that arboreal folivores prefer to eat the leaves of particular species of eucalypts or even from particular trees within a species; however, they failed to explain why animals feed on some trees but reject others.

Recent studies have identified a previously unknown class of plant secondary metabolites in eucalypts, the formylated phloroglucinol compounds (FPCs), which strongly deter feeding by marsupial folivores in laboratory experiments (Lawler *et al.*, 1998a, 1999, 2000; Pass *et al.*, 1998; Moore and Foley, 2000). It would appear that FPCs act by stimulating serotenergic systems, leading to nausea (Lawler *et al.*, 1998b). Furthermore, Lawler *et al.* (2000) found that the concentration of FPCs varies among individual trees of the same species and at the same location. Some trees contain concentrations that are either higher or lower than those known to deter feeding in the laboratory. Given these observations, it is possible that variation in the foliar concentrations of FPCs in the wild could control the distribution and abundance of marsupial folivores and create the landscape-level patchiness in folivore populations found by large-scale surveys (e.g. Braithwaite *et al.*, 1983).

There are several reasons why a strong effect of FPCs on feeding in laboratory trials might not scale-up to strong effects on tree selection in the wild. First, the concentrations of FPCs in stands of eucalypts are normally distributed (Lawler *et al.*, 2000), so few trees in a population have either very low or very high concentrations of FPCs. In contrast, deriving relationships between FPC concentration and feeding in the laboratory depends on selecting trees with a wide range of FPC concentrations (Lawler *et al.*, 1998a, 2000). This range of concentrations may not be available to any individual wild animal. Second, free-ranging animals can choose the foliage they eat. In contrast, captive animals are typically offered foliage from only a single tree or a limited selection of trees (Lawler *et al.*, 1998a, 2000). Finally, many factors apart from the concentrations of plant secondary metabolites may influence the selection of trees by free-ranging animals. Trees could be selected in relation to their nutrient concentrations, location in a territory, proximity to dens, security from predators, or the opportunities they provide to interact with conspecifics. Separating the contributions of all factors to the pattern of tree use in the field is likely to be particularly difficult. No study has yet demonstrated that concentrations of defined plant secondary metabolites, with a demonstrated mode of action, influence food plant use by wild herbivores.

In this study, we examined whether FPC concentrations influence the use of individual trees by an arboreal folivore with a eucalypt diet, the common brushtail possum *Trichosurus vulpecula*. Given the strong effect of FPCs on feeding in the laboratory, we expected that high concentrations of FPCs would make some trees unsuitable for feeding but that other factors might determine whether possums use trees with low FPC concentrations. Therefore, our initial analysis focused on detecting whether possums avoid trees whose foliage contains a high concentration of FPCs.

### MATERIALS AND METHODS

The study was conducted on a 12 ha study site near Mt Fox in tropical northeast Queensland (145°46’E, 18°50’S) on basalt-derived soils of high fertility. The vegetation was open eucalypt woodland with a grassy ground layer. The dominant tree species was
Eucalyptus crebra (44% of all trees on the site) followed by E. (= Corymbia) clarksoniana (22%), E. tereticornis (16%), E. tesselaris (16%) and E. (= Corymbia) intermedia (2%). This area has a wet–dry tropical climate. The study was conducted from the late wet to the early dry season between May and July 2000.

Nine brushtail possums (seven males, two females) were radio-tagged and then located and observed at least once each night over 22 nights, yielding 27–33 observations per possum. A hand-held spotlight fitted with a red filter was used to minimize disturbance while allowing observations. The first observations were not made until at least 30 min after nightfall to allow animals time to move from their dens into trees in which they might feed. Upon locating an animal, we recorded the date, time and weather, the tree species, the possum’s position in the tree and the nature of its activity. Each tree was labelled and its position determined to within 2 m using a Garmin differential GPS. Each day foliage samples were collected from the same positions in the canopy that animals were observed on the previous night. Leaves, including the petiole, were carefully removed from the branch and about 100 g put in a labelled paper bag inside a plastic bag, stored on ice until the day’s end, then frozen until analysis. The samples were divided into ‘young’ (not fully expanded) and ‘mature’ leaves when leaves of both classes were present.

Leaf samples were freeze-dried, then ground to pass a 1 mm sieve (Tecator Cyclotec). Measuring FPCs is slow and expensive, so we analysed the foliages from only the two main species used by the possums – Eucalyptus crebra and E. tereticornis – which together represented 60% of all observations and 74% of trees used on more than one occasion. The number of samples was further reduced by randomly rejecting half of the samples from those trees used only once by each possum. The remaining samples (29 E. crebra and 21 E. tereticornis) were analysed in duplicate, for FPCs, nitrogen and in vitro digestibility. We analysed both young and mature foliage for nitrogen and in vitro digestibility, but determined FPCs only in the mature foliage. All results were expressed relative to dry matter corrected for residual dry matter content at the time of each analysis.

Formylated phloroglucinol compounds were extracted according to the procedure of Wallis et al. (2003). Briefly, we obtained a crude extract by refluxing 1.5 g of dried ground leaf with 20% acetone in light petroleum spirit (40–60°C bp) for 5 h in a soxhlet apparatus. About 20 mg of this extract was dissolved in 20% methanol in acetonitrile containing 0.3 g l⁻¹ of 2-ethyl phenol as an internal standard. The mixture was sonicated, filtered and then 15 µl was injected onto a Wakosil 250 × 4 mm GL 3C18RS (SGE) column maintained at 37°C with a flow rate of 0.75 ml min⁻¹ on a Waters Alliance Model HPLC. The FPCs were eluted under gradient conditions with 0.1% TFA acid in acetonitrile (A) and 0.1% TFA in water (B) as follows: 60% A/40% B for 5 min, linear gradient to 90% A/10% B at 60 min, hold for 10 min and return to starting conditions over 10 min. The specific FPC compounds present in extracts of each of the eucalypt species were first identified by Fourier transform ion cyclone resonance mass spectrometry (Eschler et al., 2000). We then confirmed these compounds by high-performance liquid chromatography (HPLC) of authentic standards. These were purified from Eucalyptus foliage as described by Eschler and Foley (1999) and Takasaki et al. (1994) or, in the case of grandinol, synthesized directly (Matsumoto et al., 2001).

Foliar nitrogen was determined with a semi-micro Kjeldahl procedure with selenium as a catalyst and verified against an ammonium sulphate standard. In vitro dry matter digestibility was measured in duplicate using a pepsin/cellulase procedure in Ankom filter bags.
RESULTS

Use of tree species

Two species, *E. crebra* and *E. tereticornis*, accounted for 60% of all observations of tree use by *T. vulpecula* (31% and 29%, respectively; *n* = 262). The frequency of use differed from that expected from their proportional representation on the site (*G* = 400.3, d.f. = 4, *P* < 0.001). *Eucalyptus crebra*, which accounted for 44% of trees on the site, was used less than expected and *E. tereticornis* (16% of trees on the site) was used more than expected. The use of other species matched their frequencies of occurrence.

Secondary plant metabolites

*Eucalyptus crebra* contained two main FPCs, grandinol (a monoformylated compound; Matsumoto *et al.*, 2001) and jensenone (a diformylated compound; Eschler *et al.*, 2000). The FPC profile of *E. tereticornis* was more complex. Four FPCs – macrocarpal-A, sideroxylonal-A, sideroxylonal-C and macrocarpal-G – were able to be identified and quantified for use in further statistical analysis. This left many unidentified and unquantified compounds. However, the peak areas of three of these four compounds were strongly correlated with the total peak areas from the HPLC traces, so these compounds were representative of the overall variation in FPCs (rank correlations: macrocarpal-G, *r* = 0.886, *P* < 0.001; macrocarpal-A, *r* = 0.799, *P* < 0.001; sideroxylonal-C, *r* = 0.646, *P* = 0.002; sideroxylonal-A, *r* = 0.111, *P* = 0.633; *n* = 21 in each case).

The concentrations of grandinol and jensenone in *E. crebra* varied from 0.8 to 7.0 and 3.0 to 19.0 mg·g⁻¹ of dry leaf, respectively, giving a six-fold range in the total content of FPCs. For *E. tereticornis*, concentrations of sideroxylonal-A ranged from 0 to 3.3 mg·g⁻¹; for sideroxylonal-C the range was 0 to 1.3 mg·g⁻¹, for macrocarpal-A 0 to 4.0 mg·g⁻¹ and for macrocarpal-G 0 to 18.0 mg·g⁻¹ of dry leaf. The total FPC concentrations for both species combined varied from 0 to 26 mg·g⁻¹ of dry leaf.

Possums rarely used trees with high FPC concentrations, while their use of trees with low FPC concentrations was variable (Fig. 1). Thus, the relationship between FPC con-

![Fig. 1. The relationship between foliar FPC content and tree use by nine focal common brushtail possums, *T. vulpecula* (*n* = 50 observations). The maximum values of FPC content in each tree use category are shown as solid circles.](image)
centration and tree use was triangular, making conventional regression and correlation techniques inappropriate for its analysis.

Data of this form are typically analysed in two ways. The first is to compare observations with theoretically derived expectations of the form and position of limits on the possible data (Enquist et al., 1995; Guo et al., 1998). The second approach is quantile regression, which attempts to characterize the edge of a scatter of points (Blackburn et al., 1992; Scharf et al., 1998). Although laboratory studies have indicated a linear relationship between foliage consumption and FPC content (Lawler et al., 1998a; Wallis et al., 2002), we had no way to translate that information into a theoretical expectation of the form and position of a limit on tree use by possums in the field. Consequently, there was no basis for analysing our data by the techniques of Enquist et al. (1995) or Guo et al. (1998). The quantile regression approach of Blackburn et al. (1992) has been criticized because it always produces a line within the data rather than defining the edge of the data (Enquist et al., 1995). Even so, it could still have been used to show there was a relationship at or near the edge of our data. However, we regarded this approach unsuitable because in our data set sample sizes declined with increasing tree use. This was a consequence of the foraging behaviour of the possums, which concentrated their activity in relatively few trees, leaving many that they hardly used. This covariation in sample size and tree use could produce a trend in minimum and maximum values of FPC content and variance of the sample, as a sampling artefact, because extreme values are more likely to be found in large samples. Thus, even if possums were not actively selecting trees based on FPC content, we expected a pyramidal distribution of data with very high and low FPC values in the low use, large sample size categories. In contrast, we expected relatively less variation (i.e. close to the overall mean FPC content) in trees falling in the high use, small sample size categories. To account for this sampling bias, we developed a bootstrap randomization procedure to generate a null distribution against which to test the rank correlation between tree use category and the maximum and minimum of FPC content in each tree use category (Sokal and Rohlf, 1995). Because the data were categorical, we chose Spearman’s rank-sum correlation coefficient ($r_s$) to describe the relationships at the edges. Trees were randomly allocated to tree use categories in the same proportions as the observed data set and a rank correlation was calculated. This was done for both the upper and lower edge of the data scatter using the maximum and minimum values in each category, respectively. This procedure was iterated 60,000 times to define the distribution of $r_s$-values possible from random allocation of trees within a single population. The significance of the observed correlations was determined directly from the proportion of randomly generated correlations that were equal to or stronger than those observed. These procedures were repeated for both the nitrogen and the in vitro digestibility data.

This procedure demonstrated a significant negative correlation between foliar FPC concentration and tree use along the upper edge of the FPC versus tree use relationship, but not along the lower edge (Table 1). In contrast, there was no relationship between tree use and either foliar nitrogen content or in vitro digestibility (Table 1) for either young or mature foliage.

**Factors contributing to deviation from the FPC content: tree use limit**

Although neither nitrogen nor in vitro digestibility had a significant effect on tree use in the above analyses, there was still substantial variation in the data. This was especially true for
the low tree use categories, where many trees were used less than might have been expected from their FPC content alone (Fig. 1). We further tested the importance of foliar nitrogen and \textit{in vitro} digestibility on deviation from the FPC–tree use limit. Having demonstrated that the limit to the data (Fig. 1) was statistically significant, we used linear least-squares regression to numerically describe that limit and calculated expected FPC content at the limit for each tree use category. We determined the deviation from the limit for each data point by subtracting the FPC value from the limiting value for that category. Then we used multiple regression to test the effect of foliar nitrogen and \textit{in vitro} digestibility on that deviation. Initially, each of these factors was regressed against the residual from the FPC–tree use limit and then the factor with greatest descriptive power (i.e. $r^2$) was entered first into a stepwise regression model. Again, tree use was not significantly influenced by either the \textit{in vitro} digestibility ($F_{1,28} = 0.927$, $P = 0.344$) or the nitrogen concentration ($F_{1,28} = 1.396$, $P = 0.247$) of the foliage.

**DISCUSSION**

Recent research examining the feeding preferences of captive marsupial folivores (including \textit{T. vulpecula}) identified FPCs as highly potent feeding deterrents (Lawler \textit{et al.}, 1998a,b, 1999, 2000; Pass \textit{et al.}, 1998; Wallis \textit{et al.}, 2002). In all cases, variation in FPC concentrations explained more than 60% of the variation in food intake. Given these unequivocal findings and the fact that we studied mostly the same FPCs, we predicted a negative relationship between foliar FPC concentrations and tree use in free-living brushtail possums. As expected, possums avoided trees with high FPC concentrations. It follows that sites in which a high proportion of trees have high FPC concentrations will be relatively unsuitable for \textit{T. vulpecula}. Thus, the distribution of foliar FPC concentrations may be a

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$^a$ Correlation between tree use and either the minimum or maximum value of a foliar constituent within that tree use category.

$^b$ Probability of an equal or stronger correlation within 60,000 randomized distributions.

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major determinant of variations in density of possum populations, as proposed by Lawler et al. (2000).

This result was demonstrable only by using a novel statistical approach. While possums used some trees rarely, they used a few heavily, making those trees disproportionately important to the population. This correlation between tree use and sample size precluded the use of traditional statistics. Simple correlations between use and the extreme values of foliar composition were over 10 times more likely to reject the null hypothesis (at \( P < 0.05 \)) than was our randomization procedure and are likely to interpret random patterns caused by the sampling regime as ecologically significant relationships. This finding probably applies to many other studies of foraging, where animals favour a few among many potential food items. While the trees visited frequently by \( T. \) vulpecula never had high concentrations of FPCs, there were also trees with low concentrations of these compounds that possums rarely visited. This was unexpected because, in our experience, trees with low FPC concentrations are uncommon. If FPCs were the only determinants of feeding, we would expect these trees to be focal points of home ranges. Some of these trees may have been important to other, non-focal possums that behaviourally excluded focal animals, but we consider it unlikely as other possums were never observed in these trees.

Clearly, other factors, such as variations in predation risk, also play a role in determining tree use by free-ranging brushtail possums. Although we remain uncertain about which other factors influence tree selection, our results suggest that FPCs set strong limits on which trees may be used, so other factors influence which trees are used from the set with suitable FPC concentrations.

In this study, we linked tree use with foliage chemistry. Proposing a causal relationship from this link explicitly assumes that possums ate most foliage from the trees in which they spent the most time. We could not rigorously test this assumption. However, including trees used for non-feeding activities would decrease, rather than increase, our chances of observing a relationship between tree use and foliar FPC concentration. Thus, our result is conservative and the underlying relationship between feeding and FPC concentrations may be stronger than observed. This aside, observations of \( T. \) vulpecula at a nearby (\( \sim 1 \) km) site indicated that, apart from den trees, possums rarely visited trees from which they did not feed (C. Hodkinson, unpublished data). We minimized the chances of including non-food trees by restricting observations to a time when possums were not courting or mating. Similarly, we limited the risk of confounding feeding and den trees by making observations well after nightfall. Finally, by sampling foliage from the sites within the canopy that possums were seen and in the day following the observation, we reduced any effects of temporal and spatial variation in foliar chemistry within trees. However, the precision of the results and the strength of the relationship between tree use and FPC concentration would both be greater if it were possible to quantify foliage intake rather than measure tree use as a surrogate. Measuring foliage intake by free-ranging animals remains the next challenge in understanding the relationships of plant defences to animal foraging ecology.

Our results exemplify the difficulties in scaling from the laboratory to the field but they also offer the basis for a myriad of other studies. The simple linear relationships uncovered by laboratory studies became complex and non-linear in the field. If overall habitat quality for marsupial arboreal folivores depends mainly on its nutrient availability, as suggested by Braithwaite et al. (1983) and Norton and Neave (1990), then it is necessary to understand this variable before attempting to describe other sources of variation. Thus, our findings
provide an opening for studies of other potentially important factors, including social and spatial issues.

Nitrogen is considered one of the most common limiting nutrients in herbivore nutrition (Ullrey et al., 1981; Robbins, 1993) and a key to the abundance of arboreal marsupials (Braithwaite et al., 1983). The concentrations of nitrogen and fibre in plant material are broadly inversely related. As a leaf matures, the proportion of structural material, which is rich in fibre but poor in nitrogen, increases (Robbins, 1993). Thus, in the absence of plant secondary metabolites, a folivore should select young foliage that is both rich in nitrogen and highly digestible. We found no link between either the nitrogen content or the in vitro digestibility of foliage and the tree preferences of possums. This need not suggest that nitrogen and digestibility are unimportant, but rather that the effects of FPCs are more important at the scale of home ranges and choices of individual animals. If this is true, then relationships between nutrient concentrations, such as nitrogen, and animal abundance are probably relevant at larger scales. The moisture content of foliage might also affect tree selection by folivores (Munks et al., 1996). There is circumstantial evidence for this. Soderquist and MacNally (2000) found more common brushtail possums in moist gullies than in surrounding terrain. Likewise, the koalas that survived a drought in central Queensland, which killed 63% of animals, all lived in moist gullies (Gordon et al., 1988). Since the present study followed several months of high rainfall, it is unlikely that water determined food choice. Nevertheless, foliar water content may be a determinant of food choice and animal abundance over a longer temporal scale.

The scale of the present study was an individual’s home range. What is the relevance of data from this scale to explaining the distribution and abundance of arboreal folivores at larger scales, such as across the eucalypt woodlands of Australia? We have shown that free-ranging possums tend to avoid trees with high FPC concentrations and instead focus their activities in trees with low concentrations of these plant secondary metabolites. This indicates that the density of trees with low concentrations of foliar FPCs may place a proximate limit on the local density of possums. We predict that the distribution of FPC concentrations in foliage would also explain much of the patchiness of folivore distribution at larger scales, because those eucalypt marsupial folivores tested in the laboratory (common ringtail and brushtail possums and the koala) all respond to FPCs (Lawler et al., 1998a,b, 1999, 2000; Pass et al., 1998). However, this relationship may break down when nutrients are scarce. Regardless of FPC content, eucalypts growing on extremely poor soils may produce foliage with insufficient nutrients to maintain folivores. If so, this would explain the correlation between soil nutrients and folivore density at large scales (Braithwaite et al., 1983) at which plant secondary metabolites may be irrelevant to herbivory. But what about landscapes of higher fertility? When leaves are worth eating, plants should protect them and it is in this context that we expect the interplay of herbivory and plant secondary metabolites.

If foliar plant secondary metabolites such as FPCs effectively limit herbivory, plants with high concentrations of plant secondary metabolites within a population should experience lower herbivory and thus greater reproductive success than those with low concentrations. Recent studies suggest that variation in plant secondary metabolites is under genetic control and highly heritable (Hamilton et al., 2001). Possums and eucalypts have a long association, dating back 5–10 million years to the late Tertiary Period (Archer, 1987). Given this, we might expect eucalypts to have been selected for uniformly high foliar FPC concentrations. In reality, FPCs are highly variable within eucalypt populations (a range of six-fold in this
study). What mechanism maintains this variation? There are three main possibilities. First, the selection pressure exerted by marsupial folivores might be weak. This is unlikely given the ability of marsupial folivores, such as brushtail possums introduced to New Zealand (Payton, 2000) and various koala populations, to defoliate and kill their food trees (Martin and Handasyde, 1999). Second, FPCs may be under different selection pressures than suggested by our study. For instance, phenolics may act primarily as photoprotectants, with herbivore deterrence being secondary (Close and McArthur, 2002). Finally, FPCs may be costly, leading to a trade-off between defence and the cost of producing and maintaining that defence under conditions of variable herbivore pressure. The type and magnitude of costs to the tree of maintaining high FPC concentrations are unknown, but are probably significant in maintaining the variation we observe.

Many previous studies have attempted to relate food plant use to concentrations of plant secondary metabolites. The strength of the present research is that it demonstrates this link for a compound in which we know both the detection and the feedback mechanisms that marsupials use to avoid intoxication (Moore and Foley, 2000). Lawler et al. (1998b) showed that FPCs stimulate the serotonergic system leading to nausea. Thus, administering serotonin receptor antagonists can largely reverse the depression in intake on high FPC diets. It would appear that folivores cannot detect FPCs, but the concentrations of FPCs in eucalypt leaves are strongly and positively correlated to concentrations of aromatic terpenes (Lawler et al., 2000). Once animals learn to associate the odour of leaf terpenes with the consequences of feeding, they use the odour as a guide to choosing leaves. The present study completes the link between this mechanistic explanation of diet selection and the reality of food plant use by free-ranging animals. Clearly, we need now to combine the statistical approaches described here with better methods of measuring foliar chemistry on a larger scale. Recently, we successfully used airborne remote sensing to measure FPCs and foliar nitrogen concentrations in the canopies of individual eucalypts (Z. Huang, B. Turner, S. Dury, W. Foley and I. Wallis, unpublished data). This makes it possible to map foliar chemistry over whole landscapes and to relate it to patterns of animal abundance.

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