Vol 435|26 May 2005|doi:10.1038/nature03551

LETTERS

Tree use by koalas in a chemically complex landscape

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Although defence against herbivores is often argued to be the main action of plant secondary metabolites (PSMs)[1], very few examples have demonstrated that intraspecific variation in PSM concentrations influences foraging by wild vertebrate herbivores[2,3]. Experiments with captive animals often indicate that PSM concentrations influence how much herbivores eat from individual plants[3–7], but these experiments do not replicate the subtle trade-offs in diet selection faced by wild animals, which must avoid predators and extremes of weather, interact with conspecifics, and achieve a balanced, nutritious diet, while avoiding intoxication by PSMs. We characterized the foliar chemistry of every tree from two *Eucalyptus* species available to a population of koalas (*Phascolarctos cinereus*) and considered rates of tree visitation over a ten-year period. We show that visitation rate was most strongly influenced by tree size, but that koalas also visited trees less frequently if the foliage contained either high concentrations of deterrent PSMs known as formylated phloroglucinol compounds, or low concentrations of nitrogen. Consequently, plant chemistry restricts the use of trees by this herbivore, and thus limits the food available to koalas and potentially influences koala populations.

Although feeding experiments with captive animals often show that the intake of foliage by herbivores is a linear function of PSM concentrations[4,6,8], more-complex relationships may exist between levels of plant defence and rates of herbivory by wild animals. For example, high PSM concentrations may limit the potential nutritional value of a plant by restricting herbivores' intake, and detoxification of PSMs may entail time, energetic or nutritional costs[9,10]. Beyond the point at which the net gain from eating a plant is exceeded by the PSM-related costs, herbivory may be prevented entirely, but herbivores may be less discriminating among plants with PSM concentrations below such a threshold. Furthermore, wild herbivores may counter the effects of plant toxins by eating mixed diets, thereby distributing detoxification costs across multiple detoxification pathways[10] or by selecting nutrient-rich foliage[9,11–13].

Koalas are highly specialized folivores of *Eucalyptus*, primarily of the subgenus *Symphyomyrtus*[14], in which a class of lipophilic phenolic compounds known as formylated phloroglucinol compounds (FPCs) are widespread[15]. Individual *Eucalyptus* trees are large and long-lived, and so represent clearly defined feeding patches[16] in which the presence of a koala can be clearly and unambiguously observed. We considered tree use by koalas in a *Eucalyptus* woodland dominated by three species: *Eucalyptus globulus* (38% of *Eucalyptus* trees), *E. viminalis* (29%) and *E. ovata* (32%), each of which display intraspecific variation in FPC concentrations[17]. The amount of *E. globulus* and *E. viminalis* foliage that koalas eat in captivity is inversely related to FPC concentration, but concentrations in *E. ovata* never reach levels sufficient to deter koalas from feeding[8], so we did not consider that species in this study. Only 16% of 1,264 observations of koala use of *Eucalyptus* trees were in *E. ovata*, so this species accounts for only a small proportion of tree use in this woodland.

We described two key aspects of the foliar chemistry of all 857 *E. globulus* and *E. viminalis* trees present within the 7.6-hectare *Eucalyptus* woodland: total FPC concentration (indicating defence) and total nitrogen concentration (indicating nutritional quality) of the foliage. *Eucalyptus* contains extremely low nitrogen concentrations[18] and many studies have concluded that nitrogen is of particular importance to the koala[19–23]. Regression analysis showed no significant relationship between FPC and nitrogen concentrations in *E. globulus* and only a very weak, positive relationship in *E. viminalis* ($r^2 = 0.019$, $P < 0.01$).

Having characterized the foliar chemistry of the entire populations of both study species, we adopted a new approach to investigating the relationship between tree attributes (both chemical and morphological) and koala visitation rates. Conventional regression techniques were inappropriate for modelling the relationship between tree visitation and chemical or morphological attributes, because the expected relationship was triangular, rather than linear[24]. Although we predicted that trees with high FPC concentrations would receive low rates of koala visitation, we expected variable rates of visitation to trees with low FPC concentrations. Even if trees possess low levels of defence, they may be visited only rarely if an unmeasured attribute makes them unattractive to folivores, or simply if the requirements of the folivore population are met by other equally or more suitable trees.

The first attribute we tested was tree size (circumference), because we expected that bigger trees would receive more koala visits, as they represent larger food patches and account for a greater proportion of the foliar biomass available to koalas. We compared the observed distribution of koala visits across trees to the expected distribution if koalas had visited trees randomly and found that koalas used trees that were on average significantly larger than expected (Table 1).

Table 1 | Observed and expected mean tree circumferences

	E. globulus	*E. viminalis*
n_{obs}	907	421
n_{avail}	484	373
$\bar{\mu}_{obs}$	205.3	111.8
$\bar{\mu}_{exp}$	127.8	62.6
95% confidence interval	121.9–133.7	57.4–68.0
$P(\bar{\mu}_{obs} \leq \bar{\mu}_{exp})$	< 0.0001	< 0.0001

Mean tree circumference ($\bar{\mu}_{obs}$, in centimetres) was calculated across all n_{obs} observed koala visits and compared to the expected mean circumference ($\bar{\mu}_{exp}$). $\bar{\mu}_{exp}$ and its 95% confidence interval were estimated by randomly selecting n_{obs} values (with replacement) from the list of n_{avail} available trees.

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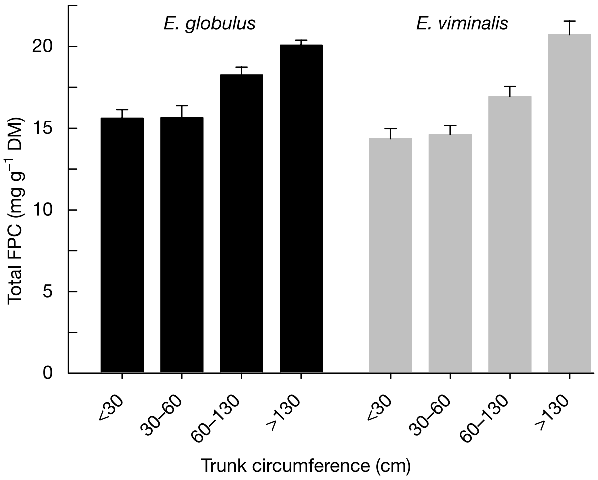

Figure 1 | FPC concentrations in four tree size classes. Mean FPC concentrations (with one standard error) in each of four size classes of tree, for *E. globulus* (black bars) and *E. viminalis* (grey). DM, dry matter basis.

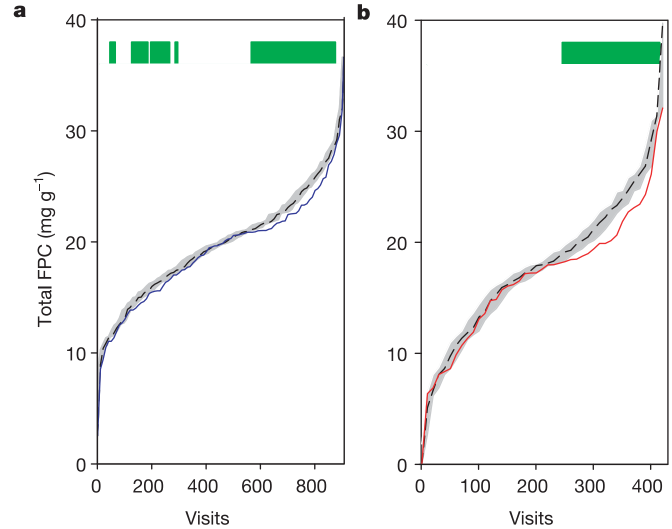

Figure 2 | Distribution of koala visits across FPC concentrations. Observed (coloured lines) and expected (dashed black lines with 95% confidence intervals determined by randomization indicated by grey shading) cumulative distribution functions of tree visitation with respect to FPC concentrations for (**a**) *E. globulus* and (**b**) *E. viminalis*. Green bars highlight visits to trees with significantly lower-than-expected FPC concentrations. Data has been constrained to the observed pattern of tree size selection.

In both species, FPC concentration was positively correlated with tree size (Supplementary Information, also Fig. 1), so by biasing their visits towards larger-than-average trees, koalas were limiting their dietary choices to a subset of trees with higher-than-average FPC concentrations. Therefore, we designed a randomization test to determine whether foliar chemistry influenced visitation rates once the distribution of visits with respect to tree size had been accounted for. We found that mean FPC concentrations were significantly lower and mean nitrogen concentrations were significantly greater than expected among trees visited by koalas (Table 2). However, the observed cumulative distributions of visits across FPC concentrations (Fig. 2) deviated most strongly from the expected distributions at high FPC concentrations, demonstrating that FPCs were only effective in reducing koalas' use of the most highly defended trees. In contrast, trees with moderately high FPC concentrations were used more than expected, and trees with median-to-low FPC concentrations were visited at the rate expected. Similarly, koala visitation rates were lower than expected for trees with low foliar nitrogen concentrations, but were as expected for trees with high concentrations of nitrogen (Fig. 3).

Although subtle, the differences observed between the foliar chemistry of trees visited by koalas and those available to the animals are the result of reduced rates of koala visitation to the most highly defended trees in the woodland, which also tend to be the largest food patches. Koalas are highly specialized folivores and as such are remarkably tolerant of PSMs. Less-specialized folivores of *Eucalyptus*, which have lower tolerances for FPCs[14], are likely to be deterred from an even larger proportion of trees. The selective herbivory that we have demonstrated may impose evolutionary pressure on the aspects of primary and secondary plant chemistry that we measured. Support for this idea comes from the fact that koala herbivory can seriously affect the fitness of these trees by causing extensive defoliation and mortality[25] and that resistance to mammalian browsing and FPC concentrations have a strong genetic basis[26]. FPC and nitrogen concentrations vary significantly both between[15,17] and within *Eucalyptus* species, including within-species variation along gradients of altitude and plant productivity[27].

Table 2 | Observed and expected foliar chemistry for all koala visits

	E. globulus		*E. viminalis*	
	FPC	Nitrogen	FPC	Nitrogen
n_{obs}	907	907	421	421
n_{avail}	484	484	373	373
$\bar{\mu}_{obs}$	19.18	12.54	16.82	16.16
$\bar{\mu}_{exp}$	19.72	12.45	17.85	15.97
95% confidence interval	19.41–20.06	12.36–12.53	17.28–18.43	15.80–16.14
$P(\bar{\mu}_{obs} \geq \bar{\mu}_{exp})$	<0.0001		<0.0001	
$P(\bar{\mu}_{exp} \leq \bar{\mu}_{obs})$		0.017		0.013

Data has been constrained to the observed pattern of tree size selection. Mean total FPC and nitrogen concentrations ($\bar{\mu}_{obs}$, in mg g^{-1}) were calculated across all n_{obs} observed koala visits and compared to the expected mean concentrations ($\bar{\mu}_{exp}$). $\bar{\mu}_{exp}$ and its 95% confidence interval were estimated by randomly selecting (with replacement) a number of trees from each of four size classes to match the number of trees of each size class visited by koalas.

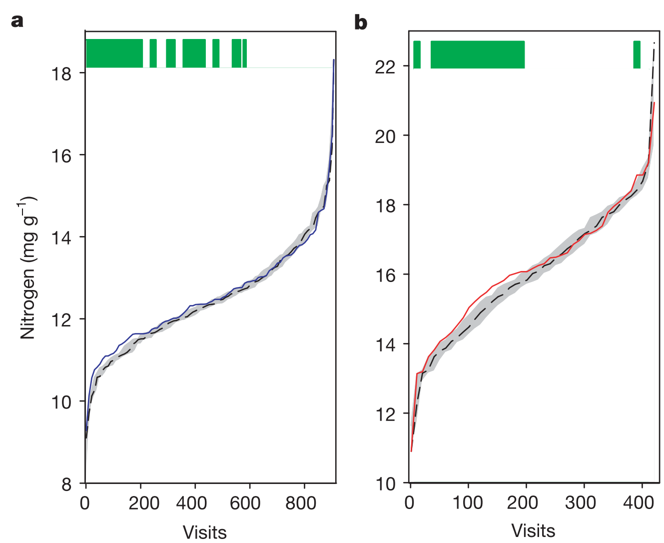

Figure 3 | Distribution of koala visits across nitrogen concentrations. Observed (coloured lines) and expected (dashed black lines with 95% confidence intervals determined by randomization indicated by grey shading) cumulative distribution functions of tree visitation with respect to nitrogen concentrations for (**a**) *E. globulus* and (**b**) *E. viminalis*. Green bars highlight visits to trees with significantly greater-than-expected nitrogen concentrations. Data has been constrained to the observed pattern of tree size selection.

Accordingly, we expect that the proportion of trees from which koalas are deterred by high FPC and low nitrogen concentrations varies between sites, providing a mechanism for foliar chemistry to influence the distribution and abundance of this vertebrate herbivore.

METHODS

Koala tree visitation. The study was conducted at the Koala Conservation Centre on Phillip Island (38° 28′ S, 145° 13′ E). Observations of diurnal tree use by individual koalas were collected at monthly intervals between 1993 and March 2004 by a community group, under the supervision of park rangers. On each occasion, the entire reserve was searched systematically for koalas, and the identities of all koalas found and of the trees occupied were recorded. Approximately 20 koalas were present in the reserve at all times throughout the study. The reserve was enclosed by a koala-proof fence; however, koalas in the reserve forage and breed naturally. Several studies have concluded that tree visitation is a reasonable measure of koala foraging[28].

Foliage collection and analysis. All trees in the woodland were individually numbered and mapped, and their circumference at a height of 130 cm recorded. In January 1997, foliage was sampled from the canopy of each *Eucalyptus* tree. Standard methods were used for processing and subsequently for collecting near infrared spectra from these samples[4,29]. Subsets of samples from each species were analysed for their FPC content and nitrogen concentration using established methods[4,17], allowing predictive calibrations to be developed for each of these constituents using Near Infrared Reflectance Spectroscopy (NIRS)[29]—details of these calibration equations are provided as Supplementary Information.

Randomization tests. Two types of randomization test were used. Initially we tested whether koalas visited trees randomly with respect to size. For each tree species, we generated a list of trees the same length as the list of observed koala visits to that species, by a process of random selection (with replacement) from all trees available. We performed 10,000 iterations of this procedure to determine whether the mean circumference of trees visited by koalas was greater than expected (a one-tailed test), by directly calculating the proportion of randomly generated distributions with means equal to or greater than that observed. Next, we modified the randomization procedure to generate 'expected' cumulative distribution functions of tree visitation with respect to both aspects of foliar chemistry, given the observed pattern of tree visitation with respect to tree size. We divided the trees into four size classes and then randomly selected (with replacement) a number of trees from each class to match the observed number of koala visits to trees of that size class. We performed 10,000 iterations of this procedure in order to generate expected cumulative visitation functions with 95% confidence intervals.

Received 12 January; accepted 15 March 2005.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We are grateful to A. Reed and 'Friends of the Koalas Inc.' for the use of their koala data; I. Lawler , K. Handasyde, I. Wallis, R. Bednarik, M. Ebbers and K. Marsh for assistance in the field and laboratory; Phillip Island Nature Park for allowing access to the Koala Conservation Centre and for providing accommodation to B.D.M.; the Australian Research Council for funding to W.J.F.; and C. Johnson, M. Jennions, J. DeGabriel and M. Symonds for comments on the manuscript.

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