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Plant secondary metabolites and vertebrate herbivores – from physiological regulation to ecosystem function

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Plant secondary metabolites can constrain the diet of vertebrates and these effects can flow through to community dynamics. Recent studies have moved beyond attempting to correlate diet choice with secondary metabolite profiles and instead focus on mechanisms that animals use to detect toxins and to regulate their intake and absorption. These include molecularly determined taste specificity, serotonin-mediated learning and the control of toxin absorption by permeability-glycoproteins. Focus on the detoxification pathways employed by specialist and generalist herbivores has facilitated explicit tests of the long-standing hypothesis that detoxification rates limit feeding. Understanding the molecular basis of differences amongst species in their tolerance of plant secondary metabolites opens many opportunities for understanding the evolutionary history of interactions between vertebrates and their food plants.

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Introduction

For more than 30 years, plant secondary metabolites (PSMs) have been seen as important regulators of feeding and dietary niche in vertebrates. Throughout this period, ecologists have had a clear set of hypotheses concerning the limitations placed on herbivores by their ability to detoxify plant toxins [1]. However, the complexities of plant chemistry and animal metabolism, and the difficulty of scaling laboratory findings to the field, mean that there is little convincing evidence of how secondary metabolites change or restrict the nutritional outcomes for wild vertebrates.

Influential theories of plant resource allocation (e.g. the carbon–nutrient balance) stimulated a focus on the abiotic factors that influence the allocation of plant resources to plant secondary metabolites that deter vertebrates. These are non-adaptive, supply-side theories that assign a minor role to herbivory. Given recent challenges to these theories [2], we can expect a re-evaluation of more demand-side, or adaptive, theories of chemical defence, such as optimal defence theory. These allow herbivory to play a driving role in shaping patterns of plant defence. This shift in focus might 'put the animal back in plant–animal interactions' [3].

A major impediment to progress in many field studies of vertebrate herbivory has been the difficulty of chemically characterizing the PSMs of interest. Consequently, rather than studying compounds that have specific chemical actions as originally envisaged [1], there has been a tendency to use general measures of broad groups of PSMs such as 'tannins' or 'total phenolics'. Although it is clear that a detailed understanding of plant chemistry is valuable [4,5], the use of broad class-wide assays persists, particularly of tannins, and some comment on the directions of those studies is worthwhile.

Tannins as determinants of diet selection — ecological versus agricultural studies

Over the past decade, studies of tannins in the diet selection and foraging of wild vertebrates have declined in ecology but flourished in agricultural systems. In ecology, colorimetric quantification of tannins has rarely yielded convincing correlations with diet selection (but see [6]). By contrast, many agricultural studies use the non-toxic polymer polyethylene glycol (PEG) to block the protein-binding action of tannins [7]. This approach is powerful because it allows natural diets instead of isolated tannins to be investigated and because the approach can be developed into functional assays [8]. For example, supplementation with PEG has revealed that condensed tannins can have positive effects on domestic ruminants, including increased wool growth and reproductive efficiency and reduced intestinal parasitism [9•,10•]. Not surprisingly, the concentration and molecular structure of the tannin is an important mediator of these effects.

Identifying and characterizing specific tannins or phenolics should be the ultimate goal, and assays that are demonstrably relevant to animal feeding [11] should be the focus of studies of tannins and wild vertebrates. Nonetheless, it seems that the use of PEG has outstripped an understanding of the interaction between PEG and plant chemistry.

Regulation of PSM intake, rather than outright avoidance, is key for vertebrates

The ubiquity of PSMs in trees and shrubs means that it is not possible for vertebrates to avoid them altogether. Some plants contain highly toxic principles (e.g. monofluoroacetate) but not all PSMs threaten rapid death. Nonetheless, even PSMs of low toxicity that are ingested by vertebrates must be removed from the body to avoid chronic illness. This implies, first, that animals should detect and regulate toxin intake; second, that animals should have detoxification capacities that match the range and volume of toxins encountered; and, third, that animals may have mechanisms that reduce the absorption of ingested PSMs from the gastrointestinal tract, so lessening the requirement for metabolically costly detoxification. These three themes have been the focus for a range of recent studies.

Detection and regulation of PSM intake

Evidence that vertebrates can regulate their intake of PSMs comes from studies in which captive animals are fed diets that contain varying concentrations of PSMs. Intake of the PSMs reaches an asymptote, which is not exceeded even when the concentration of PSM in the diet increases [12]. Animals must detect impending toxicosis and translate it into changes in feeding behaviour. Many PSMs damage cells in the stomach and small intestine, causing the release of serotonin, which stimulates the nausea centres of the brain [13]. Nausea is a powerful stimulus to learning, and animals can associate this experience with the tastes and flavours of foods to develop conditioned food aversions [13]. It remains unclear, however, how well these laboratory studies can be generalised to free-ranging herbivores [14,15]. Nonetheless, the ability of animals to learn rapidly about the negative consequences of foods and to choose diets that ameliorate these negative consequences and maximise their nutritional gain is central to understanding diet selection in chemically complex environments [16••,17].

Taste as a determinant of diet choice

Recent discoveries of new taste receptors have revealed that different bitter taste receptors detect particular bitter tastants and might be under diversifying selection to recognize bitter compounds that are specific to species' evolutionary experiences [18,19]. Because many plant toxins taste bitter, this process is probably driven by exposure to PSMs. For example, many herbivores possess inhibition taste thresholds for tannins that are considerably greater than those for alkaloids. Recently discovered taste receptors for L-enantiomeric amino acids [20] might also be relevant to the detection of toxic amino acids. Developments in sensory physiology provide a means to study how taste, separate from post-ingestive consequences, might play a role in an animal's choices between toxin-rich plants.

Detoxification rate as a constraint on feeding

The idea that the rate at which animals can detoxify and excrete PSMs acts as a constraint on their intake seems straightforward. However, testing these ideas requires an understanding of the metabolic fate of PSMs in vertebrates. Nearly all studies of this sort focus on terpenes as a model for understanding the ecological consequences of PSM detoxification. Although terpenes appear to be low-potency toxins, especially for specialized herbivores, they occur widely and comprise part of the natural diet of many herbivores. It is clear that the detoxification of terpenes and other substrates is, energetically expensive in most cases [21•,22], although there are some exceptions [23].

Behavioural studies show that captive animals that are challenged with high concentrations of PSMs eat smaller meals and spread their total feeding time out over a longer period [24]. This feeding behaviour reduces instantaneous loads on detoxification systems, but animals face an overall daily ceiling of detoxification capacity. Terpenes are detoxified by a suite of cytochrome-P450-dependent monooxygenases (CYP450 enzymes), which are rapidly inducible on exposure to terpenes *in vivo* [25]. The extent of subsequent conjugation appears to vary, with specialist herbivores relying more on extensive oxidation to produce water-soluble metabolites for excretion and generalists relying more on conjugation reactions such as glucuronidation (Figure 1; [26,27]). In addition to reducing the energetic costs of terpene excretion for specialists, the polyoxygenation strategy also frees up conjugation pathways for the disposal of phenolic compounds [28]. The trade-off, however, might be a reduced capacity for glucuronidation. This can reduce specialists' ability to detoxify PSMs that they do not normally encounter, such as nordihydroguaiaretic acid (NDGA) in creosote resin [21•], making their specialisation obligate rather than facultative.

The key question posed by these observations is the extent to which the saturation of specific detoxification pathways can limit feeding on diets that contain either a single PSM or multiple types of PSMs. In common brushtail possums feeding on *Eucalyptus* foliage, there is no evidence of the saturation of the specific enzymatic reactions that oxidise the terpene 1,8-cineole, and the capacity for detoxifying cineole far exceeds the typical daily intake [29•]. However, saturation of the conjugation pathways can occur, and Marsh *et al*. [30] has recently shown that improving the capacity for conjugation of PSMs allows feeding rates to increase. Furthermore, inhibition of CYP450 enzymes by some PSMs, including certain terpenes, might inhibit the microsomal detoxification of others [31]. Further studies that link rates of detoxification to feeding and diet selection should be a priority, as should studies that investigate how animals

Figure 1
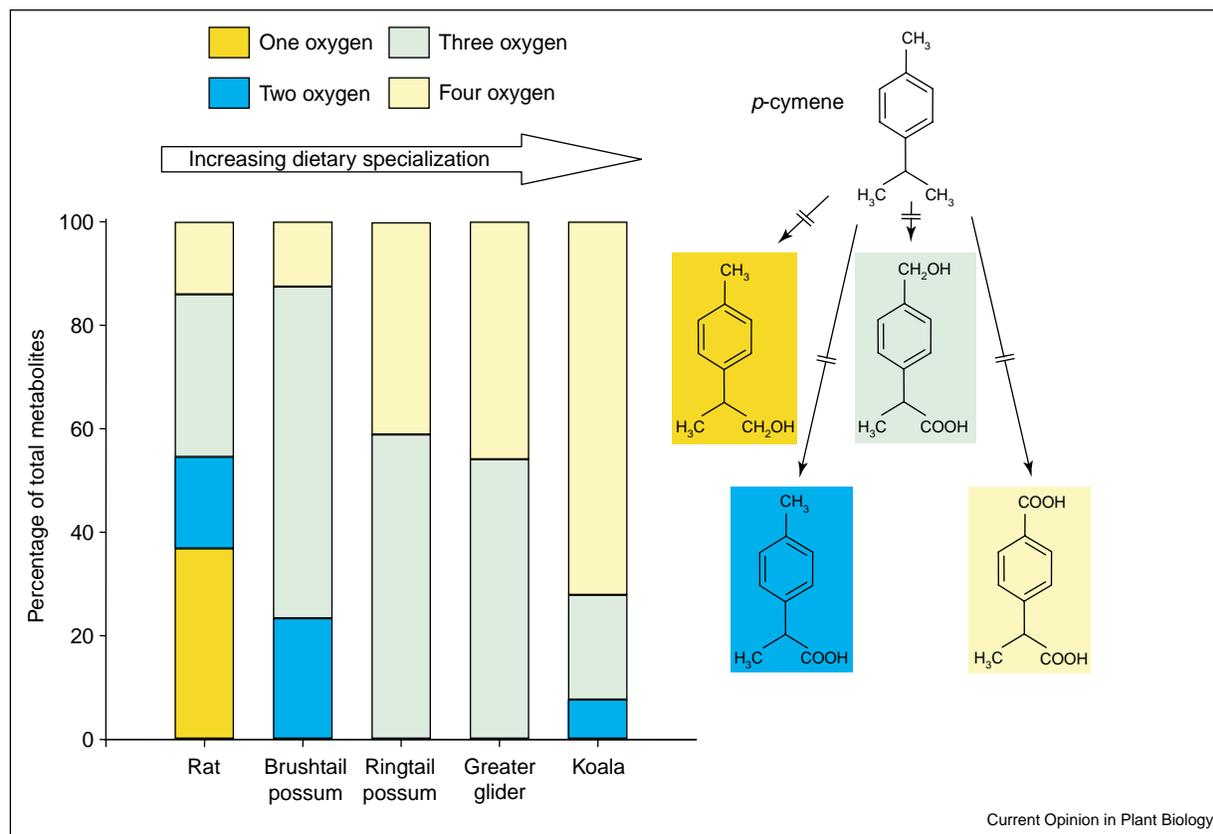
Variation in the metabolism of the terpene *p*-cymene in marsupials that have different degrees of specialization on *Eucalyptus* foliage: brushtail possum (*Trichosurus vulpecula*), ringtail possum (*Pseudocheirus peregrinus*), greater glider (*Petauroides volans*) and koala (*Phascolarctos cinereus*). Rat data are included for comparison. The data indicate the number of oxygen atoms that are added to metabolites in each species. More specialized species have a greater reliance on polyoxygenation, which creates water-soluble compounds without the need for conjugation to other molecules. Examples of the structure of a common metabolite in each class are shown on the right [26].

translate these metabolic bottlenecks into feeding decisions.

Permeability glycoprotein can limit the absorption of PSMs

The possibility that mammals can avoid absorbing PSMs that they have ingested is an exciting recent perspective. In one study, the specialist woodrat *Neotoma stephensi* absorbed a smaller proportion of ingested α-pinene than did a related woodrat that has a broader diet, *Neotoma albigula* [32]. This difference might be due to the effects of membrane-bound transport proteins, such as permeability glycoprotein (P-gp), that actively exclude many drugs and PSMs from the barrier epithelia of the small intestine, thus preventing their absorption. *In vitro* experiments confirm that the specialist woodrat has a greater intestinal P-gp capacity than the generalist species [33•]. Evaluating the importance of these effects *in vivo* will require studies that use specific P-gp inhibitors. As new P-gp inhibitors are discovered [34], we might also discover whether P-gps play a role in natural systems,

perhaps by enabling plants to improve the effectiveness of other chemical defences.

Differences between vertebrate groups in sensitivity to PSMs — evidence for directed dispersal of fruits

There are some major differences in the susceptibility of birds and mammals to the antifeedant effects of PSMs. Whereas mammals are deterred by the pain induced by irritants such as capsaicin, birds remain unaffected. By contrast, many mammals are indifferent to strongly bitter tastes but birds are not [35]. Recent research has demonstrated that the differential susceptibility of birds and mammals to capsaicin has a distinct molecular basis in the vanilloid receptor [36••]. These molecular and behavioural differences in the impact of capsaicin on birds and mammals offer significant support to the idea that fruits might direct their dispersal strategies to particular frugivores. For example, capsaicin protects *Capsicum* seeds from mammalian seed predators yet promotes their effective dispersal by avian frugivores [37].

More generally, there is an increasing recognition that PSMs have been underplayed as important factors in the evolutionary ecology of fruit dispersal by vertebrates [38] and also perhaps in pollination [39]. Progress in understanding vertebrate frugivore–plant interactions has come from determining how secondary metabolites are distributed in fruits, at various scales, in systems with well-characterised PSMs. Of particular significance is a recent study of the anthraquinone emodin in buckthorn (*Rhamnus alaternus*), which provides the first detailed evaluation of how secondary metabolites vary with other fruit traits [40], and a study of glycoalkaloids in horsenettle (*Solanum carolinense*) [41], which suggests that variations in resource availability have little effect on fruit secondary chemistry. Both of these studies suggest that nutrient-rich fruits are better protected by PSMs, which gives support to the idea that fruit defences are a compromise between attracting dispersers and deterring predators [42].

Evolutionary impact of plant secondary metabolites

If detailed examples of interactions between plants and individual herbivore species are to lead to an understanding of the role of PSMs in shaping populations and communities, ecologists must take account of the biochemical diversity that exists in natural plant populations and communities and of how this is generated and maintained. To demonstrate that herbivores play a role in this process, it must be shown in each case that the plant defence in question is effective, that the defensive trait is heritable, and that herbivory negatively impacts upon plant fitness. At present, few examples demonstrate all of these three principles, largely because of the difficulty in measuring the lifetime fitness of long-lived plants. Common-environment experiments have shown that resistance of *Eucalyptus globulus* to marsupial herbivory and of cottonwood to beaver herbivory, which are attributable to sideroxylonal and condensed tannin, respectively, have strong genetic bases [43,44]. Concentrations of monoterpenes that deter deer from feeding on western red cedar also have a genetic basis [45]. A comparison of island and mainland populations of these trees suggests that the level of defence is greater in trees that have co-evolved with deer herbivory [46]. Under weak browsing pressure, however, defence is only weakly related to the ability of young trees to escape herbivory [47]. Similarly, in a study of silver birch, vole herbivory did not affect seedling mortality, although insect herbivory did [48].

Intraspecific differences in the concentrations of deterrent PSMs contribute to the patchiness of habitats for vertebrate herbivores [49], which influences how animals forage [50•,51]. By contrast, variation in the responses of individual vertebrates or vertebrate populations to PSMs remains largely unknown. Variation in both plants and herbivores is the raw material upon which co-evolutionary processes act to shape ecological systems. We should expect individual animals to vary in their susceptibility to PSMs but unravelling these links requires a better understanding of the molecular targets of PSMs.

Population-level studies provide strong evidence for co-evolutionary relationships between plants and vertebrates [52]. Notably, Twigg *et al.* [53] have built on earlier extensive evidence of the co-evolution of monofluoroacetate-bearing vegetation and Australian vertebrates, initiating inter-population breeding studies to explore the genetic processes that are involved in developing tolerance to this highly toxic compound. The extensive use of fluoroacetate as a vertebrate poison in some parts of Australia, together with the variable resistance shown by both native and, now, introduced populations of vertebrates [54], provides a superb opportunity to understand the processes involved in past events in the co-evolution of plants and herbivores.

Conclusions

Interactions between plant chemistry and vertebrates play an important part in determining how ecosystems function. For example, the monoterpene diversity of individual Scots pine trees can influence the diversity of the associated ground-layer vegetation [55], and condensed tannins from cottonwood influence the mineralisation rates of soil nitrogen [56]. These chemical attributes are largely determined genetically and contribute to a plant's extended phenotype [57]. As with all phenomena in plant–herbivore interactions, however, animals play an equally important role in shaping community structure. In an important synthesis, Provenza and colleagues [16••] have outlined how browsing and grazing by mammalian herbivores can enhance or reduce plant chemical and species diversity across landscapes, depending on the animals' previous experience with chemically diverse diets.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Freeland WJ, Janzen DH: **Strategies in herbivory by mammals: the role of plant secondary compounds**. *Am Nat* 1974, **108**:269-289.

2. Hamilton JG, Zangerl AR, DeLucia EH, Berenbaum MR: **The carbon–nutrient balance hypothesis: its rise and fall**. *Ecol Lett* 2001, **4**:86-95.

3. Karban R, Agrawal AA: **Herbivore offense**. *Annu Rev Ecol Sys* 2002, **33**:641-664.

4. Bryant JP: **Winter browsing on Alaska feltleaf willow twigs improves leaf nutritional value for snowshoe hares in summer**. *Oikos* 2003, **102**:25-32.

5. Moore BD, Foley WJ, Wallis IR, Cowling A, Handasyde KA: ***Eucalyptus* foliar chemistry explains koala feeding preferences**. *Biol Lett* 2005, **1**:64-67.

6. Robbins CT, Hanley TA, Hagerman AE, Hjeljord O, Baker DL, Schwartz CC, Mautz WW: **Role of tannins in defending plants against ruminants —reduction in protein availability**. *Ecology* 1987, **68**:98-107.

7. Silanikove N, Perevolotsky A, Provenza FD: **Use of tannin-binding chemicals to assay for tannins and their negative post-ingestive effects in ruminants**. *Anim Feed Sci Tech* 2001, **91**:69-81.

8. Jones RJ, Palmer B: ***In vitro* digestion studies using ^{14}C-labelled polyethylene glycol (PEG) 4000: comparison of six tanniniferous shrub legumes and the grass *Panicum maximum***. *Anim Feed Sci Tech* 2000, **85**:215-221.

9. Min BR, Barry TN, Attwood GT, McNabb WC: **The effect of**
•   **condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review**. *Anim Feed Sci Tech* 2003, **106**:3-19.
An excellent up-to-date review that draws together a scattered and often confusing literature on the effects of tannins on domestic ruminants. The review highlights the many positive effects of tannins on ruminant production, which are largely achieved by reducing the rumen degradation of forage proteins. These proteins then become available to be digested in the small intestine. The mechanism of effects on the helminth parasite burden is less clear.

10. Hutchings MR, Athanasiadou S, Kyriazakis I, Gordon IJ: **Can**
•   **animals use foraging behaviour to combat parasites?** *Proc Nutr Soc* 2003, **62**:361-370.
This review provides a strong framework for understanding how parasites might influence foraging in vertebrates. It integrates foraging behaviour with the effects of PSMs on parasite loads and evaluates the possibility of self-medication with PSMs as a strategy to reduce parasite burdens.

11. Marsh KJ, Foley WJ, Cowling A, Wallis IR: **Differential susceptibility to *Eucalyptus* secondary compounds explains feeding by the common ringtail (*Pseudocheirus peregrinus*) and common brushtail possum (*Trichosurus vulpecula*)**. *J Comp Physiol [B]* 2003, **173**:69-78.

12. Lawler IR, Foley WJ, Pass GJ, Eschler BM: **Administration of a 5HT$_3$ receptor antagonist increases the intake of diets containing *Eucalyptus* secondary metabolites by marsupials**. *J Comp Physiol [B]* 1998, **168**:611-618.

13. Provenza FD, Villalba JJ, Cheney CD, Werner SJ: **Self-organization of foraging behaviour: from simplicity to complexity without goals**. *Nutr Res Rev* 1998, **11**:199-222.

14. Duncan AJ, Young SA: **Can goats learn about foods through conditioned food aversions and preferences when multiple food options are simultaneously available?** *J Anim Sci* 2002, **80**:2091-2098.

15. Ginane C, Duncan AJ, Young SA, Elston DA, Gordon IJ: **Herbivore diet selection in response to simulated variation in nutrient rewards and plant secondary compounds**. *Anim Behav* 2005, **69**:541-550.

16. Provenza FD, Villalba JJ, Dziba LE, Atwood SB, Banner RE:
•• **Linking herbivore experience, varied diets, and plant biochemical diversity**. *Small Rum Res* 2003, **49**:257-274.
A highly stimulating paper that provides a framework for integrating the learning abilities of individual animals with variations in toxin and nutrient intake. The authors argue that plant biochemical diversity that is maintained through factors such as selective feeding and associational defence increases the resilience and productivity of ecosystems by increasing options for plants, herbivores, and people deriving a living from grazing animal systems.

17. Villalba JJ, Provenza FD: **Foraging in chemically diverse environments: energy, protein, and alternative foods influence ingestion of plant secondary metabolites by lambs**. *J Chem Ecol* 2005, **31**:123-138.

18. Margolskee RF: **Molecular mechanisms of bitter and sweet taste transduction**. *J Biol Chem* 2002, **277**:1-4.

19. Shi P, Zhang JZ, Yang H, Zhang YP: **Adaptive diversification of bitter taste receptor genes in mammalian evolution**. *Mol Biol Evol* 2003, **20**:805-814.

20. Nelson G, Chandrashekar J, Hoon MA, Feng LX, Zhao G, Ryba NJP, Zuker CS: **An amino-acid taste receptor**. *Nature* 2002, **416**:199-202.

21. Sorensen JS, McLister JD, Dearing MD: **Novel plant secondary**
•   **metabolites impact dietary specialists more than generalists (*Neotoma* spp.)**. *Ecology* 2005, **86**:140-154.
A broad-ranging and integrative study that tests a fundamental prediction about dietary specialization. The authors show that woodrat species that specialize in eating juniper have a higher capacity to eliminate PSMs from juniper than do generalists. When faced with eating novel PSMs, however, the specialists eat less, have less energy to spend on activities such as locomotion and lose more body mass than do generalists. The authors argue that these results suggest that dietary specialization requires tradeoffs in other capacities.

22. Sorensen JS, McLister JD, Dearing MD: **Plant secondary metabolites compromise the energy budgets of specialist and generalist mammalian herbivores**. *Ecology* 2005, **86**:125-139.

23. McLean S, Pass GJ, Foley WJ, Brandon S, Davies NW: **Does excretion of secondary metabolites always involve a measurable metabolic cost? Fate of plant antifeedant salicin in common brushtail possum, *Trichosurus vulpecula***. *J Chem Ecol* 2001, **27**:1077-1089.

24. Wiggins NL, McArthur C, McLean S, Boyle R: **Effects of two plant secondary metabolites, cineole and gallic acid, on nightly feeding patterns of the common brushtail possum**. *J Chem Ecol* 2003, **29**:1447-1464.

25. Pass GJ, McLean S, Stupans I, Davies N: **Microsomal metabolism of the terpene 1,8-cineole in the common brushtail possum (*Trichosurus vulpecula*), koala (*Phascolarctos cinereus*), rat and human**. *Xenobiotica* 2001, **31**:205-221.

26. Boyle R, McLean S, Foley WJ, Moore BD, Davies NW, Brandon S: **Fate of the dietary terpene, *p*-cymene, in the male koala**. *J Chem Ecol* 2000, **26**:1095-1111.

27. Lamb JG, Marick P, Sorensen J, Haley S, Dearing MD: **Liver biotransforming enzymes in woodrats *Neotoma stephensi* (Muridae)**. *Comp Biochem Physiol C* 2004, **138**:195-201.

28. McLean S, Brandon S, Davies NW, Boyle R, Foley WJ, Moore B, Pass GJ: **Glucuronuria in the koala**. *J Chem Ecol* 2003, **29**:1465-1477.

29. Boyle RR, McLean S: **Constraint of feeding by chronic ingestion**
•   **of 1,8-cineole in the brushtail possum (*Trichosurus vulpecula*)**. *J Chem Ecol* 2004, **30**:757-775.
The first study to use a pharmacological approach to question whether saturation of detoxification pathways might explain how plant secondary metabolites limit feeding by vertebrates.

30. Marsh KM, Wallis IR, Foley WJ: **Detoxification rates constrain feeding in common brushtail possums (*Trichosurus vulpecula*)**. *Ecology* 2005, in press.

31. Pass GJ, McLean S: **Inhibition of the microsomal metabolism of 1,8-cineole in the common brushtail possum (*Trichosurus vulpecula*) by terpenes and other chemicals**. *Xenobiotica* 2002, **32**:1109-1126.

32. Sorensen JS, Turnbull CA, Dearing MD: **A specialist herbivore (*Neotoma stephensi*) absorbs fewer plant toxins than does a generalist (*Neotoma albigula*)**. *Physiol Biochem Zool* 2004, **77**:139-148.

33. Green AK, Haley SL, Dearing MD, Barnes DM, Karasov WH:
•   **Intestinal capacity of P-glycoprotein is higher in the juniper specialist, *Neotoma stephensi*, than the sympatric generalist, *Neotoma albigula***. *Comp Biochem Physiol A* 2004, **139**:325-333.
This exciting study provides the first data on the suggestion [32] that animals might avoid absorbing ingested secondary plant compounds through the actions of permeability glycoproteins in the intestine.

34. Zhou SF, Lim LY, Chowbay B: **Herbal modulation of P-glycoprotein**. *Drug Metab Rev* 2004, **36**:57-104.

35. Matson KD, Millam JR, Klasing KC: **Cockatiels (*Nymphicus hollandicus*) reject very low levels of plant secondary compounds**. *Appl Anim Behav Sci* 2004, **85**:141-156.

36. Jordt SE, Julius D: **Molecular basis for species-specific**
•• **sensitivity to 'hot' chili peppers**. *Cell* 2002, **108**:421-430.
The burning pain of capsaicin is due to the activation of vanilloid receptors (VR1). However, amino acid substitutions in the VR1 receptor of chickens relative to that of rats renders chickens, and presumably other birds, insensitive to capsaicin.

37. Tewksbury JJ, Nabhan GP: **Seed dispersal — directed deterrence by capsaicin in chillies**. *Nature* 2001, **412**:403-404.

38. Tewksbury JJ: **Fruits, frugivores and the evolutionary arms race**. *New Phytol* 2002, **156**:137-139.

39. Tadmor-Melamed H, Markman S, Arieli A, Distl M, Wink M, Izhaki I: **Limited ability of Palestine sunbirds *Nectarinia osea* to cope with pyridine alkaloids in nectar of tree tobacco *Nicotiana glauca***. *Funct Ecol* 2004, **18**:844-850.

40. Izhaki I, Tsahar E, Paluy I, Friedman J: **Within population variation and interrelationships between morphology, nutritional content, and secondary compounds of *Rhamnus alaternus* fruits**. *New Phytol* 2002, **156**:217-223.

41. Cipollini ML, Paulk E, Mink K, Vaughn K, Fischer T: **Defense tradeoffs in fleshy fruits: effects of resource variation on growth, reproduction, and fruit secondary chemistry in *Solanum carolinense***. *J Chem Ecol* 2004, **30**:1-17.

42. Schaefer HM, Schmidt V, Winkler H: **Testing the defence trade-off hypothesis: how contents of nutrients and secondary compounds affect fruit removal**. *Oikos* 2003, **102**:318-328.

43. Bailey JK, Schweitzer JA, Rehill BJ, Lindroth RL, Martinsen GD, Whitham TG: **Beavers as molecular geneticists: a genetic basis to the foraging of an ecosystem engineer**. *Ecology* 2004, **85**:603-608.

44. O'Reilly-Wapstra JM, McArthur C, Potts BM: **Linking plant genotype, plant defensive chemistry and mammal browsing in a *Eucalyptus* species**. *Funct Ecol* 2004, **18**:677-684.

45. Vourc'h G, Russell J, Martin JL: **Linking deer browsing and terpene production among genetic identities in *Chamaecyparis nootkatensis* and *Thuja plicata* (Cupressaceae)**. *J Hered* 2002, **93**:370-376.

46. Vourc'h G, Martin JL, Duncan P, Escarre J, Clausen TP: **Defensive adaptations of *Thuja plicata* to ungulate browsing: a comparative study between mainland and island populations**. *Oecologia* 2001, **126**:84-93.

47. Vourc'h G, Vila B, Gillon D, Escarre J, Guibal F, Fritz H, Clausen TP, Martin JL: **Disentangling the causes of damage variation by deer browsing on young *Thuja plicata***. *Oikos* 2002, **98**:271-283.

48. Prittinen K, Pusenius J, Koivunoro K, Rousi M, Roininen H: **Mortality in seedling populations of Silver Birch: genotypic variation and herbivore effects**. *Funct Ecol* 2003, **17**:658-663.

49. Brenes-Arguedas T, Coley PD: **Phenotypic variation and spatial structure of secondary chemistry in a natural population of a tropical tree species**. *Oikos* 2005, **108**:410-420.

50. Moore BD, Foley WJ: **Tree use by koalas in a chemically**
• **complex landscape**. *Nature*, in press. (Published on-line [doi:10.1038/nature03551].)
A good example of how near-infrared spectroscopy can be used to make large-scale measurements of plant chemistry and so allow the effects of PSMs to be understood in the complex foraging environments of a free-ranging herbivore.

51. Scrivener NJ, Johnson CN, Wallis IR, Takasaki M, Foley WJ, Krockenberger AK: **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* 2004, **6**:77-87.

52. Mangione AM, Dearing MD, Karasov WH: **Interpopulation differences in tolerance to creosote bush resin in desert woodrats (*Neotoma lepida*)**. *Ecology* 2000, **81**:2067-2076.

53. Twigg LE, Martin GR, Eastman AF, King DR, Kirkpatrick WE: **Sensitivity of some Australian animals to sodium fluoroacetate (1080): additional species and populations, and some ecological considerations**. *Aust J Zool* 2003, **51**:515-531.

54. Twigg LE, Martin GR, Lowe TJ: **Evidence of pesticide resistance in medium-sized mammalian pests: a case study with 1080 poison and Australian rabbits**. *J Appl Ecol* 2002, **39**:549-560.

55. Iason GR, Lennon JJ, Pakeman RJ, Thoss V, Beaton JK, Sim DA, Elston DA: **Does chemical composition of individual Scots pine trees determine the biodiversity of their associated ground vegetation?** *Ecol Lett* 2005, **8**:364-369.

56. Schweitzer JA, Bailey JK, Rehill BJ, Martinsen GD, Hart SC, Lindroth RL, Keim P, Whitham TG: **Genetically based trait in a dominant tree affects ecosystem processes**. *Ecol Lett* 2004, **7**:127-134.

57. Whitham TG, Young WP, Martinsen GD, Gehring CA, Schweitzer JA, Shuster SM, Wimp GM, Fischer DG, Bailey JK, Lindroth RL *et al.*: **Community and ecosystem genetics: a consequence of the extended phenotype**. *Ecology* 2003, **84**:559-573.