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 tannis 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., mono-fluoracetate) 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 toxicity 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 obligatory 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
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].

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**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].
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 inheritable, 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 monoterpenene 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.

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**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


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 associationist defence increases the resilience and productivity of ecosystems by increasing options for plants, herbivores, and people deriving a living from grazing animal systems.


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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.


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.


