J Chem Ecol (2006) 32: 1247–1266
DOI 10.1007/s10886-006-9082-3

REVIEW ARTICLE

The Detoxification Limitation Hypothesis: Where Did it Come From and Where is it Going?

Karen J. Marsh · Ian R. Wallis · Rose L. Andrew · William J. Foley

Received: 18 December 2005 / Revised: 2 March 2006 /
Accepted: 3 March 2006 / Published online: 23 May 2006

Abstract The detoxification limitation hypothesis is firmly entrenched in the literature to explain various aspects of the interaction between herbivores and plant toxins. These include explanations for the existence of specialist and generalist herbivores and for the prevalence of each of these. The hypothesis suggests that the ability of mammalian herbivores to eliminate plant secondary metabolites (PSMs) largely determines which plants, and how much, they can eat. The value of the hypothesis is that it provides a clear framework for understanding how plant toxins might limit diet breadth. Thus, it is surprising, given its popularity, that there are few studies that provide experimental support either for or against the detoxification limitation hypothesis. There are two likely reasons for this. First, Freeland and Janzen did not formally propose the hypothesis, although it is implicit in their paper. Second, it is a difficult hypothesis to test, requiring an understanding of the metabolic pathways that lead to toxin elimination. Recent attempts to test the hypothesis appear promising. Results suggest that herbivores can recognize mounting saturation of a detoxification pathway and adjust their feeding accordingly to avoid intoxication. One strategy they use is to ingest a food containing a toxin that is metabolized by a different pathway. This demonstrates that careful selection of food plants is a key to existing in a chemically complex environment. As more studies characterize the detoxification products of PSMs, we will better understand how widespread this phenomenon is.

Keywords Detoxification limitation hypothesis · Mammalian herbivores · Plant secondary metabolites · Diet mixing · *Trichosurus vulpecula* · Chemical defense · Food choice · Foraging

K. J. Marsh (✉) · I. R. Wallis · R. L. Andrew · W. J. Foley
School of Botany and Zoology,
Australian National University,
Canberra, ACT 0200, Australia
e-mail: karen.marsh@anu.edu.au

Introduction

Free-ranging mammalian herbivores can usually feed from a wide variety of plants, but most do not forage at random. Rather, they make careful choices about what they eat. Ultimately, these choices need to provide the animal with its nutritional requirements for maintenance, growth, and reproduction, without causing harm. It is well known that captive herbivores can select a balanced diet if given a variety of foods containing different individual nutrients (Wang and Provenza, 1997; Aranda et al., 2000; Scott and Provenza, 2000) or different concentrations of a given nutrient (Shariatmadari and Forbes, 1993; Gous and Swatson, 2000). No doubt, free-ranging herbivores can do the same. There is an important difference, however, between captive and wild animals: the latter must obtain their nutrients from plants that often have potentially toxic chemicals. Put simply, herbivores need to eat plants, but plants have developed many chemicals, known as plant secondary metabolites (PSMs), that act as defensive agents. Not surprisingly, given the diversity of plants, PSMs are extremely diverse and widespread and often occur in sufficient quantities to harm, or even kill, any animal that might eat it. As Freeland and Janzen (1974) point out, however, it is much easier to find animals that eat plants known to contain toxins than to find animals that can avoid them entirely. It seems that herbivores cannot reliably avoid ingesting PSMs, and instead have evolved a suite of mechanisms to counter their effects (Fig. 1).

There is no doubt that PSMs can influence feeding by mammalian herbivores, reducing food intake, altering dietary preferences, and sometimes even killing the animal (Foley et al., 1999). However, because of the difficulties in studying these interactions with wild animals, most of the evidence comes from domestic or captive animals. For example, cattle ingesting large amounts of alkaloids from rangeland plants or cardiac glycosides from oleander can die (Galey et al., 1998; Pfister et al., 2001). A less extreme example is of lambs that choose to eat less of a diet containing terpenes than one free of terpenes (Villalba and Provenza, 2005). Another scenario is of researchers feeding natural diets, but with only a partial understanding of the PSMs that the animal faces. *Eucalyptus* foliage, which is well known as a rich source of tannins, phenolics, and terpenes (Moore et al., 2004), to list just a few PSMs, is a prime example. Increasing concentrations of formylated phloroglucinol compounds (FPCs) in eucalypt foliage restrict feeding by marsupial folivores, but at any concentration of FPCs, there is typically wide variation in food intake (Wallis et al., 2002; Marsh et al., 2003; Moore et al., 2005). This implies that compounds other than FPCs also influence feeding.

The depression of food intake by PSMs is not uniform. There are other instances where animals have shown almost no response to extremely high dietary PSM concentrations. For example, common brushtail possums (*Trichosurus vulpecula*) maintained their food intake when the dietary concentration of rutin (a glycoside of quercetin) increased from 0 to 27% of dry matter (Marsh et al., 2006). Perhaps even more bizarre is the decline in feeding when brushtail possums are given a diet containing small amounts of free amino acids (DeGabriel et al., 2002; Marsh et al., 2005). In this case, the nutrients (amino acids) appear to be behaving as toxins.

The seemingly impossible complexity of plant–animal interactions may explain why the detoxification limitation hypothesis, emanating from a review by Freeland and Janzen (1974) some 30 years ago, has become so popular with so little conclusive testing. The hypothesis seems reasonable on an intuitive level, but

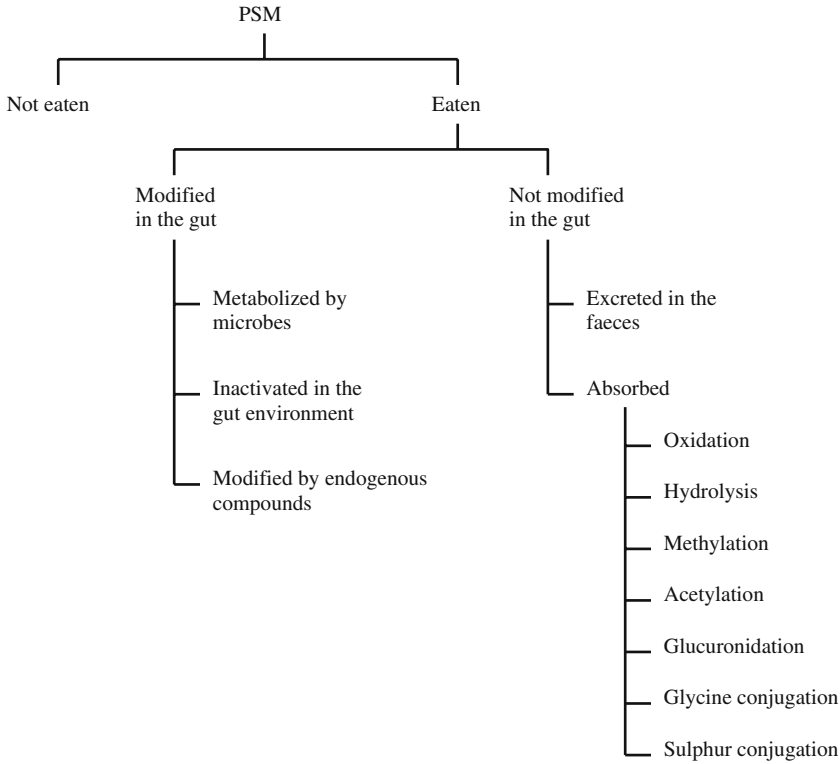

Fig. 1 Schematic of the possible fates of PSMs encountered by herbivores. Availability of alternative foods and the current detoxification capacity of the herbivore are some examples of factors that may influence whether a herbivore chooses to ingest a particular PSM. Because these factors can change, it may choose differently at different times. However, once a PSM has been ingested, its fate will depend on the particular PSM–herbivore combination

further understanding of the processes involved, as well as more specific testing, will further strengthen and refine it.

The Fate of Plant Secondary Metabolites

Animals have many defenses against PSMs (Fig. 1), the first being avoidance of plants that contain them. This may be a learned response (Provenza, 1996) or an initial reaction to the aversive sensory properties of the PSM (Foley et al., 1999). The second line of defense is to retain the PSM in the gastrointestinal tract and perhaps modify it there. This strategy might be a physiological response of the animal, or it might occur through a synergistic association with microbes. The responses of animals that ingest tannins provide examples of both mechanisms. Mule deer (*Odocoileus hemionus*) respond to tannins by producing proline-rich salivary proteins that bind them (Robbins et al., 1991), whereas koalas (*Phascolarctos cinereus)* have a symbiotic relationship with tannin-degrading bacteria (Osawa et al., 1993).

However, a great many PSMs have features that enable ready absorption from the gut—they are lipid-soluble, nonpolar, and nonionic at physiological pH (McLean and Duncan, 2006). Unless detoxified and eliminated from the body, these compounds will eventually reach harmful concentrations, although there are instances where metabolites are more toxic than the original compound (Tamási et al., 2003). For excretion in the urine—the typical mode of removal for metabolites with a molecular weight less than 500—detoxification processes convert the molecule to one that is water-soluble, contains polar groups, and is ionic at physiological pH (McLean and Duncan, 2006). Larger molecules are usually excreted in the bile and may even be reactivated to the original compound in the gut (Dearing et al., 2005).

Biotransformation (usually leading to detoxification) of PSMs occurs mainly in the liver in two phases that may occur independently or simultaneously and involve many enzymes. Phase I enzymes typically catalyze reactions such as oxidation, reduction, or hydrolysis, whereas those in phase II conjugate PSMs to polar molecules, known as conjugates. In the context of the detoxification limitation hypothesis, it is important to realize that any herbivore will potentially have many metabolic pathways in both phases to cope with the chemical diversity it might encounter (Dearing et al., 2005; McLean and Duncan, 2006). For example, there are more than 17 families of cytochrome P450 enzymes involved in oxidation reactions (Lin and Lu, 2001) and a similarly diverse range of enzymes that conjugate compounds to glucuronic acid (Radominska-Pandaya et al., 1999).

The particular sequence of reactions leading to the elimination of a compound is called the detoxification pathway. As with any chemical reaction, a pathway may become saturated for various reasons, including a depletion of enzyme or cosubstrate, resulting in the accumulation of the PSM. Thus, the time required for detoxification depends on the rate of detoxification, the concentration of PSM in the plant, the amount ingested, the proportion absorbed, and the interactions between PSMs and the physiological state of the animal, to name just a few (McLean and Duncan, 2006).

What is the Detoxification Limitation Hypothesis?

The detoxification limitation hypothesis has its origins in the seminal paper by Freeland and Janzen (1974). The paper emphasizes the complexity of the interactions between animals and PSMs, but, interestingly, the authors do not propose a hypothesis that they specifically call "the detoxification limitation hypothesis." Instead, they finish their review with a section entitled "Hypothetical feeding behavior" in which they summarize their expectations as follows:

> Generalist herbivores should: (1) treat new foods with extreme caution; (2) be able to learn quickly to eat or reject particular foods; (3) have the capacity to seek out and eat plants containing highly specific classes of nutrients; (4) have to ingest a number of different staple foods over a short period of time; (5) preferentially feed on the foods with which they are familiar, and continue to feed on them for as long as possible; (6) prefer to feed on foods that contain only minor amounts of toxic plant secondary compounds; and (7) have searching strategies and a body size that neither maximize the number of types of foods

> that are potentially available nor maximize the total amount of food eaten, but rather compromise between these two functions.

The hypothesis as we know it was more formally delivered in a later review. Freeland (1991) suggested that:

> Feeding behavior of herbivorous mammals is thus dependent on the individual mammal's capacity to detoxify and/or tolerate the biological effects of particular individual or combinations of plant secondary metabolites. Metabolites that cannot be detoxified/tolerated are likely to be avoided, while those that can be successfully detoxified are likely to be consumed. The rate at which a particular plant metabolite is detoxified is of obvious importance in determining how much a mammal eats per unit time.

The hypothesis itself has been given different names by different researchers. Some mention its predictions without giving it a name (Provenza, 1995; Foley et al., 1999; Burritt and Provenza, 2000), whereas others call it "Freeland and Janzen's hypothesis" (Iason, 2005), the "toxin dilution hypothesis" (Behmer et al., 2002; Singer et al., 2002; Duncan et al., 2003; Miura and Ohsaki, 2004), the "toxin avoidance hypothesis" (Dearing and Schall, 1992; Cassini, 1994), or the "detoxification limitation hypothesis" (Dearing and Cork, 1999; Sorensen and Dearing, 2003; Wiggins et al., 2006). It appears that the first study to propose the name "detoxification limitation hypothesis" was Dearing and Cork (1999).

Although it is more than 30 years and more than 500 citations since Freeland and Janzen, few studies have provided conclusive experimental support either for or against the hypothesis. Many researchers acknowledge that the detoxification limitation hypothesis can potentially explain aspects of the feeding behavior of herbivores (e.g., Freeland and Winter, 1975; Freeland et al., 1985; Freeland and Saladin, 1989; Dearing and Cork, 1999; Foley et al., 1999; Hagele and Rowell-Rahier, 1999; Burritt and Provenza, 2000; Behmer et al., 2002; Singer et al., 2002; Wiggins et al., 2003; Miura and Ohsaki, 2004; Rogosic et al., 2006). None of these studies, however, had the information required to rigorously test the hypothesis. This aside, if we are to understand the influence of PSMs on animal feeding, and thus their ecology, there is need for a hypothesis, and by far the most popular is the detoxification limitation hypothesis.

What Does the Detoxification Limitation Hypothesis Predict about Animal Feeding?

There appear to be three main ways that detoxification limitations might influence an animal's feeding behavior. The first suggests that feeding rates depend on the rates at which herbivores can detoxify PSMs. If rates of ingestion exceed rates of detoxification, then PSMs will accumulate. The consequences of a link between feeding and detoxification rates are twofold. First, the herbivore will need to alter its rate of feeding with changing concentrations of a PSM in the food to keep the rate of ingestion of the PSM stable (Fig. 3). Second, because detoxification systems are dynamic, an animal must adjust its rate of feeding to suit its current detoxification state, which depends on a myriad of factors discussed by Freeland and Janzen (1974)

and elsewhere (e.g., Foley et al., 1999; Dearing et al., 2005). For example, exposure to a PSM often induces the production of specific enzymes involved in detoxification (Pass et al., 1999; Boyle and McLean, 2004). An animal should alter its feeding behavior whenever the rate of detoxification changes (Fig. 2).

The second prediction of the detoxification limitation hypothesis is that specialist herbivores should be better at detoxifying the PSMs they encounter than should generalists. Specialist herbivores rely on few plants for most of their food, whereas generalist herbivores tend to eat many different plants, even when one is abundant. Generalist herbivores are much more common than specialists, probably because they do not have to rely on a limited food source for all of their lives (Freeland and Janzen, 1974). Where specialist and generalist herbivores occur sympatrically, however, such as koalas and possums in the eucalypt forests of southeastern Australia, the dietary niches of each may indicate their differing abilities to detoxify the PSMs in their food.

Finally, the detoxification limitation hypothesis suggests that because generalist herbivores are less efficient than specialists at detoxifying the PSMs that are found in a group of related plants, they must obtain their nutrients from a wider variety of plants (Sorensen and Dearing, 2003). This variety of foods, which we will refer to as "diet mixing," is thought to increase the amount that a generalist herbivore can eat. For diet mixing to succeed, the detoxification limitation hypothesis assumes that the PSMs ingested from different plants are metabolized by separate pathways. However, even the detoxification of an individual PSM is complex, with many using several pathways for detoxification. This is illustrated in Table 1, showing the detoxification pathways that brushtail possums use for six PSMs. It shows that some PSMs share pathways, and so, when ingested simultaneously, may interact. For example, two PSMs partially overlap when they are both absorbed into the bloodstream, transformed by different processes during phase I, but then conjugated with the same molecule, such as glucuronic acid, in phase II. In contrast, an animal may ingest a food rich in benzoic acid, which is rapidly absorbed and detoxified by conjugation with glycine. It might simultaneously ingest a food rich in tannins, which combine with salivary proteins and remain in the gut pending excretion (Robbins et al., 1991). Discounting any interaction in the gut, there appears little competition between the two PSMs for detoxification. The response in feeding should

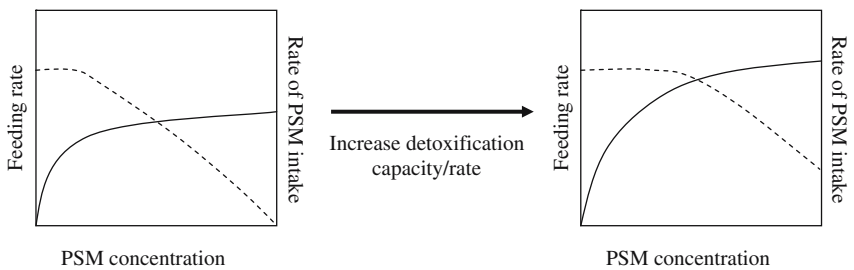

Fig. 2 The hypothetical rate of feeding and of PSM ingestion by a herbivore, for which food intake is limited by the rate of PSM detoxification. If the detoxification rate increases, we would expect that the rate of PSM ingestion would also be able to increase, allowing food intake (or the rate of feeding) to be maintained at the maximum to a higher PSM concentration. The dashed lines show changing feeding rates as PSM concentrations increase, and the solid lines show how this is linked to rates of PSM intake

Table 1 The percentage of PSMs ingested by brushtail possums that were metabolized by a given pathway in 24 hr

	1,8-Cineole	*p*-Cymene	Benzoic acid	Salicin	Orcinol	Rutin
Hydrolysis (I)				80		71
Oxidation (I)	33	51		22		71
Glycine conj. (II)		trace	90	10		
GA conj.[a] (II)	16	28	5	63	66	69

Most of the tested PSMs were metabolized by multiple mechanisms, and in some cases, both phase I and II reactions occurred (reaction type indicated in parentheses). Some columns add to more than 100% because the same molecule underwent multiple modifications. Adapted from Marsh et al., (2006).

[a] GA = glucuronic acid.

presumably correlate with the degree of competition for detoxification between the PSMs.

Can Detoxification Limitations Influence Feeding Rates?

There is little evidence either supporting or refuting any of the predictions of the detoxification limitation hypothesis. This does not discount the importance of PSMs and detoxification limitations in the interactions between herbivores and plants, but

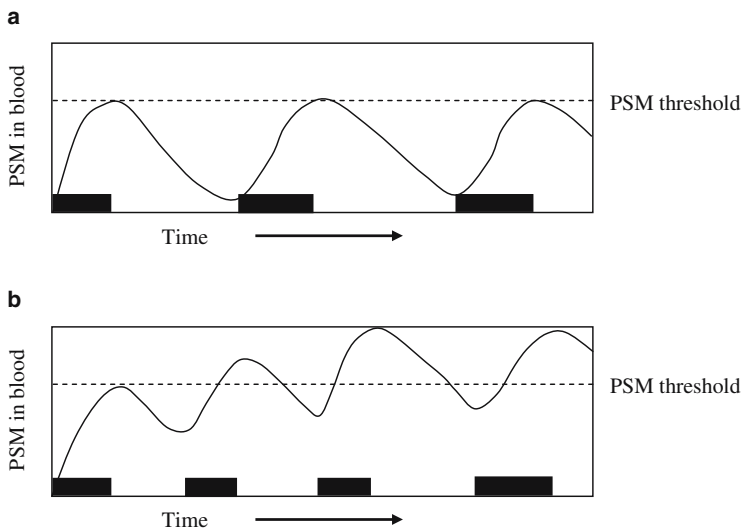

Fig. 3 (a) The detoxification limitation hypothesis suggests that the feeding behavior of a herbivore should aim to keep the amount of unmetabolized PSM in the blood below a threshold amount (dashed line). (b) If a herbivore ingests a PSM faster than it can detoxify it, then the concentration in the blood will exceed the threshold and poisoning could occur. The amount of unmetabolized PSM present at any time is a result of a combination of the rate of ingestion, the degree and rate of absorption, and the rate of detoxification. The black bars indicate times at which the herbivore is feeding, and the solid line tracks the hypothetical amount of unmetabolized PSM in its blood as it ingests a PSM with its food, and subsequently detoxifies it

rather demonstrates that it is difficult to elucidate their contribution to feeding. For example, slow rates of detoxification may explain why many mammalian herbivores ingest threshold amounts of PSMs (e.g., Pfister et al., 1997; Wang and Provenza, 1997; Lawler et al., 2000; Mangione et al., 2000; Stapley et al., 2000). In order to show that the feeding decisions of herbivores depend on the rate of detoxification, however, it is necessary to know how the herbivore of interest, in a certain physiological state, detoxifies a particular compound.

The "drink-driving" laws in many countries illustrate clearly the idea that the rate of ingestion of a substance determines toxicity (Fig. 3). It is possible to imbibe alcoholic drinks and drive a motor vehicle as long as the rate of metabolism of the alcohol equals the rate of ingestion. Ingesting alcohol at a faster rate, so that the blood alcohol concentration exceeds 50–80 mg per 100 ml, makes one unfit to drive, whereas raising the blood alcohol content to 300–400 mg per 100 ml may be lethal. This system appears simple because it involves a single toxin for which the pathway of detoxification is well known. However, recommended alcohol intakes differ for males and females, and tolerance to alcohol can differ widely among individuals in a population and among populations (Norberg et al., 2003). Other enzymes for drug metabolism show similar interindividual variability (Mulder, 1995; Lin and Lu, 2001), and different mammalian species may metabolize a particular drug at different rates and sometimes in differing ways (Walton et al., 2001), illustrating the complexity of detoxification. It is apparent that a defined experimental system is necessary to test the detoxification limitation hypothesis. Only then can we answer simple questions about interactions between herbivores and toxins, before tackling ones that are more complex. These might include questions about how toxins interact with each other and with other systems, such as the gut microbes.

Study Systems for Testing the Detoxification Limitation Hypothesis

A system for testing an animal's feeding response to any toxin has several requirements. First, it requires a species that is easy to keep in captivity, usually in a metabolism cage, and is easy to maintain on an artificial diet. The diet should provide all of the animal's nutrient requirements, be free of plant toxins, and be easily manipulated to provide, for example, different concentrations of nutrients or plant cell walls. Finally, the toxin should be a substance that the species typically encounters in the wild. It is necessary to know its mode of detoxification in the animals in question, and it must be possible to obtain enough toxin for well-replicated feeding experiments. The factor that usually prevents researchers from proving the detoxification limitation hypothesis is not knowing detoxification pathways.

Proof of the Detoxification Limitation Hypothesis

There appears to be only one study of mammalian herbivores that satisfies the requirements for a study system and shows that the rate of detoxification influences the rate of feeding. In this study, Marsh et al., (2005) fed common brushtail possums a basal diet consisting mainly of fruit and cereals, and they manipulated the concentrations of both benzoate and glycine. Benzoate is eaten by wild brushtail possums, and it is also known that a variety of animals, including brushtail possums, detoxify it by conjugation with glycine (Bridges et al., 1970; Awaluddin and

McLean, 1985). Furthermore, the rate of conjugation of benzoate depends on the supply of glycine (Griffith and Lewis, 1923; Amsel and Levy, 1969; Gregus et al., 1993). The key finding of Marsh et al., (2005) was that possums eating a diet containing both benzoate and glycine detoxified the benzoate faster and ate more than when eating a similar diet without the glycine. In other words, the rate of detoxification determined the rate of feeding. Thus, plant toxins do not have a fixed effect on feeding, but rather, their effect will depend on the animal's current detoxification state.

There is also at least one study in invertebrates showing that feeding depends on the rate of detoxification. In this study with tobacco hornworms (Snyder and Glendinning, 1996), piperonyl butoxide slowed the detoxification process by inhibiting cytochrome P450 enzymes, which are important in phase I reactions. The hornworms responded by eating less of a diet containing the plant toxin nicotine.

Both of the previous examples illustrate extremely simple cases that show a link between rates of detoxification and feeding rates. There is a need for more manipulative studies to examine a wide range of apparently simple interactions, but also to delve into complexities, such as interactions among plant toxins, and to separate effects that are not attributable to the rates of detoxification. By this, we mean that rates of feeding may depend on the concentrations of PSMs in plants but be independent of the rates of detoxification. One example is of plants that contain bitter or spicy compounds, for which the main consequence of ingestion is the stimulation of capsaicin receptors that cause a burning sensation (Jakubas and Mason, 1991; Pass and Foley, 2000). Another is of plants containing compounds that stimulate nausea or satiety receptors causing feeding to stop. For example, McLean et al., (2004) propose that some of the compounds found in eucalypt foliage are not absorbed from the gut, but cause the enterochromaffin cells to release serotonin, which leads to nausea and the cessation of feeding.

Another important condition that we know little about is the interaction between physiological state and detoxification limitations. In particular, animals with higher nutrient requirements, such as those living in the cold or reproducing, must eat more, but avoid physiological damage. This may be complicated. For example, a nocturnal animal lactating during the summer may have to eat much more in a short night, with less opportunity to spread feeding to aid detoxification. Stapley et al., (2000) examined the tolerance to a plant toxin in the face of higher nutrient requirements by comparing feeding in brushtail possums housed at 4°C or at 18°C. As expected, those in the cold ate more of a basal diet than did those living in the warm environment. However, both groups of possums ate the same amount of a diet containing jensenone, a toxin from eucalypt foliage. This indicates that possums habituated to the cold did not acquire any mechanisms for detoxifying this particular PSM faster, suggesting that similar animals in the wild would need to seek different options.

How Do Animals Overcome the Limitations Imposed by Plant Toxins?

If plant toxins can reduce feeding and even kill certain animals, then it is clear that herbivores must have mechanisms to circumvent them. The strategies of specialist and generalist herbivores may differ, but still be equally effective ways of obtaining the required nutrients without succumbing to intoxication. The detoxification

limitation hypothesis suggests that the ability to detoxify large quantities of PSMs from a single source defines a specialist herbivore. No studies, however, have shown that a specialist herbivore is able to detoxify the PSMs in its food faster than a generalist herbivore that also eats that food. Although specialist wood rats (*Neotoma* spp.) ingest more juniper PSMs than do generalists (Dearing et al., 2000), they cope with them by absorbing less from the gut, rather than detoxifying them faster (Sorensen and Dearing, 2003). But the overall result is the same—the specialist herbivore can ingest more PSMs without toxic effects. The folivores of *Eucalyptus* provide another example. The specialist koala oxidizes the terpenes, 1,8-cineole and *p*-cymene, more than does the generalist possum (Boyle et al., 1999, 2001), but it is unknown whether this speeds the rate of detoxification. Instead, what it may do is keep other pathways free for the detoxification of PSMs ingested simultaneously (Boyle et al., 2001) and thus minimize competition.

Because limitations of detoxification may force generalist herbivores to obtain their nutrient requirements from a variety of plants and plant parts, these animals should be skilled at selecting diets that spread the PSM load over their repertoire of detoxification pathways. As a consequence, presenting a herbivore with several foods containing PSMs that use different detoxification pathways should allow them to eat more. Although Freeland and Janzen (1974) specifically targeted mammalian herbivores in their predictions, the detoxification limitation hypothesis has attracted as much attention from those explaining why generalist invertebrate herbivores mix their diets (e.g., Hagele and Rowell-Rahier, 1999; Behmer et al., 2002; Singer et al., 2002; Miura and Ohsaki, 2004).

This appealing field of research has been the most common way in which researchers have attempted to test the detoxification limitation hypothesis (Freeland and Winter, 1975; Freeland et al., 1985; Freeland and Saladin, 1989; Dearing and Cork, 1999; Hagele and Rowell-Rahier, 1999; Burritt and Provenza, 2000; Behmer et al., 2002; Singer et al., 2002; Wiggins et al., 2003; Miura and Ohsaki, 2004; Marsh et al., 2006; Rogosic et al., 2006). However, it has not proved particularly fertile, probably because many experiments do not satisfy the stringent requirements needed for the study system. Although most of these studies show that generalist herbivores can eat more when offered a choice between two distinct diets, it is difficult to interpret them because they generally feed PSMs whose detoxification remains undescribed. Simply providing animals with foods with a variety of flavors or textures stimulates them to eat more than they would if given just one of these foods (Rolls et al., 1981; Treit et al., 1983; Clifton et al., 1987; DiBattista and Sitzer, 1994; Ginane et al., 2002). This indicates that different sensations alone can influence feeding behavior and reiterates the importance of devising robust systems when testing diet mixing aspects of the detoxification limitation hypothesis.

The importance of understanding modes of detoxification is borne out in the results of Burritt and Provenza (2000). Lambs did not eat more when offered two diets containing sparteine (a quinolizidine alkaloid) and saponins (triterpenoid glycosides extracted from Quillaja bark) or quebracho condensed tannins and saponins than when offered a single diet with one of the additives. The obvious interpretation is that all compete directly for detoxification, leaving no scope for a change in feeding behavior. This interpretation, however, requires knowing the detoxification pathways involved.

We set forth to test diet mixing in relation to limitations on detoxification (Marsh et al., 2006), taking advantage of pharmacological studies of PSM metabolism in

common brushtail possums (Table 1). By knowing the detoxification pathways, we could roughly predict the degree of competition for detoxification that might occur when characterized compounds were ingested simultaneously. Thus, we could predict how possums should respond when offered a choice between two PSMs, compared to the amount they ate of a diet containing only one of the compounds. For example, we expected possums to eat more if allowed to choose between diets containing PSMs metabolized by distinct pathways than if offered two diets with the same PSM or containing different PSMs that compete directly for detoxification (Marsh et al., 2006). The results were appealing in that the feeding responses of possums usually matched our predictions. For instance, possums did not eat any more than when given the choice between diets containing either 1,8-cineole or *p*-cymene (Marsh et al., 2006), which are both detoxified by oxidation followed by conjugation with glucuronic acid (Table 1). In contrast, giving possums a choice between the basal diet supplemented with 1,8-cineole or with benzoate (no competition for detoxification; Table 1) enabled them to eat significantly more (Marsh et al., 2006), indicating the benefits of diet mixing.

Problems with Understanding the Role of Detoxification in Diet Mixing

Although many of the predictions in the diet mixing experiment with possums were correct, others did not match the experimental findings. For example, possums offered a choice between salicin and orcinol, two compounds that are excreted as conjugates with glucuronic acid (Table 1), ate more food than possums offered either of the compounds singly (Marsh et al., 2006). Thus, there did not appear to be competition for detoxification. Do these findings refute the detoxification limitation hypothesis, or do they indicate a poor understanding of the system? We feel that the latter explanation is probably true.

There are many difficulties in understanding the links between diet mixing and detoxification limitations. First, it is necessary to understand the meaning of "competition," which implies competition for a limiting resource. This means that there can be no competition when unlimited supplies of a resource, such as an enzyme or cosubstrate, exist. Brushtail possums detoxify *p*-cymene mainly by oxidizing it, but conjugate some with glucuronic acid and a trace with glycine (Boyle et al., 1999). This implies that *p*-cymene would compete strongly for detoxification with compounds that also undergo oxidation, but would have little influence on the rates of detoxification of compounds that are conjugated with glycine. However, if there is a shortage of glycine, then this conjugation step may become limiting. Thus, it may be difficult to measure whether two PSMs compete for detoxification because a series of processes eliminate most PSMs, and the limiting step needs to be identified for each.

Further complicating the picture is that there may be limited competition for detoxification between two compounds that appear to be metabolized by the same process. This is because a finer-scale definition of detoxification pathways would focus on the individual enzymes and enzyme families that carry out a broad class of reactions. For example, more than 17 families of cytochrome P450 enzymes have been characterized (Lin and Lu, 2001). Thus, oxidation reactions may involve quite separate cytochrome P450 enzymes and thereby reduce competition. If one knows the chemical pathways that detoxify a PSM, then *in vitro* experiments, such

as those of Pass and McLean (2002), can probably convey much information about competition.

Another problem is that some compounds can inhibit the detoxification of others, without ever competing for enzymes or cosubstrates. For example, furocoumarins inhibit cytochrome P450 enzymes (Baumgart et al., 2005) and can thus inhibit the detoxification of compounds requiring oxidation by these specific enzymes.

On an even broader scale, all pathways are fuelled by energy, and depletion of cellular energy could act as a limit to detoxification. Gregus et al., (1996) showed that a depletion of liver ATP reserves can slow the rate of detoxification of benzoate. Thus, even compounds that do not compete for any common enzymes or cosubstrates might still influence the rates of detoxification of other PSMs. Detoxification pathways may never truly be independent of others, but instead may be separated by differing degrees. This might explain why possums fed with PSMs that did not compete for detoxification still ate less than they did of a basal diet free of PSMs (Marsh et al., 2006).

How Are Detoxification Limitations Detected?

Measuring changes in food intake is a simple way of showing that a toxin influences feeding. However, this finding tells us nothing about the workings of the PSM—why it reduces feeding and how the animal knows it is there. One crucial area that previous discussions of the detoxification limitation hypothesis do not address is how animals detect excessive intake of one toxin, and then make the decision to eat food containing a different toxin. If an animal ingests a PSM at a rate that exceeds its detoxification capacity, it must trigger a feedback signal that stops the animal eating that food. Presumably, other signals exist that tell an animal whether it has met its caloric or protein needs. These signals interact so that the animal obtains its nutritional needs without experiencing toxicosis.

The details of these signals are not well known. Although several have been proposed, experimental evidence is mostly lacking. One likely signal is nauseous feedback, stimulated by PSM-linked increases in neurotransmitter activity in the gut or brain. Provenza et al. have drawn attention to this possibility through an extensive series of papers suggesting that aversive learning is a basis to herbivores choosing their diets (e.g., Provenza et al., 1992, 1998; Provenza, 1995, 1996). In support of this concept, Aldrich et al., (1993) found that a nonselective dopamine antagonist, metoclopramide (which in humans attenuates nausea and vomiting), allowed sheep to eat more endophyte-infected tall fescue. Further support comes from the study of Lawler et al. (1998) with marsupials fed with the *Eucalyptus* PSM jensenone. Injecting the human antinausea/antivomiting drug, ondansetron, a selective 5-HT_3 receptor antagonist, enabled these animals to ingest more jensenone, suggesting that the signal monitoring the consumption of jensenone involved the release of serotonin (5-HT). Both Lawler et al., (1998) and McLean et al., (2004) speculated that damage to cells in the stomach or small intestine led to the release of serotonin from enterochromaffin cells, which bound to 5-HT_3 receptors locally, initiating a cascade of events that triggered nausea.

Because nausea is effective at conditioning aversions to foods or flavors (Provenza, 1995), it might signal impending toxicoses in other situations. If so, it might be a more widespread signal that lets animals recognize their limits and make

appropriate feeding decisions. Marsh et al., (2005), however, showed that ondansetron failed to attenuate the reduction in feeding by brushtail possums fed with benzoate-rich diets. This suggests that other mechanisms, apart from nausea, signal detoxification limitations in that system. One possible mechanism is a change in blood pH because of the formation of organic acids from conjugation reactions (Foley et al., 1995).

However, before placing too little, or too much, emphasis on the emetic system, it is important to note that the emetic system is complex, and little is known about the neurochemistry of nausea. Nausea and vomiting can be triggered by input to the emetic center from the chemoreceptor trigger zone (CTZ), neurotransmitters in the gastrointestinal tract, the cerebrocortical pathway (responsible for learned associations), or the vestibular pathway (body positional changes such as in motion sickness; Rhodes and McDaniel, 2001). Furthermore, serotonin acting at the 5-HT_3 receptor is only one of many neurotransmitter–receptor combinations that can lead to nausea and/or vomiting. Other combinations that elicit emetic responses to stimuli include substance P and tachykinin NK_1 receptors (Gardner et al., 1996), acetylcholine and muscarinic receptors (Takeda et al., 1993), dopamine and D_2 receptors (Harrington et al., 1983), as well as histamine and H_1 receptors (Takeda et al., 1993). The gastrointestinal tract, the CTZ, and the emetic center are all rich in these receptors (Rhodes and McDaniel, 2001). New selective drugs that target specific receptors are appearing as knowledge increases. We suggest that more studies, targeting a variety of potential receptors, will help us to understand how widespread nausea might be in regulating the ingestion of PSMs by animals.

The Cost of Detoxification

Presumably, if there were no costs of detoxification, then there would be few limitations to the processes. Instead, arguments about limitations of detoxification imply that it is costly to animals. There is good evidence that this is true. Cork (1981) calculated that koalas use about 25% of their fasting glucose entry rate to excrete glucuronide conjugates. In research with sheep, Lowry et al., (1993) showed that the conjugation of phenolic acids with glycine, to form hippuric acid, resulted in them losing almost 20% of their digestible nitrogen intake in urine. These detoxification costs are a cost of foraging, little different from increases in search times. Thus, from an optimal foraging perspective, one might predict that animals should eat a food only when the nutritional benefits outweigh the costs of detoxification.

If there is a cost of detoxification that influences an animal's fitness, then there needs to be a currency to measure it. The complexities of detoxification mean that researchers have largely avoided calculating the energy exchanges in individual chemical reactions, in favor of integrative measures, such as whole-body energy expenditure. For example, Iason and Murray (1996) gave sheep intraruminal infusions of orcinol and found that their basal metabolic rate (BMR) increased by 5%. This was rather small compared with voles (*Microtus pennsylvanicus*) consuming diets containing gallic acid, whose BMR increased by up to 22% (Thomas et al., 1988). More recently, Sorensen et al., (2005) used wheel-running behavior and measures of energetics to quantify detoxification costs in two species of wood rats (*Neotoma* sp.). Both the BMR and locomotor activity of the specialist

species of wood rat dropped as they ate more juniper, whereas the generalist reduced only locomotor activity.

These studies illustrate that detoxification consumes energy that the animal could use for another function. They also illustrate the strengths and the weaknesses of using energy as the currency for measuring the cost of detoxification. The obvious advantage of using energy is that it allows the integration of the costs of many different processes. The main disadvantage is that free-living animals can make subtle metabolic changes that are difficult to measure and may obscure the costs of detoxification. The drop in BMR and activity by wood rats is one example. Another is the research of White and Lawler (2002), who used the change in the heat increment of feeding on browse, relative to that of feeding on hay, to estimate the energy cost of detoxification for muskoxen (*Ovibos moschatus*). For some species of browse, they found that reductions in ruminal methane production (and its subsequent loss by eructation) partially compensated for the energy cost of detoxifying secondary compounds. The opportunity for tradeoffs and compensations in energy metabolism across different species of browse makes it a poor currency for measuring the cost of detoxification.

We suggest that measuring whole-body protein turnover might be a better way of estimating the costs of detoxification. Whole-body protein turnover measures the cost of the synthesis and turnover of proteins and is strongly related to metabolic rate because protein synthesis is energetically expensive (Waterlow, 2006). Whole-body protein turnover is more integrative of the range of processes that occur during detoxification than simple measurements of nitrogen or energy balance because it includes intermediary metabolism, amino acid activation, and associated processes such as RNA turnover (Waterlow, 2006). For example, protein is required for synthesis of cytochrome P450 and other detoxification enzymes. That many of the enzymes involved in detoxifying PSMs are inducible suggests that they are costly to maintain.

Changes in protein turnover should translate directly into changes in protein requirements and, so, are easier to incorporate into models of diet selection than are measurements of energy loss. They can also integrate many different detoxification processes. Animals can excrete some phenolic glycosides with a minimal apparent energy cost (McLean et al., 2001), but there may be costs that are not immediately obvious and need to be paid for later. For example, these compounds may increase protein requirements through damage to the kidney's filtration processes (Garner et al., unpublished data), through uncoupling of oxidative phosphorylation (Singleton and Kratzner, 1969), and through acid–base adjustments arising from the excretion of organic acids of detoxified metabolites (Foley et al., 1995).

Changes in whole-body protein turnover during various types of immune challenges suggest major costs of maintaining the immune system (Klasing and Calvert, 2000). These appear to have an evolutionary basis because there are direct links between the increased protein requirements of these challenges and compelling models of diet selection (Lee et al., 2006). PSMs have complex effects on the immune systems of mammals (Allen et al., 2003). This indicates that we need to view detoxification costs more broadly, and that separating detoxification costs from other potential costs, such as immune responses, is probably artificial. Accordingly, we recommend that effort be given to measuring whole-body protein turnover of animals fed with diets containing either purified PSMs or, more usefully, complex mixtures typical of those found in most browse plants.

Applying the Detoxification Limitation Hypothesis to Wild Herbivores

This review of the detoxification limitation hypothesis has so far considered the case of the captive animal given a limited choice of foods. In contrast, wild animals face situations that are not nearly so simple. The feeding behavior of wild herbivores depends on many factors in addition to forage quality. These include social interactions, nutritional status, and the need for a home range with other qualities, such as a den or shelter. Clearly, determining whether wild herbivores feed according to the predictions of the detoxification limitation hypothesis is difficult. Even so, in this section, we outline some of the factors that researchers might consider when studying how free-living herbivores might mix several plant toxins in their diet. Foremost among them is the spatial distribution of plant defenses, which are presumably important because they influence costs, such as search times.

There is growing evidence that the spatial distribution of defense variation affects feeding choices (Hjalten et al., 1993; Alm Bergvall and Leimar, 2005). Nevertheless, few studies provide evidence that the spatial patterns required for such behaviors exist in nature (Covelo and Gallardo, 2004; Brenes-Arguedas and Coley, 2005). In the case of diet mixing, it is essential for variation to occur at a relevant scale for herbivores—presumably within a home range. A patchy distribution could increase the cost of diet mixing because animals would be unlikely to encounter chemically different food plants in the same patch or stand. Herbivores might prefer areas where diverse food is available, such as between patches, or where different food species overlap.

Another interesting difference in the environments of captive and wild herbivores is that those of the latter may provide mechanisms that enable the animal to cope with toxins. For thousands of years, humans have used a variety of methods to render plant toxins harmless. Herbivorous animals sometimes do the same. Perhaps the most obvious is removing toxic plant parts before ingestion. Other techniques include eating substances that are not normally considered food to negate the effects of plant toxins. One of the best examples is of parrots practicing geophagy—the ingestion of soil. By carefully selecting soils rich in particular clays, with a high capacity for binding plant toxins, the birds avoid absorbing the toxins from the gut (Gilardi et al., 1999). The abundance and distribution of such resources clearly impinge on the nutritional ecology of the herbivores that can use them.

Although the amount eaten of a plant should ultimately depend on an animal's ability to detoxify PSMs, its initial selection may be driven by other factors. The amount of feeding an individual plant attracts probably depends on the characteristics of the surrounding matrix of plants (Atsatt and O'Dowd, 1976; Milchunas and Noy-Meir, 2002; Alm Bergvall et al., 2006). Thus, the nutritional and toxic characteristics of a plant may influence how herbivores use neighboring plants (Villalba and Provenza, 2005). From a plant's perspective, the benefits and costs of defense need to consider both the individual and the community it inhabits (Tuomi and Augner, 1993). Likewise, the foraging strategy of the animal should account for this. One example is that sheep were more likely to graze a relatively unpalatable shrub when the surrounding plants were palatable (Rousset and Lepart, 2003).

To understand the role of detoxification in nature, there is a need for detailed studies of the feeding behavior and population dynamics of wild animals, in relation to the distribution of PSMs in their food plants. One approach might be to use data from feeding studies with captive animals to predict the palatability of wild plants

and then to follow this up by comparing the expected patterns of use with the observed patterns. Recently developed methods for studying spatial autocorrelation (Double et al., 2005) can detect the scale and intensity of patchiness in PSM distributions (Andrew et al., unpublished data), which can then be related to herbivore behavior.

In summary, the initial testing of the detoxification limitation hypothesis on wild herbivores requires a simple system consisting of a specialist herbivore eating a diet whose PSMs are well characterized. An example of this approach is that used by Marsh et al. (unpublished data), who fitted koalas with radiocollars equipped with microphones, enabling the constant monitoring of both the animals' location and the amount that they ate. By sampling foliage from all of the trees in the area, they could then examine a koala's feeding pattern in relation to the chemistry of its environment. They found that the chemistry of foliage influenced the choice of trees by koalas, as well as the amount of food eaten.

Conclusions

Some 30 years after Freeland and Janzen's seminal paper on the interactions between herbivores and plant toxins, the hypothesis it gave rise to, detoxification limitations, is entrenched in the literature but remains inadequately tested. There are thousands of plant toxins and a wide variety of metabolic systems to render them less potent, making the study of the interactions complex. Some PSMs require similar modes of detoxification and are said to compete, whereas the animal metabolizes others by pathways that appear to be independent. In between these modes, however, are detoxification pathways that compete to differing degrees and are much harder to understand. In fact, because all are fueled by energy, it may be that no pathway is truly independent of another. It is impossible to study all of the systems of detoxification, and there is little to gain from trying. However, further investigations of the detoxification limitations of herbivores feeding on PSM-rich diets, and how they detect these, are likely to advance our understanding of the interactions between animals and plants.

References

ALDRICH, C. G., RHODES, M. T., MINER, J. L., KERLEY, M. S., and PATERSON, J. A. 1993. The effects of endophyte-infected tall fescue consumption and use of a dopamine antagonist on intake, digestibility, body temperature, and blood constituents in sheep. *Anim. Sci.* 71:158–163.

ALLEN, C. T., PEDEN-ADAMS, M. M., EUDALY, J., and KEIL, D. E. 2003. Subchronic exposure to ellagic acid impairs cytotoxic T-cell function and suppresses humoral immunity in mice. *Immunopharm. Immunot.* 25:409–422.

ALM BERGVALL, U. and LEIMAR, O. 2005. Plant secondary compounds and the frequency of food types affect food choice by mammalian herbivores. *Ecology* 86:2450–2460.

ALM BERGVALL, U., RAUTIO, P., KESTI, K., TUOMI, J., and LEIMAR, O. 2006. Associational effects of plant defences in relation to within- and between-patch food choice by a mammalian herbivore: neighbor contrast susceptibility and defense. *Oecologia* 147:253–260.

AMSEL, L. P. and LEVY, G. 1969. Drug biotransformation interactions in man. II. A pharmacokinetic study of the simultaneous conjugation of benzoic and salicylic acids with glycine. *J. Pharm. Sci.* 58:321–326.

ARANDA, A., SANCHEZ-VAZQUEZ, F. J., ZAMORA, S., and MADRID, J. A. 2000. Self-design of fish diets by means of self-feeders: validation of procedures. *J. Physiol. Biochem.* 56:155–166.

ATSATT, P. R. and O'DOWD, D. J. 1976. Plant defense guilds. *Science* 193:24–29.
AWALUDDIN, A. B. and MCLEAN, S. 1985. Conjugation of benzoic acid in marsupials. *Aust. J. Zool.* 33:693–698.
BAUMGART, A., SCHMIDT, M., SCHMITZ, H.-J., and SCHRENK, D. 2005. Natural furocoumarins as inducers and inhibitors of cytochrome P450 1A1 in rat hepatocytes. *Biochem. Pharmacol.* 69:657–667.
BEHMER, S. T., SIMPSON, S. J., and RAUBENHEIMER, D. 2002. Herbivore foraging in chemically heterogeneous environments: nutrients and secondary metabolites. *Ecology* 83:2489–2501.
BOYLE, R. and MCLEAN, S. 2004. Constraint of feeding by chronic ingestion of 1,8-cineole in the brushtail possum (*Trichosurus vulpecula*). *J. Chem. Ecol.* 30:757–775.
BOYLE, R., MCLEAN, S., FOLEY, W. J., and DAVIES, N. W. 1999. Comparative metabolism of dietary terpene, *p*-cymene, in generalist and specialist folivorous marsupials. *J. Chem. Ecol.* 25:2109–2126.
BOYLE, R., MCLEAN, S., FOLEY, W., DAVIES, N. W., PEACOCK, E. J., and MOORE, B. 2001. Metabolites of dietary 1,8-cineole in the male koala (*Phascolarctos cinereus*). *Comp. Biochem. Physiol. C* 129:385–395.
BRENES-ARGUEDAS, T. and COLEY, P. D. 2005. Phenotypic variation and spatial structure of secondary chemistry in a natural population of a tropical tree species. *Oikos* 108:410–420.
BRIDGES, J. W., FRENCH, M. R., SMITH, R. L., and WILLIAMS, R. T. 1970. The fate of benzoic acid in various species. *Biochem. J.* 118:47–51.
BURRITT, E. A. and PROVENZA, F. D. 2000. Role of toxins in intake of varied diets by sheep. *J. Chem. Ecol.* 26:1991–2005.
CASSINI, M. H. 1994. Behavioral mechanisms of selection of diet components and their ecological implications in herbivorous mammals. *J. Mammal.* 75:733–740.
CLIFTON, P. G., BURTON, M. J., and SHARP, C. 1987. Rapid loss of stimulus-specific satiety after consumption of a second food. *Appetite* 9:149–156.
CORK, S. J. 1981. Digestion and metabolism in the koala (*Phascolarctos cinereus* Goldfuss): an arboreal folivore. Ph.D. thesis. University of New South Wales, Australia.
COVELO, F. and GALLARDO, A. 2004. Green and senescent leaf phenolics showed spatial autocorrelation in a *Quercus robur* population in northwestern Spain. *Plant Soil* 259: 267–276.
DEARING, M. D. and CORK, S. 1999. Role of detoxification of plant secondary compounds on diet breadth in a mammalian herbivore, *Trichosurus vulpecula*. *J. Chem. Ecol.* 25:1205–1219.
DEARING, M. D. and SCHALL, J. J. 1992. Testing models of optimal diet assembly by the generalist herbivorous lizard *Cnemidophorus murinus*. *Ecology* 73:845–858.
DEARING, M. D., MANGIONE, A. M., and KARASOV, W. H. 2000. Diet breadth of mammalian herbivores: nutrient versus detoxification constraints. *Oecologia* 123:397–405.
DEARING, M. D., FOLEY, W. J., and MCLEAN, S. 2005. The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. *Annu Rev. Ecolog. Evol. Syst.* 36:169–189.
DEGABRIEL, J., FOLEY, W. J., and WALLIS, I. R. 2002. The effect of excesses and deficiencies in amino acids on the feeding behavior of the common brushtail possum (*Trichosurus vulpecula*). *J. Comp. Physiol. B* 172:607–617.
DIBATTISTA, D. and SITZER, C. A. 1994. Dietary variety enhances meal size in golden hamsters. *Physiol. Behav.* 55:381–383.
DOUBLE, M. C., PEAKALL, R., BECK, N. R., and COCKBURN, A. 2005. Dispersal, philopatry, and infidelity: dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). *Evolution* 59:625–635.
DUNCAN, A. J., GINANE, C., GORDON, I. J., and ORSKOV, E. R. 2003. Why do herbivores select mixed diets?, pp. 195–208, *in* L. t'Mannetje, L. Ramírez-Avilés, C. A. Sandoval-Castro, and J. C. Ku-Vera (eds.). Matching Herbivore Nutrition to Ecosystems Biodiversity. Proceedings of the VI International Symposium on the Nutrition of Herbivores. Universidad Autónoma de Yucatán, Factad de Medicina Veterinaria y Zootecnia, Merida, Mexico.
FOLEY, W. J., MCLEAN, S., and CORK, S. J. 1995. Consequences of biotransformation of plant secondary metabolites on acid–base metabolism in mammals—a final common pathway. *J. Chem. Ecol.* 21:721–743.
FOLEY, W. J., IASON, G. R., and MCARTHUR, C. 1999. Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: How far have we come in 25 years?, pp. 130–209, *in* H.-J. G. Jung and G. C. J. Fahey (eds.). Nutritional Ecology of Herbivores. Proceedings of the Vth International Symposium on the Nutrition of Herbivores. American Society of Animal Science, Savoy, USA.
FREELAND, W. J. 1991. Plant secondary metabolites: Biochemical coevolution with herbivores,

pp. 61–81, *in* R. T. Palo and C. T. Robbins (eds.). Plant Defenses Against Mammalian Herbivory. CRC Press, Boca Raton, FL, USA.
FREELAND, W. J. and JANZEN, D. H. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. *Am. Nat.* 108:269–289.
FREELAND, W. J. and SALADIN, L. R. 1989. Choice of mixed diets by herbivores: the idiosyncratic effects of plant secondary compounds. *Biochem. Syst. Ecol.* 17:493–497.
FREELAND, W. J. and WINTER, J. W. 1975. Evolutionary consequences of eating: *Trichosurus vulpecula* (*Marsupialia*) and the genus Eucalyptus. *J. Chem. Ecol.* 1:439–455.
FREELAND, W. J., CALCOTT, P. H., and ANDERSON, L. R. 1985. Tannins and saponin—interaction in herbivore diets. *Biochem. Syst. Ecol.* 13:189–193.
GALEY, F. D., HOLSTEGE, D. M., JOHNSON, B. J., and SIEMENS, L. 1998. Toxicity and diagnosis of oleander (*Nerium oleander*) poisoning in livestock, pp. 215–219, *in* T. Garland and A. C. Barr (eds.). Toxic Plants and Other Natural Toxicants. CABI Publishing, Oxon, UK.
GARDNER, C. J., ARMOUR, D. R., BEATTIE, D. T., GALE, J. D., HAWCOCK, A. B., KILPATRICK, G. J., TWISSELL, D. J., and WARD, P. 1996. GR205171: a novel antagonist with high affinity for the tachykinin NK1 receptor, and potent broad-spectrum anti-emetic activity. *Regul. Pept.* 65:45–53.
GILARDI, J. D., DUFFEY, S. S., MUNN, C. A., and TELL, L. A. 1999. Biochemical functions of geophagy in parrots: detoxification of dietary toxins and cytoprotective effects. *J. Chem. Ecol.* 25:897–922.
GINANE, C., BAUMONT, R., LASSALAS, J., and PETIT, M. 2002. Feeding behavior and intake of heifers fed on hays of various quality, offered alone or in a choice situation. *Anim. Res.* 51:177–188.
GOUS, R. M. and SWATSON, H. K. 2000. Mixture experiments: a severe test of the ability of a broiler chicken to make the right choice. *Br. Poult. Sci.* 41:136–140.
GREGUS, Z., FEKETE, T., VARGA, F., and KLAASSEN, C. D. 1993. Dependence of glycine conjugation on availability of glycine—role of the glycine cleavage system. *Xenobiotica* 23:141–153.
GREGUS, Z., FEKETE, T., HALASZI, E., and KLAASSEN, C. D. 1996. Does hepatic ATP depletion impair glycine conjugation *in vivo? Drug Metab. Dispos.* 24:1347–1354.
GRIFFITH, W. H. and LEWIS, H. B. 1923. Studies in the synthesis of hippuric acid in the animal organism. V. The influence of amino-acids and related substances on the synthesis and rate of elimination of hippuric acid after the administration of benzoate. *J. Biol. Chem.* 57:1–24.
HAGELE, B. F. and ROWELL-RAHIER, M. 1999. Dietary mixing in three generalist herbivores: nutrient complementation or toxin dilution? *Oecologia* 119:521–533.
HARRINGTON, R. A., HAMILTON, C. W., BROGDEN, R. N., LINKEWICH, J. A., ROMANKIEWICZ, J. A., and HEEL, R. C. 1983. Metoclopramide—an updated review of its pharmacological properties and clinical use. *Drugs* 25:451–494.
HJALTEN, J., DANEALL, K., and LUNDBERG, P. 1993. Herbivore avoidance by association: vole and hare utilization of woody-plants. *Oikos* 68:125–131.
IASON, G. R. 2005. The role of plant secondary metabolites in mammalian herbivory: ecological perspectives. *Proc. Nutr. Soc.* 64:1–9.
IASON, G. R. and MURRAY, A. H. 1996. The energy costs of ingestion of naturally occurring nontannin plant phenolics by sheep. *Physiol. Zool.* 69:532–546.
JAKUBAS, W. J. and MASON, J. R. 1991. Role of avian trigeminal sensory system in detecting coniferyl benzoate, a plant allelochemical. *J. Chem. Ecol.* 17:2213–2221.
KLASING, K. C. and CALVERT, C. C. 2000. The care and feeding of an immune system: an analysis of lysine needs, pp. 253–264, *in* G. E. Lobley, A. White and J. C. MacRae (eds.). Proceedings of the VIII International Symposium on Protein Metabolism and Nutrition. Wageningen University Press, Wageningen, Netherlands.
LAWLER, I. R., FOLEY, W. J., PASS, G. J., and ESCHLER, B. M. 1998. Administration of a 5HT3 receptor antagonist increases the intake of diets containing *Eucalyptus* secondary metabolites by marsupials. *J. Comp. Physiol. B* 168:611–618.
LAWLER, I. R., FOLEY, W. J., and ESCHLER, B. M. 2000. Foliar concentration of a single toxin creates habitat patchiness for a marsupial folivore. *Ecology* 81:1327–1338.
LEE, K. P., CORY, J. S., WILSON, K., RAUBENHEIMER, D., and SIMPSON, S. J. 2006. Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. *Proc. R. Soc. Lond., B Biol. Sci.* 273:823–829.
LIN, J. H. and LU, A. Y. H. 2001. Interindividual variability in inhibition and induction of cytochrome P450 enzymes. *Annu. Rev. Pharmacol. Toxicol.* 41:535–567.
LOWRY, J. B., SUMPTER, E. A., MCSWEENEY, C. S., SCHLINK, A. C., and BOWDEN, B. 1993. Phenolic acids in the fibre of some tropical grasses; effect on feed quality and their metabolism by sheep. *Aust. J. Agric. Resour.* 44:1123–1133.

MANGIONE, A. M., DEARING, M. D., and KARASOV, W. H. 2000. Interpopulation differences in tolerance to creosote bush resin in desert woodrats (*Neotoma lepida*). *Ecology* 81:2067–2076.
MARSH, K. J., FOLEY, W. J., COWLING, A., and WALLIS, I. R. 2003. Differential susceptibility to *Eucalyptus* secondary compounds explains feeding by the common ringtail (*Pseudocheirus peregrinus*) and common brushtail possum (*Trichosurus vulpecula*). *J. Comp. Physiol. B* 173:69–78.
MARSH, K. J., WALLIS, I. R., and FOLEY, W. J. 2005. Detoxification rates constrain feeding in common brushtail possums (*Trichosurus vulpecula*). *Ecology* 86:2946–2954.
MARSH, K. J., WALLIS, I. R., MCLEAN, S., SORENSEN, J. S., and FOLEY, W. J. 2006. Conflicting demands on detoxification pathways influence how brushtail possums choose their diets. *Ecology* (in press).
MCLEAN, S. and DUNCAN, A. J. 2006. Pharmacological perspectives on the detoxification of plant secondary metabolites: implications for ingestive behavior of herbivores. *J. Chem. Ecol.* (this volume).
MCLEAN, S., PASS, G. J., FOLEY, W. J., BRANDON, S., and DAVIES, N. W. 2001. 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.* 27:1077–1089.
MCLEAN, S., BRANDON, S., DAVIES, N. W., FOLEY, W. J., and MULLER, H. K. 2004. Jensenone: biological reactivity of a marsupial antifeedant from *Eucalyptus*. *J. Chem. Ecol.* 30:19–36.
MILCHUNAS, D. G. and NOY-MEIR, I. 2002. Grazing refuges, external avoidance of herbivory and plant diversity. *Oikos* 99:113–130.
MIURA, K. and OHSAKI, N. 2004. Diet mixing and its effect on polyphagous grasshopper nymphs. *Ecol. Res.* 19:269–274.
MOORE, B. D., WALLIS, I. R., PALÁ-PAÚL, J., BROPHY, J. J., WILLIS, R. H., and FOLEY, W. J. 2004. Antiherbivore chemistry of *Eucalyptus*—cues and deterrents for marsupial folivores. *J. Chem. Ecol.* 30:1743–1769.
MOORE, B. D., FOLEY, W. J., WALLIS, I. R., COWLING, A., and HANDASYDE, K. A. 2005. *Eucalyptus* foliar chemistry explains selective feeding by koalas. *Biol. Lett.* 1:64–67.
MULDER, G. J. 1995. Polymorphism in drug conjugation in man: is it a factor of concern in drug toxicity or efficacy? *Eur. J. Pharm. Sci.* 3:57–59.
NORBERG, A., JONES, W. A., HAHN, R. G., and GABRIELSSON, J. L. 2003. Role of variability in explaining ethanol pharmacokinetics—research and forensic applications. *Clin. Pharmacokinet.* 42:1–31.
OSAWA, R., BIRD, P. S., HARBROW, D. J., OGIMOTO, K., and SEYMOUR, G. J. 1993. Microbiological studies of the intestinal microflora of the koala, *Phascolarctos cinereus*. 1. Colonization of the cecal wall by tannin–protein complex degrading enterobacteria. *Aust. J. Zool.* 41:599–609.
PASS, G. J. and FOLEY, W. J. 2000. Plant secondary metabolites as mammalian feeding deterrents: separating the effects of the taste of salicin from its post-ingestive consequences in the common brushtail possum (*Trichosurus vulpecula*). *J. Comp. Physiol. B* 170:185–192.
PASS, G. J. and MCLEAN, S. 2002. Inhibition of the microsomal metabolism of 1,8-cineole in the common brushtail possum (*Trichosurus vulpecula*) by terpenes and other chemicals. *Xenobiotica* 32:1109–1126.
PASS, G. J., MCLEAN, S., and STUPANS, I. 1999. Induction of xenobiotic metabolising enzymes in the common brushtail possum, *Trichosurus vulpecula*, by *Eucalyptus* terpenes. *Comp. Biochem. Physiol. C* 124:239–246.
PFISTER, J. A., PROVENZA, F. D., MANNERS, G. D., GARDNER, D. R., and RALPHS, M. H. 1997. Tall larkspur ingestion: Can cattle regulate intake below toxic levels? *J. Chem. Ecol.* 23:759–777.
PFISTER, J. A., PANTER, K. E., GARDNER, D. R., STEGELMEIER, B. L., RALPHS, M. H., MOLYNEUX, R. J., and LEE, S. T. 2001. Alkaloids as anti-quality factors in plants on western US rangelands. *J. Range Manag.* 54:447–461.
PROVENZA, F. D. 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *J. Range Manag.* 48:2–17.
PROVENZA, F. D. 1996. Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. *J. Anim. Sci.* 74:2010–2020.
PROVENZA, F. D., PFISTER, J. A., and CHENY, C. D. 1992. Mechanisms of learning in diet selection with reference to phytotoxicosis in herbivores. *J. Range Manag.* 45:36–45.
PROVENZA, F. D., VILLALBA, J. J., CHENEY, C. D., and WERNER, S. J. 1998. Self-organization of foraging behavior: from simplicity to complexity without goals. *Nutr. Res. Rev.* 11:199–222.
RADOMINSKA-PANDAYA, A., CZERNIK, P. J., and LITTLE, J. M. 1999. Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metab. Rev.* 31:817–899.

RHODES, V. A. and MCDANIEL, R. W. 2001. Nausea, vomiting, and retching: Complex problems in palliative care. *CA Cancer J. Clin.* 51:232–248.
ROBBINS, C. T., HAGERMAN, A. E., AUSTIN, P. J., MCARTHUR, C., and HANLEY, T. A. 1991. Variation in mammalian physiological responses to a condensed tannin and its ecological implications. *J. Mammal.* 72:480–486.
ROGOSIC, J., ESTELL, R. E., SKOBIC, D., MARTINOVIC, A., and MARIC, S. 2006. Role of species diversity and secondary compound complementarity on diet selection of Mediterranean shrubs by goats. *J. Chem. Ecol.* (this volume).
ROLLS, B. J., ROWE, E. A., ROLLS, E. T., KINGSTON, B., MEGSON, A., and GUNARY, R. 1981. Variety in a meal enhances food intake in man. *Physiol. Behav.* 26:215–221.
ROUSSET, O. and LEPART, J. 2003. Neighbourhood effects on the risk of an unpalatable plant being grazed. *Plant Ecol.* 165:197–206.
SCOTT, L. L. and PROVENZA, F. D. 2000. Lambs fed protein or energy imbalanced diets forage in locations and on foods that rectify imbalances. *Appl. Anim. Behav. Sci.* 68:293–305.
SHARIATMADARI, F. and FORBES, J. M. 1993. Growth and food intake responses to diets of different protein contents and a choice between diets containing two concentrations of protein in broiler and layer strains of chicken. *Br. Poult. Sci.* 34:959–970.
SINGER, M. S., BERNAYS, E. A., and CARRIERE, Y. 2002. The interplay between nutrient balancing and toxin dilution in foraging by a generalist insect herbivore. *Anim. Behav.* 64:629–643.
SINGLETON, V. L. and KRATZNER, F. H. 1969. Toxicity and related physiological activity of phenolic substances of plant origin. *J. Agric. Food Chem.* 17:497–512.
SNYDER, M. J. and GLENDINNING, J. I. 1996. Causal connection between detoxification enzyme activity and consumption of a toxic plant compound. *J. Comp. Physiol. A* 179:255–261.
SORENSEN, J. S. and DEARING, M. D. 2003. Elimination of plant toxins by herbivorous woodrats: revisiting an explanation for dietary specialization in mammalian herbivores. *Oecologia* 134:88–94.
SORENSEN, J. S., MCLISTER, J. D., and DEARING, M. D. 2005. Plant secondary metabolites compromise the energy budgets of specialist and generalist mammalian herbivores. *Ecology* 86:125–139.
STAPLEY, J., FOLEY, W. J., CUNNINGHAM, R., and ESCHLER, B. 2000. How well can common brushtail possums regulate their intake of *Eucalyptus* toxins? *J. Comp. Physiol. B* 170:211–218.
TAKEDA, N., MORITA, M., HASEGAWA, S., HORII, A., KUBO, T., and MATSUNAGA, T. 1993. Neuropharmacology of motion sickness and Emesis—a review. *Acta Oto-laryngol.* 10–15.
TAMÁSI, V., VERECZKEY, L., FALUS, A., and MONOSTORY, K. 2003. Some aspects of interindividual variations in the metabolism of xenobiotics. *Inflamm. Res.* 52:322–333.
THOMAS, D. W., SAMSON, C., and BERGERON, J. 1988. Metabolic costs associated with the ingestion of plant phenolics by *Microtus pennsylvanicus*. *J. Mammal.* 69:512–515.
TREIT, D., SPETCH, M. L., and DEUTSCH, J. A. 1983. Variety in the flavor of food enhances eating in the rat: a controlled demonstration. *Physiol. Behav.* 30:207–211.
TUOMI, J. and AUGNER, M. 1993. Synergistic selection of unpalatability in plants. *Evolution* 47:668–672.
VILLALBA, J. J. and PROVENZA, F. D. 2005. Foraging in chemically diverse environments: energy, protein, and alternative foods influence ingestion of plant secondary metabolites by lambs. *J. Chem. Ecol.* 31:123–138.
WALLIS, I. R., WATSON, M. L., and FOLEY, W. J. 2002. Secondary metabolites in Eucalyptus melliodora: field distribution and laboratory feeding choices by a generalist herbivore, the common brushtail possum. *Aust. J. Zool.* 50:1–13.
WALTON, K., DORNE, J. L., and RENWICK, A. G. 2001. Uncertainty factors for chemical risk assessment: interspecies differences in glucuronidation. *Food Chem. Toxicol.* 39:1175–1190.
WANG, J. and PROVENZA, F. D. 1997. Dynamics of preference by sheep offered foods varying in flavours, nutrients and a toxin. *J. Chem. Ecol.* 23:275–288.
WATERLOW, J. C. 2006. Protein Turnover. CABI Publishing, Wallingford, UK.
WHITE, R. G. and LAWLER, J. P. 2002. Can methane suppression during digestion of woody and leafy browse compensate for energy costs of detoxification of plant secondary compounds? A test with muskoxen fed willows and birch. *Comp. Biochem. Phys. A* 133:849–859.
WIGGINS, N. L., MCARTHUR, C., MCLEAN, S., and BOYLE, R. 2003. Effects of two plant secondary metabolites, cineole and gallic acid, on nightly feeding patterns of the common brushtail possum. *J. Chem. Ecol.* 29:1447–1464.
WIGGINS, N. L., MCARTHUR, C., and DAVIES, N. W. 2006. Diet switching in a generalist mammalian folivore: fundamental to maximising intake. *Oecologia* 147:650–657.