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Review

Translating physiological signals to changes in feeding behaviour in mammals and the future effects of global climate change

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Abstract. Mammals cannot avoid ingesting secondary metabolites, often in significant amounts. Thus, their intake must be regulated to avoid intoxication. Three broad mechanisms have been described by which this can be achieved. These are conditioned aversions mediated by nausea, non-conditioned aversions and the recognition of limits to detoxification. Although there is some overlap between these, we know little about the way that mechanisms of toxin avoidance interact with regulation of nutrient intake and whether one has priority over the other. Nonetheless, regulation of meal length and inter-meal length allows the intake of some plant secondary metabolites to be matched with an animal's capacity for detoxification and its nutritional requirements. Toxicity itself is not a fixed limitation and recent work suggests that ambient temperature can be a major determinant of the toxicity of plant secondary metabolites, largely through effects on liver function. These effects are likely to be of major importance in predicting the impact of global climate change on herbivores.

Additional keywords: conditioned aversions, diet selection, heat dissipation limit hypothesis, herbivore, plant secondary metabolites, temperature-dependent toxicity.

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Introduction

Browsing mammals, and oftentimes grazers, are exposed to foods containing large quantities of plant secondary metabolites (PSMs). Traditionally, PSMs have been considered to be either directly toxic to herbivore metabolism, to interfere with digestion of other dietary components either directly or indirectly, or to be relatively inert. These distinctions are not absolute but they remain useful ways to think about the diversity of compounds that mammals consume. Of course, different foods contain different mixtures and concentrations of PSMs and so diet selection must ensure that animals meet their nutritional requirements while avoiding potential toxic effects. Regulating the intake of both nutrients and PSMs is likely to occur during individual feeding bouts as well as over longer timeframes such as one or multiple days (e.g. DeGabriel *et al*. 2009; Nersesian *et al*. 2012), but how the effects of PSMs are integrated into a broader framework of diet selection remains largely unknown.

The impacts and effects of PSMs have been studied intensively both by animal scientists aiming to improve production, and by ecologists interested in the evolution and function of natural ecosystems. Elsewhere (Foley *et al*. 2007), we have pointed out the surprising lack of cross-fertilisation between these two groups and the benefits of a broader perspective are clear. For example, until recently ecologists tended to regard PSMs as entirely negative, yet there is significant evidence from animal science of a range of positive effects of PSMs for herbivore metabolism (Forbey *et al*. 2009) and ecosystem resilience (Provenza *et al*. 2003). These differing perspectives are probably largely attributable to the fact that wild species are generally exposed to less nutritious foods that contain more PSMs. There are also interesting parallels between the regulation of intake of dietary toxins by mammalian herbivores and the ingestion of psychoactive 'recreational' drugs by humans. Hagen *et al*. (2013) challenged existing theories of human drug use and hypothesised that their intake is closely regulated to balance costs and fitness through beneficial effects of low concentrations – and similar phenomena have been proposed for herbivores (e.g. Forbey *et al*. 2009). This theme is particularly important in animal science, but it remains true that wild animals are exposed to a significantly greater diversity and higher concentrations of PSMs than either domestic animals or humans.

There are several detailed reviews that have described the effects of PSMs on mammalian herbivores and the mechanisms by which they detoxify and excrete these parts

of their diet (Foley *et al*. 1999; Dearing *et al*. 2005; Iason 2005). In the present paper, we aim to review ideas about the links between the ingestion of PSMs and feeding behaviour to explain how physiological signals of toxicity are translated into behaviour. PSMs do not exist in isolation and animals must obtain sufficient nutrients while monitoring the effects of PSMs (which may be neutral, positive or negative) both at individual meals, but also over longer periods. Our knowledge of how this occurs is rudimentary and largely based on the experimental addition of single isolated PSMs to simple artificial diets.

Toxicity is not a fixed limitation for herbivores, and Provenza and colleagues have highlighted some of the physiological and metabolic factors that might change the toxicity of PSMs either temporally or among individual animals (e.g. Provenza (1995); Provenza *et al*. (1994*a*, 2003)). In addition, a rapidly expanding body of research shows that herbivore gut microbiota play an important role in the detoxification of PSMs, thus mediating toxicity in herbivores (Dearing *et al*. 2005). More recently, global change has also been recognised as an increasingly important influence, through the effects of ambient temperature on toxicity of PSMs to herbivores (Dearing 2013) and because elevated atmospheric CO_2 and temperature can alter PSM concentrations in plants (Hovenden and Williams 2010). Incorporating these effects into foraging models is a major challenge for the future. We will develop these emerging themes further by describing new ideas and theories about the role of gut microbes and climate change in mediating the toxicity of PSMs to herbivores.

Mechanisms by which secondary compounds are regulated

Several studies have shown that herbivorous mammals are capable of regulating their intake of secondary metabolites quite precisely. Doing so requires close integration of the effects of the PSM paired with rapid feedback that leads to behavioural changes. This process must be replicated over several time scales – first, over a matter of minutes to regulate meal lengths and, second, over longer scales for food quality to be recognised for future feeding bouts. The importance of regulation during a single meal argues for a signal to be transmitted to the appetite centre by the nervous system.

In past reviews (e.g. Torregrossa and Dearing 2009; DeGabriel *et al*. 2010), several mechanisms have been identified by which the ingestion of secondary metabolites is regulated. These are (1) conditioned aversions in which toxic compounds lead to stimulation of the emetic system and the resultant illness is associated with the taste or smell of the food, (2) non-conditioned aversions where deterrent tastes such as 'bitter' or 'hot' limit intake even though no acute toxic effects that may reinforce long-term aversion are involved, and (3) recognition of limits to detoxification, in which animals monitor the rate of metabolism and excretion of PSMs and adjust feeding rates so as not to exceed some threshold dose.

Conditioned aversions

Conditioned aversions play a central role in current theories of regulation of intake by mammalian herbivores (Provenza 1995; Villalba and Provenza 2000; Duncan *et al*. 2005; Ginane *et al*. 2005; Iason 2005; Duncan *et al*. 2006). Animals can learn to associate the flavours of foods with a variety of post-ingestive consequences lying along a continuum from slight nausea (mild satiety) to strong nausea (malaise; Provenza 1995), and this allows them to modify their feeding on those foods in the future. Nausea and vomiting are the most powerful conditioning stimuli and have been the major focus of studies of conditioned aversions in herbivores.

In particular, it has been suggested that ingested PSMs cause the release of neurotransmitters, such as serotonin (5-hydroxytryptamine; 5-HT), which are involved in nausea and vomiting. Serotonin has long been known to play an important role in regulating food intake (Blundell 1984; Leibowitz and Alexander 1998) via actions on 5-HT_1, 5-HT_2 and 5-HT_6 receptors (Blundell 1992). The intensity of the effect is modulated by nutritional factors, including ratios of macronutrients in the diet. Nonetheless, agonistic drugs that target these receptors have been widely targeted as appetite suppressants (Garfield and Heisler 2009). In non-pathological states, increased bioavailability of serotonin is a key signal involved in the cessation of individual meals. Recent studies have indicated that serotonin modulates neurons containing the melanocortin pathway agonists and antagonists, which are a key part of the appetite regulation system (Garfield and Heisler 2009). Although activation of serotonin pathways generally can lead to a cessation of food intake, increases in food intake following antagonism of the serotonin receptors is much rarer (Blundell 1984).

Serotonin is abundant in the gut enterochromaffin (EC) cells and is secreted in response to a variety of stimuli. EC cells are distributed throughout the gastrointestinal tract, although the highest numbers are located in the small intestine and rectum. Under normal conditions, serotonin stimulates vagal afferents and the enteric nervous system to increase motility of the gut and its secretory processes and thus plays a key role in the transit of material through the intestine (Spiller 2007; Grundy 2008). However, larger amounts of serotonin can also initiate nausea via vagal stimulation.

Lithium chloride is widely used as an emetic agent in experimental studies of conditioned aversions because it is effective in inducing nausea and vomiting, but Lawler *et al*. (1999) showed that naturally occurring PSMs from *Eucalyptus* foliage were also powerful emetic agents in marsupials. The mechanisms of nausea and vomiting have been intensively studied in humans and those laboratory species that vomit, and several drugs are available that attenuate the emetic response. For example, common brushtail and common ringtail possums ate more of a diet containing the *Eucalyptus* formylated phloroglucinol compound, jensenone, when given ondansetron (a selective 5-HT_3 receptor antagonist; Lawler *et al*. 1998), and a similar response was seen in brushtail possums that were dosed orally with vofopitant, a specific tachykinin NK_1 receptor antagonist (DeGabriel *et al*. 2010). Sheep ate more endophyte-infected tall fescue when given the dopamine D_2 (and possible serotonin 5-HT_4) receptor antagonist metoclopramide (Aldrich *et al*. 1993), and sheep given diets containing lithium chloride ate more food when they were also provided with a combination of diphenhydramine,

metoclopramide and dexamethasone than when they were not (Provenza *et al*. 1994*b*, 1994*c*).

These studies confirm that there are multiple pathways by which PSMs may cause malaise related to nausea and vomiting. Both serotonin and substance P, the neurotransmitter associated with tachykinin NK_1 receptors, are found in EC cells (Sundler *et al*. 1977), and the current working hypothesis is that PSM-induced damage to or stimulation of these cells releases these neurotransmitters, which can in turn stimulate the emetic centre. Even in the absence of vomiting, conditioned aversions can form though these pathways (Lawler *et al*. 1998). The EC cells in the gut release additional serotonin post-PSM ingestion, resulting in increased gut contractions to promote faster removal of gut contents. Increased levels of free serotonin in the blood can activate 5-HT_3 receptors in the brain to stimulate vomiting. Note that our hypothesis for the action of PSM ingestion on serotonin metabolism does not yet take account of key elements such as the re-uptake of serotonin by specific transporters (serotonin transporter, SERT), which also control its diffusion and action (Spiller 2007). Whether PSMs also affect serotonin re-uptake remains to be investigated.

Non-conditioned aversions

Not all PSMs stimulate conditioned aversions, but this is not a prerequisite for them to restrict feeding. For example, bitter and hot sensations are detected pre-ingestively (before swallowing) and can reduce intake without conditioning long-term aversions. This effect is familiar to humans that have consumed a very hot curry. Although capsaicin restricts the rate of ingestion, most of us are still prepared to eat curry again at subsequent meals. These effects occur widely in natural systems (Tewksbury and Nabhan 2001).

Extensive molecular work on both the bitter receptors and the capsaicin receptors has elucidated the way that these sensations are signalled. In particular, molecular differences in the capsaicin receptor explain the relatively higher tolerance of birds for plants such as peppers (*Capsicum* spp.) compared with mammals (Jordt and Julius 2002; Furness *et al*. 2013; Janssen and Depoortere 2013). This work has also revealed the presence of both taste receptors in the gut, along with capsaicin-sensitive neurons that can release substance P, arguing against a purely pre-ingestive role for bitter and hot substances affecting feeding.

Along a similar vein, it has recently been demonstrated that exposure to volatile compounds (dietary terpenes) can result in the upregulation of biotransforming enzymes in the nasal epithelium. The upregulation of two biotransformation enzymes, cytochrome P450 2B and glutathione-S-transferase, in the nasal epithelium was correlated with their expression in the liver of the white-throated woodrat, *Neotoma albigula* (Skopec *et al*. 2013). Pygmy rabbits (*Brachylagus idahoensis*) also consume a terpene-rich diet, and mastication is a way to reduce monoterpene (White *et al*. 1982) and tannin (Provenza and Malechek 1984; McArthur *et al*. 1991) concentrations in herbivore ingesta. The presence of bitter-tasting or volatile compounds may also be used as a cue signalling the presence of other, less volatile compounds that may initiate a stronger emetic response (e.g. Lawler *et al*. 1998). However, at present we lack data that encompass the elaborate connections linking sensory systems, monitoring processes and feedback mechanisms that make possible the regulated intake of dietary toxins.

Recognising detoxification limitations

Several studies have shown that animals can closely match feeding rates to their capacity to detoxify ingested PSMs. For example, Marsh *et al*. (2005) found that common brushtail possums ate more of a diet containing benzoate when it was offered with supplemental glycine, even though glycine is a non-essential amino acid. Glycine is, however, used to form benzoylglycine (hippuric acid), which is excreted in the urine. Despite this, glycine conjugation does not appreciably increase the water solubility of benzoic acid, although this is the major perceived benefit of most other conjugations (e.g. with glucuronic acid). Accordingly, the 'glycine deportation hypothesis' (Beyoğlu *et al*. 2012) has been proposed to argue that the conjugation of glycine with benzoates serves to regulate the amount of glycine in the central nervous system where it (and other amino acids that can be conjugated with benzoate) functions as a neurotransmitter. Imbalances in brain amino acids can trigger aversive responses to food (DeGabriel *et al*. 2002; Maurin *et al*. 2005) and this may explain why diets rich in either benzoates or glycine are aversive to animals (Marsh *et al*. 2005; Badenhorst *et al*. 2014).

Marsh *et al*. (2006*b*) also showed that possums could eat more when allowed to choose from diets containing PSMs that were detoxified by complementary biotransformation pathways, but not when choosing from those detoxified by competing pathways (those with shared biotransformation enzymes or substrates). McLean and colleagues (Boyle *et al*. 2005; McLean *et al*. 2007) have also shown that the timing and duration of meals of possums fed diets containing 1,8-cineole are strongly correlated with changes in the plasma concentration of cineole and its metabolites.

Although these studies of the metabolism of benzoate and cineole support detoxification capacity as a major determinant of feeding behaviour, the complexity of most natural diets and the biotransformation pathways involved makes these ideas difficult to test more generally. Nonetheless, these observations suggest that mammals can recognise limitations to their detoxification capacity and regulate their feeding or choose alternative diets that allow them to escape these constraints. However, it is unclear whether these behavioural responses depend on the same physiological feedback that is involved in the development of conditioned aversions. In other words, do accumulations of secondary metabolites in blood trigger responses such as nausea that allow animals to recognise impending toxicoses and respond behaviourally?

Using anti-emetic drugs to determine whether nausea is a signal of PSM ingestion is often difficult with non-domesticated animals (but see Lawler *et al*. 1998; Pass and Foley 2000; Marsh *et al*. 2005). The drugs are expensive and must also be administered simultaneously with feeding for them to have an effect. As a consequence, Marsh (2006) explored whether urinary markers of serotonin metabolism might provide a less invasive way of studying feedback from a wider range of PSMs.

This is a first step in testing how limitations to detoxification might be recognised.

The main route of serotonin metabolism is oxidative deamination to form 5-hydroxyindoleacetaldehyde, and then further oxidation to form 5-hydroxyindoleacetic acid (5-HIAA; Deacon 1994). 5-HIAA is water-soluble and is therefore excreted almost entirely in urine (Deacon 1994). Since the measurement of 5-HIAA is routine, Marsh (2006) analysed 5-HIAA concentrations in urine of brushtail possums in response to diets containing different PSMs (Fig. 1). Compared with those eating the basal diet, possums ingesting jensenone, 1,8-cineole or sodium benzoate had elevated urinary concentrations of 5-HIAA. In contrast, excretion of 5-HIAA on diets containing the simple phenolic compound, orcinol, was not significantly different from the basal level, although there was significant variation in 5-HIAA excretion between separate basal collections and between individuals. In other mammals, changes in 5-HIAA excretion reflect the amount of serotonin released from enterochromaffin cells and the severity and pattern of serotonin-dependent emetic responses to chemotherapeutic agents (Cubeddu 1996; Cubeddu *et al*. 1992; Cubeddu and Hoffmann 1993; Castejon *et al*. 1999). Thus, the release of serotonin from enterochromaffin cells may be a more common result of PSM ingestion than has been realised previously. These data are consistent with the suggestion that serotonin might be a unifying signal for feeding modification in response to a wide range of PSMs.

For one of the PSMs fed, sodium benzoate, possums showed no increased feeding response following injections of the 5-HT_3 receptor antagonist, ondansetron (Marsh *et al*. 2005). The discrepancy might be due to the difficulty of matching injection times with feeding times, and so, adding ondansetron in the diet, along with PSMs of interest, may be a more effective test of the role of serotonin and 5-HT_3 receptors in influencing food intake. Excesses of nutrients and/or toxins are known to stimulate the emetic systems of many livestock species (Provenza *et al*. 1994*c*). Lithium chloride feeding aversions have been demonstrated in sheep, with the strength of the aversion driven by concentration, time until the onset of malaise and previous experience. However, such feeding aversions can also occur in the presence of amino acid imbalance. Although the central nervous system and serotonin appear to be involved, the specific mechanism of response remains unclear (Provenza 1995).

In contrast to these responses to rapid and acute increases in serotonin, humans and laboratory animals do not always experience nausea and vomiting in conjunction with 24-h increases in urinary 5-HIAA excretions. Cubeddu *et al*. (1992) showed that the pattern of nausea and vomiting matched the pattern of 5-HIAA excretion in patients undergoing treatment for cancer with cisplatin or dacarbazine. When a large amount of serotonin was released in a short time, there was a correspondingly large emetic response. When the same amount of serotonin was released over a longer time period, a mild

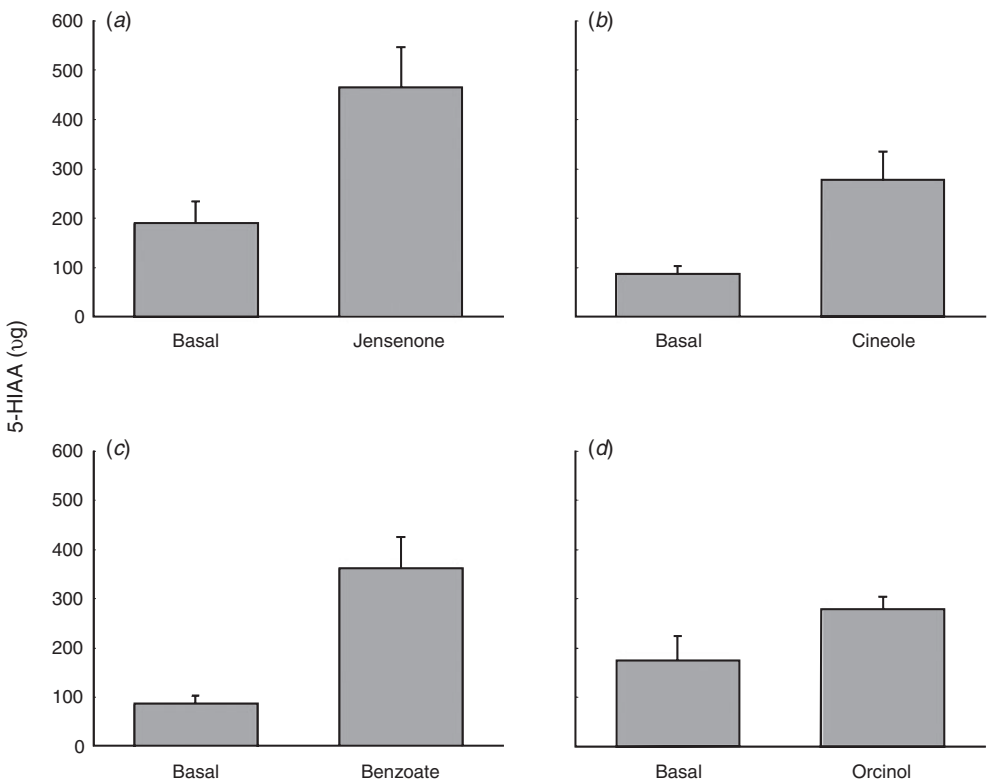

Fig. 1. The excretion of 5-hydroxyindoleacetic acid (5-HIAA) by common brushtail possums in 24 h when eating a basal diet to which no plant secondary metabolites had been added, or the same diet containing (*a*) jensenone ($n = 6$), (*b*) cineole ($n = 3$), (*c*) sodium benzoate ($n = 3$) and (*d*) orcinol ($n = 3$).

emetic response occurred, spread over the release time (Cubeddu *et al*. 1992). Thus, the time course of serotonin release may be as important in affecting feeding as the total amount released, and shorter collection periods may give an indication of the duration and severity of the emetic signal through the serotonin pathway for different PSMs. These characteristics of the emetic signal also suggest an obvious link between conditioned aversions and limits to detoxification, and refinement of protocols for measuring 5-HIAA excretion could allow the role of serotonin to be investigated in a wide variety of herbivores.

Do the mechanisms governing appetite and preventing toxicoses interact?

Notwithstanding the likelihood of cross-talk between these mechanisms (particularly between conditioned aversions and limits to detoxification), there is good evidence from studies of both wild and domestic species that the three mechanisms listed above are commonly and widely used to regulate intake of PSMs in the diet. As the complex neural mechanisms regulating appetite are uncovered, it is likely that this simple list will be modified. For example, there is now strong evidence that taste receptors in the gut play a vital role in communication among the gut lumen, epithelium and nerve afferents that signal the appetite centre and so affect food intake (Depoortere 2014). It is quite possible that they also play a role in sensing and modifying the intake of PSMs.

Although the sensory systems of the gut are being rapidly elaborated, we need to know how these are functionally integrated with other information from throughout the body to regulate appetite. In particular, a major research need is to understand how the neural pathways regulating appetite interact with those that regulate ingestion of toxins. Are these systems separate or is there cross-talk between them? Does regulation of toxin intake override normal appetite controls? Little evidence is available on this critical point, although Currie *et al*. (2010) showed that serotonin can act within the paraventricular nucleus of the hypothalamus to block the stimulatory effect of the neuropeptide ghrelin on appetite. Stapley *et al*. (2000) exposed common brushtail possums to cold (4°C) conditions, which led them to significantly increase the amount they ate of a basal diet, compared with control possums housed at 22°C. However, when the formylated phloroglucinol compound, jensenone, was added to the diet, animals could not maintain those higher levels of food intake demanded by thermoregulation at 4°C. These preliminary data suggest that regulating the intake of toxins did take precedence over increasing appetite to provide energy for thermoregulation, but clearly more targeted experiments need to be conducted.

PSMs do not exist in isolation

We have described some of the ways by which animals can recognise impending toxicosis. However, when the effect of a PSM is not directly toxic and the effect is, for example, reduced digestion of a dietary protein, it is hard to conceive of a mechanism that could allow animals to link undesirable outcomes to particular foods that are separate from the existing mechanisms that allow nutrient intakes to be regulated. Tannins provide a good example of this problem. Certainly, some tannins are sufficiently astringent for animals to avoid them. However, many other tannins might only exert their effects through binding with dietary or endogenous proteins, thus reducing protein digestion. If other, co-occurring, smaller molecular-weight phenolics act as toxins, then a conditioned aversion could even result (Provenza *et al*. 1994*a*). As the food matrix becomes more complicated, the ability of animals to associate subtle PSM effects with specific dietary constituents should become harder.

Interactions among PSMs and between PSMs and nutrients are widely predicted and can be demonstrated using artificial diets to which varying concentrations of nutrients and PSMs are added. In contrast, interactions are rarely tested or predicted when herbivores are fed natural diets. Furthermore, the mechanisms by which these interactions occur and the means that animals use to detect and 'titrate' nutrients against the costs imposed by PSMs remain unknown.

Nonetheless, there is good evidence from studies of wild species that animals can detect interactions between tannins and proteins. The Geometric Framework allows interactions between PSMs and nutrients to be observed far more easily than with other approaches. For example, Felton *et al*. (2009) showed that wild spider monkeys (*Ateles chamek*) tightly controlled their intake of protein each day, even though their consumption of energy varied markedly. Importantly, the data showed that it was the available protein intake and not the total protein intake that was regulated. Available protein adjusts total crude protein downward to allow for the effects of tannins and fibre on protein digestion, providing an integrated estimate of plant protein availability to animals.

The detoxification-limitation hypothesis (Marsh *et al*. 2006*a*) predicts that the ingestion and metabolism of PSMs should incur a measurable cost on animals, imposing a 'tax' on gross nutrient intake. Most attempts to estimate the cost of detoxification have used energy as a currency (Au *et al*. 2013), but most studies that attempt to manipulate detoxification capacity have supplemented animals with protein. Invariably, mammals ingest more PSMs if supplemented with protein (Villalba *et al*. 2002; Villalba and Provenza 2005; Nersesian *et al*. 2012) and wild animals sometimes simultaneously select high-protein and low-PSM diets (Moore *et al*. 2010; Ulappa *et al*. 2014) but these observations alone do not explain why this occurs. Au *et al*. (2013) argued that protein was a better currency than energy in which to measure detoxification costs, and showed that whole-body protein turnover rates were significantly higher in common brushtail possums when their diets were supplemented with benzoate (which is removed from the body via conjugation with amino acids).

Protein is involved in the detoxification process in several ways. Protein is used to synthesise enzymes that are used in detoxification (Whitlock and Denison 1995; Pass *et al*. 1999). Furthermore, excretion of PSMs often involves the loss of nitrogenous compounds either as conjugates (that are excreted in the urine or bile) or resulting from muscle catabolism following disruption of acid–base balance (Foley *et al*. 1995). Protein might also aid in repair of tissues that have been damaged by toxic actions (McLean *et al*. 2004). Quantifying the cost of detoxification in terms of protein should allow

better linkages to be made to existing models of nutritional choice and to field studies explaining reproductive success in free-ranging browsers (DeGabriel *et al*. 2009; McArt *et al*. 2009).

Pfister *et al*. (2012) found that the protein to energy ratio of diets influenced cattle consumption of a chemically defended diet (pine needles), where animals on a low-protein diet were unable to tolerate high concentrations of terpenes. This study, along with similar studies by Villalba *et al*. (2002) and Campbell *et al*. (2007), demonstrated that body condition and protein intake are important factors in the ability of herbivores to tolerate the consumption of toxic diets, presumably because they counter the additional demands for protein and energy.

Translating physiological effects to behavioural responses

The ingestion of PSMs is tightly regulated by herbivore physiological responses, which manifest as behavioural responses that act to assist in the regulation of PSM intake. Therefore, behavioural observations of how herbivores interact with their diet can provide a window of insight into the physiological effects of PSMs.

Behavioural observations of foraging herbivores have indicated that herbivores use pre-and post-ingestive behaviours to regulate PSM intake. Pre-ingestive assessment and/or avoidance of dietary PSMs can be achieved through olfaction. For example, brushtail possums spent more time pre-ingestively sniffing diets containing higher concentrations of the volatile PSM 1,8-cineole, and stopped feeding sooner after the initiation of ingestion, than did those fed low-cineole diets (Wiggins *et al*. 2003). These behaviours offer support for the theory that neutrally mediated interactions between the senses (such as taste and smell) and the viscera allow herbivores to pre-ingestively assess the consequences of food ingestion (Provenza 1995). Post-ingestive behavioural regulation of PSM intake has been demonstrated in mammalian herbivores through the close examination of daily feeding patterns (Torregrossa and Dearing 2009).

The identification of feeding patterns that herbivores use in response to PSMs not only provides important insight into how total intake is regulated, but it also offers a useful proxy for inferring the physiological effects of PSM ingestion. It is becoming increasingly apparent that the regulation of meal length and inter-meal length (i.e. the time between meals) is an important behavioural strategy used to mitigate the effects of chemically defended diets. This behavioural regulation has been shown to occur in a range of mammals from a diversity of systems (e.g. Pfister *et al*. 1997; Wiggins *et al*. 2006*a*; Marsh *et al*. 2007; Dziba and Provenza 2008; Torregrossa *et al*. 2011), and is proposed to be driven by physiological limitations to the rate of detoxification (Marsh *et al*. 2006*a*). Measured daily feeding patterns provide support for this theory; a herbivore's first meal for the day is usually the largest (in intake and in length; Wiggins *et al*. 2003) because there has been sufficient time to detoxify and/or eliminate PSMs encountered in the previous day's foraging activities. Subsequent meals are smaller (in amount eaten and duration) when herbivores continue to feed on dietary PSMs, with the effect exacerbated with increasing concentrations of PSMs (Wiggins *et al*. 2003; Marsh *et al*. 2007), and inter-meal length increases over the course of daily feeding activities, thought to be in response to blood plasma PSM concentrations reaching a physiological threshold (Dziba *et al*. 2006; Wiggins *et al*. 2006*b*; Torregrossa *et al*. 2011). The observed meal patterns occur when herbivores ingest PSMs known to stimulate emetic pathways, supporting the argument that feedback signals from nausea are an important mechanism used by mammalian herbivores to avoid toxicity. Relatively few studies have simultaneously measured the physiological and behavioural responses of herbivores to PSMs, yet they provide a powerful link between the physiological regulation of blood plasma concentrations of PSMs and the associated behavioural feeding responses (Boyle *et al*. 2005; Dziba *et al*. 2006; McLean *et al*. 2008).

This detailed examination of the link between the physiological effects of PSMs and associated behavioural responses can assist with understanding PSM-mediated effects on foraging herbivores. While physiological measurements on free-ranging or wild herbivores may prove challenging, behavioural surveys can provide insight into PSM-mediated interactions. Free-ranging herbivores must deal with multiple factors when foraging, from encountering variations in the distribution of PSMs at various spatial scales (Wiggins *et al*. 2006*c*; Frye *et al*. 2013) through to trading-off the nutritional reward of ingesting a PSM-rich diet with the risk of predation (Kirmani *et al*. 2010; Nersesian *et al*. 2011). Thus, the tightly linked physiological and behavioural responses of herbivores to dietary PSMs have complex and far-reaching implications beyond the nutritional value of the diet (Forbey *et al*. 2013).

Toxicity is not static

Microbial and metagenomic interactions

Many studies have recognised that gut microbial populations can significantly influence the toxicity of ingested secondary compounds. Pioneering studies in ruminant herbivores have demonstrated spectacular examples of detoxification of PSMs, but also examples of toxification, and foregut microbes have been described as the 'first line of defence'. These studies have been reviewed in several places (Foley *et al*. 1999; McSweeney *et al*. 2001; Dearing *et al*. 2005). More recently, Kohl *et al*. (2014) showed that faecal transplants from experienced woodrats (*Neotoma lepida*) to naïve conspecifics facilitated a greater intake of creosote bush (*Larrea tridentata*) toxins. Further study of faecal metagenomics may provide a powerful and accessible means of detecting individual variation among mammals in their metabolism of PSMs, with recent work by Kohl *et al*. (2014) demonstrating that faeces can provide a useful proxy for composition of gut microbial community in wild herbivore populations.

As next-generation sequencing methods for describing gut microbial populations have improved, there has been an increasing awareness that gut microbes might play a greater role in the disposition of xenobiotics than first thought. Furthermore, it is clear that the metagenomic profile of individuals can vary in ways that enhance or diminish the activity of xenobiotics, including drugs. These insights are derived largely from studies of drug metabolism in human and laboratory rodents and have spawned a new area of study

known as 'pharmacometagenomics', and they are highly relevant for understanding PSMs as well. It is clear that insights into xenobiotic metabolism have to involve more than just DNA-based measurements of microbial diversity but complementing this with transcriptomics and flow cytometry can illustrate the wide-ranging effects of ingested xenobiotics.

Significant variation among humans in the metabolism of common drugs such as acetaminophen (paracetamol) has now been attributed to metagenomic differences. This suggests that the role of gut microbes in modifying drug metabolism may be far subtler than direct modification of the compound as has been observed in the past. First, there is emerging evidence that gut microbes can alter the expression of host genes involved in xenobiotic metabolism (Bjorkholm *et al*. 2009) and, second, metabolites produced by gut microbes can affect the host's capacity to process xenobiotics. For example, the production of microbial *p*-cresol in some individuals reduces the capacity of the host to produce sulfur metabolites as conjugates for excretion (Clayton *et al*. 2009) and may contribute to the variation among humans in hepatic toxicity of acetaminophen (Haiser and Turnbaugh 2013). In addition, there is evidence of interactions of microbial metabolism of common drugs with dietary protein. For example, in gnobiotic mice, dietary protein reduced the *in vivo* microbial metabolism of digoxin, with significant increases in drug concentration in the serum and urine (Haiser *et al.* 2013). These types of interactions are likely to be important in the metabolism of PSMs in herbivores.

Temperature-dependent toxicity

Ambient temperature is an extrinsic factor that is not traditionally included in theories of diet selection in mammalian herbivores (Stephens and Krebs 1986; Raubenheimer *et al*. 2009; Owen-Smith *et al*. 2010). Nonetheless, temperature plays a critical role in modifying PSM toxicity in mammalian herbivores such that warmer environmental temperatures, even temperatures within the herbivore's thermoneutral zone (the range of ambient temperatures where metabolic rates are lowest), increase toxicity and reduce animals' dietary options (Dearing 2013).

The evidence for temperature-dependent toxicity (TDT) stems from pharmacological, agricultural and physiological studies across two orders of mammals, including humans (reviewed in Dearing 2013). One study with birds (Chatelain *et al*. 2013) also found that starlings, *Sturnus vulgaris*, ate more toxic prey at temperatures below their thermoneutral zone. Pharmacological studies on laboratory mice and rats are by far the most numerous that have documented the effects of temperature on toxicity. In laboratory rodents, the effect of increasing ambient temperature from 26°C to 36°C is comparable to increasing the dose from two- to eight-fold for dozens of different chemicals, including natural compounds, drugs, and environmental contaminants (Keplinger *et al*. 1959). This stunning difference in the toxicity of a compound with respect to ambient temperature is likely the result of a reduction in liver metabolism when the organism experiences higher temperatures (Ben Zvi and Kaplanski 1980; Settivari *et al*. 2009). In some instances, this effect is compounded because PSM ingestion itself leads to increased body temperatures (Dearing *et al*. 2008) and even hyperthermia (Rusyniak and Sprague 2006). PSMs can increase heat production by decoupling oxidative phosphorylation in mitochondria (Ravanel 1986) or limit the abilities of animals to dissipate heat by promoting peripheral vasoconstriction (Aldrich *et al*. 1993). Toxin tolerance appears to be greatest at temperatures just below the lower critical temperature (Fig. 2). Furthermore, the effects are manifested in animals after both acute and chronic changes in ambient temperature (Gordon and Ramsdell 2005).

More natural examples of TDT also exist. One example in agricultural systems consists of domestic horses and cattle and the fungus-infected grass tall fescue (*Lolium arundianceum*). Fescue toxicosis is a syndrome caused by the ingestion of ergot alkaloids produced by the fungal endophyteus *Neotyphodium coenophialium* infecting tall fescue (Aldrich *et al*. 1993; Cross *et al*. 1995). The toxicity of these fungal alkaloids is temperature-dependent such that symptoms are exacerbated in mammals when they are at higher ambient temperatures (Osborn *et al*. 1992; Aldrich *et al*. 1993; Settivari *et al*. 2008*a*, 2008*b*). A second example from Australia is the observation (Oliver and King 1983) that the dose of sodium monofluroacetate (1080) required to kill three species of mammals was reduced when it was consumed at high ambient temperatures. This included the common brushtail possum, which naturally encounters this PSM in plants from the family Fabaceae.

It is interesting to note that cattle reduce body temperature by seeking shade and loafing chest-deep in ponds and that during heatwaves koalas sometimes descend to shady tree hollows at ground level, hug the trunks of certain tree species that are cooler than the ambient temperature (Briscoe *et al*. 2014), and even sit in water when available. There is no shortage of evidence of such behavioural as well as physiological strategies that may enable animals to lose excess heat, but it remains to be shown that animals use these as a strategy to improve their tolerance of PSMs. Further studies on

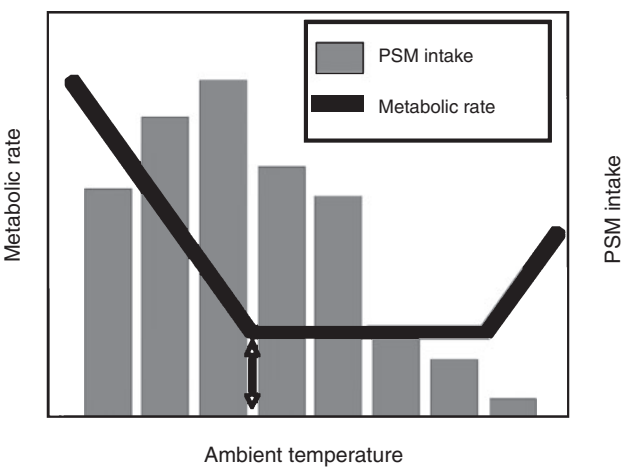

Fig. 2. The hypothetical relationship between ambient temperature, metabolic rate (solid line) and ingestion of plant secondary metabolites (PSMs, bars). Herbivores are predicted to have the greatest intake of PSMs at ambient temperatures below the lower critical temperature (arrow). Redrawn from Dearing (2013) with permission.

ecologically and evolutionary relevant systems are needed to determine the extent of TDT and to evaluate its impact on native herbivores and other endotherms.

The underlying mechanisms by which elevated temperatures intensify the effects of toxins in mammals are speculative. Liver size tends to decrease with acclimation to warm temperatures (Toloza *et al*. 1991; Settivari *et al*. 2008*b*), and the reduction in organ size could reduce an animal's capacity to metabolise toxins. However, changes in liver size cannot fully account for TDT because it is observed in animals not acclimated to the experimental temperature, i.e. animals with similar liver sizes (Keplinger *et al*. 1959). Temperature-dependent changes in toxicity seem to be primarily mediated by a reduction in overall liver metabolism at higher temperatures (Ben Zvi and Kaplanski 1980). For example, a recent study that explored the influence of temperature on the hepatic response of rats to ergot alkaloids using transcriptomic approaches found reduced hepatic gene expression of ~200 genes in rats exposed to warmer temperatures (Settivari *et al*. 2009). The types of genes that were reduced in expression included not only detoxification genes but also those related to nutrient metabolism. Thus, the liver does not seem to function as well at higher ambient temperatures, even though core body temperature often remains constant (Gordon 1993). The liver is one of the most sensitive organs to heat stress and an increase in oxidative damage may be the cause of the reduction in function at higher environmental temperatures (Flanagan *et al*. 1995; Zhang *et al*. 2003). However, results of a microarray experiment by Bhusari *et al.* (2007), in which mice were exposed to ergot alkaloids under heat stress conditions indicated that TDT may be both species- and PSM-specific. In that study, more genes were upregulated (33) than downregulated (8), and the upregulated genes were involved with protection against reactive oxygen species, lipid metabolism and xenobiotic metabolism and detoxification.

The heat dissipation limit hypothesis (Speakman and Krol 2010) abandons the traditional assumption that energetic constraints always act on animals from the supply side (i.e. animals' capabilities are limited by their ability to obtain sufficient energy to meet the demands of movement, thermogenesis and reproduction). Instead, it suggests that animals are often constrained by their ability to expend energy, because this expenditure generates heat, and with it a risk of damaging hyperthermia. As the liver can make a substantial contribution (up to 25%) to thermogenesis (Dewasmes *et al*. 2003), it is likely that liver metabolism is regulated within the abilities of animals to dissipate the heat it generates. The additional energy expenditure required to detoxify PSMs may generate additional heat over the digestion of meals without PSMs. Reduced liver metabolism increases the toxicity of PSMs by reducing the capacity for these compounds to be safely detoxified before being circulated systemically. This effect may be compounded if the consequences of exceeding this dose are also more serious at high temperatures.

The concept and mechanisms underlying TDT raise many questions. Can mammals ameliorate the impacts of TDT through the selection of cool microclimates or timing of feeding? Does TDT explain seasonal dietary shifts in wild herbivores? Modelling of species' climatic preferences on the basis of their current distributions suggests significant range contractions for many herbivorous species, for example, koalas (Adams-Hosking *et al*. 2012), in the face of global change. However, the current climatic distribution of animals does not necessarily exemplify the true constraints that they face. There is need for more sophisticated mechanistic, rather than correlative, models that integrate the physiological constraints of animals, such as limitations imposed by nutritional requirements and toxin tolerance, with climate data to predict how the demography of individual species will be affected (e.g. Forbey *et al*. 2013).

If PSMs directly interfere with the ability of mammals to maintain a constant body temperature, then the heat dissipation limit hypothesis (Speakman and Krol 2010) would predict a serious risk of hyperthermia if heat is generated beyond the animal's ability to dissipate it, particularly at higher ambient temperatures. Dearing *et al*. (2008) observed hyperthermia in woodrats (*Neotoma* spp.) fed juniper under hot (29°C) temperatures compared with control diets, and ergot alkaloids associated with fescue toxicoses also promote hyperthermia in cattle at high ambient temperatures (Settivari *et al*. 2009).

Several mechanisms are available by which PSMs can influence thermoregulation. First, a variety of compounds including several phenolics known from *Eucalyptus* have been shown to possess uncoupling activity in mitochondria (Mathiesen *et al*. 1996; Spiridonov *et al*. 2003). Adenosine triphosphate synthesis (oxidative phosphorylation) is powered by proton motive force across the inner mitochondrial membrane (from the outside-in), which is maintained by the active (and energy-consuming) export of protons in the opposite direction by the electron transport chain. Uncouplers make the membrane 'leaky' to protons so that the proton gradient is reduced and ATP synthesis is compromised. Metabolic rate then increases to compensate for the increasingly inefficient production of ATP, and energy from the electron transport chain is dissipated as heat, leading to hyperthermia if it cannot be dissipated peripherally (Saling *et al*. 2011).

Controlled uncoupling mediated by uncoupling proteins (e.g. UCP1, thermogenin) plays an important role in thermogenesis in many mammals. At least one common PSM from *Eucalyptus*, the formylated phloroglucinol compound, macrocarpal G, may be more potent than the model uncoupler, 2,4-dinitrophenol (Spiridonov *et al*. 2003). Second, many compounds can interfere with heat loss or retention by directly affecting the distribution of blood between the core and peripheral circulation. The liver is the main site of detoxification, at least in hindgut-fermenting marsupial herbivores, and so most ingested xenobiotics have access to gut and liver cells. Furthermore, because liver is a major heat-generating organ, accounting for up to 25% of thermogenesis in placentals (Dewasmes *et al*. 2003), and is likely playing a more important role in marsupials (Villarin *et al*. 2003), there is ample scope for PSMs to interfere with animals' thermal budgets.

Temperature may also influence toxicity in ectothermic animals. Clements *et al*. (2009) provided evidence of temperature influencing the gut microbiota of fish, which can alter the detoxification abilities and resulting foraging decisions of marine vertebrates. Rising sea temperatures are likely to play

an important role in this interaction. For other ectotherms, faster metabolic rates associated with higher temperatures may allow greater tolerance of xenobiotics. For example, green anole lizards (*Anolis carolinensis*) showed greater survival after exposure to a natural pyrethrin at higher than at lower temperatures (Talent 2005). Rising ambient temperatures and the advent of more extreme-weather days suggests that thinking about PSMs in this kind of framework might help us predict some of the more subtle effects of global change on both wild and domestic herbivores.

Conclusions and future directions

Although there is some understanding of the way that herbivorous mammals detect impending toxicity and modify their intake accordingly, this is largely based on feeding captive animals artificial diets to which purified secondary compounds have been added. Our understanding of nutrient interactions is largely derived from modifying these diets with additional protein or energy. There is an urgent need to focus on natural foods and free-ranging animals to understand the extent to which the principles described here apply in these more natural situations. It is likely the simple models of regulation that we have described will need to be broadened and more holistically integrated when animals are faced with multiple compounds from multiple foods. In particular, we need a better understanding of the interactions between the mechanisms to avoid toxicity and those that regulate nutrient intake, and this must include the role of microbes.

Although there have been some attempts to model the effects of PSMs on food intake in herbivores (e.g. Illius and Jessop 1995), we predict that future efforts will have to account for ambient temperature as well as plant characteristics and animal state. The potential effect of ambient temperature on toxicity is complex as we have described but may possibly be captured by models of thermal stress and a broader integration of bioclimatic variables with animal performance (e.g. Kearney *et al*. 2013). In this respect, ecologists and animal production scientists continue to have much to learn from each other.

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