CHAPTER FIVE

The herbivore's prescription: a pharm-ecological perspective on host-plant use by vertebrate and invertebrate herbivores

JENNIFER SORENSEN FORBEY
Boise State University

MARK D. HUNTER
University of Michigan

5.1 Introduction
Plants, and the organisms that eat them, constitute the majority of terrestrial multicellular diversity (Speight et al., 2008). Indeed, co-evolutionary interactions between herbivores and plants are thought by some to be 'the major zone of interaction responsible for generating terrestrial organic diversity' with plant secondary metabolites (PSMs) playing a central role in co-evolutionary processes (Ehrlich & Raven, 1964). As typically described, plants gain fitness advantages and the potential for evolutionary radiation from mutation or recombination events that generate novel PSMs that deter herbivores (or other attackers and competitors, e.g. pathogens). In turn, counter-adaptations, or offences (Karban & Agrawal, 2002; Sorensen & Dearing, 2006), by herbivore populations favour cladogenesis in the consumers and exert further selection pressure for novel PSMs (Janzen, 1980). Antagonistic interactions between plants and herbivores are, therefore, seen as a driving force behind the great diversity of PSMs that occur in plant populations (Rosenthal & Berenbaum, 1992; Gershenzon et al., Chapter 4).

The broad acceptance of a co-evolutionary arms race between plants and herbivores, with antagonism as the pivotal interaction, has led to the general view that PSMs are toxins that must be avoided, tolerated or overcome by consumers (Speight et al., 2008). Perhaps not surprisingly, many ecologists and evolutionary biologists have simply come to regard PSMs as barriers to consumption, with those barriers overcome to varying degrees by the generalist and specialist herbivore populations that consume plants (Shipley et al., 2009). However, the development of a tri-trophic perspective of plant-herbivore–enemy interactions (Price et al., 1980) paved the way for a deeper understanding of the role of PSMs in the ecology and evolutionary biology of herbivores. Various important points have been made about the importance of herbivore presence and interactions with plant chemistry, including the role of herbivores in maintaining plant diversity and the potential for herbivores to provide plant fitness advantages by improving plant fitness (Hunter & Schultze, 1993; Vanden Bossche & Schultze, 1995). Thus, herbivore pressure is often seen as a major factor affecting plant chemistry and the evolution of PSMs.

We propose explore the role of herbivores in shaping plant chemistry and evolution. Herbivores are not only agents of selection but also important components of the ecological system. By manipulating plant chemistry, herbivores can affect the fitness of other herbivores, pathogens, and plants. This interaction can have significant implications for plant diversity and ecosystem functioning.

5.2 Pharamaceutical Potential
Humans have been utilizing plant-based remedies for thousands of years, and the development of modern medicine has relied heavily on natural products. The potential of plant-based compounds as pharmaceuticals is vast, and the discovery of new active compounds is ongoing. It is estimated that only a small fraction of the world's plant species have been screened for medicinal properties, and many more await discovery. The search for new drugs continues to be a major focus in modern medicine, and the potential of plant-based compounds offers promise for addressing many of the world's most pressing health challenges.

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herbivores. Variation within and among plant populations is now seen to provide the potential for 'enemy free space' for herbivores (Jeffries & Lawton, 1984; Bernays & Graham, 1988) and a template upon which interactions between herbivores, higher trophic levels and the abiotic environment can occur (Hunter & Price, 1992). Moreover, we recognise a variety of external stressors, including predation, disease and abiotic conditions, that may be ameliorated by some level of PSM consumption (Calvert et al., 1979; Hunter & Schultz, 1993; De Roode et al., 2008; Forbey et al., 2009), whereas the same PSMs can impose fitness costs if consumption rates are too high (Rossiter et al., 1988; van Zandt & Agrawal, 2004). These examples demonstrate that PSMs are neither inherently good nor inherently bad for herbivores. Rather, their net effects on herbivore performance should reflect dose, environmental contingency and the complex biotic and abiotic interactions within which consumption takes place (Hunter et al., 1992).

We propose that the field of pharmacology offers the insight needed to explore the interaction between PSM dose and environmental conditions (level of external stress) and establish the cost–benefit function in these interactions. We begin by describing the criteria needed to demonstrate that PSMs are actually exploited by herbivores for therapeutic effects (e.g. self-medication). The dose–response relationship, the therapeutic window and dose regulation are core concepts in pharmacological research that offer useful methods for assessing the balance between costs and benefits of PSMs. The remainder of the chapter provides examples from vertebrate and invertebrate systems in which the behaviour and performance of herbivores to external stressors and PSMs are viewed from the perspective of pharmacology. We conclude with some suggestions for integrating pharmacology and ecology, termed pharm-ecology (Forbey & Foley, 2009), into future areas of research.

5.2 Pharmacological perspective
Humans have long recognised that drugs, including PSMs, have both therapeutic and toxic properties. Given the potential for toxicity, commercially marketed drugs undergo rigorous tests that require decades of research and considerable financial resources to establish the 'safe' and effective dose (Amir-Aslani & Mangematin, 2010). As such, every prescription comes with the knowledge that the benefit of that drug to mitigate an external stress outweighs the potential toxicity. This balance between benefit and toxicity allows humans to select the best therapeutic dose for a specific ailment.

Is it possible that ecological and evolutionary interactions between plants, herbivores and external stressors can lead to the selection of therapeutic doses of PSMs in natural systems as they have in human–drug interactions? To answer this question we need first to establish criteria that promote
self-medication (Singer et al., 2009), or the intentional use of PSMs for therapeutic purposes, by herbivores. The first criterion is that the external stress has a fitness cost. Although a decrease in lifetime reproductive fitness is the best measure of the cost of a stressor, this is often difficult to measure in long-lived species. Measurements that indicate a compromised state of homeostasis may provide valid proxies for fitness. Changes in body mass, metabolism, concentrations of stress hormones (e.g. cortisol), individual survival, growth rate, reproductive output etc. can provide useful endpoints of stress responses. However, these endpoints should eventually be validated to determine if they reflect true changes in fitness. The second criterion is that intake of the PSM decreases the fitness of the consumer (i.e. is toxic) at some dose or in the absence of the stressor. The third criterion is that the consumer intentionally consumes PSMs when the external stressor is present and experiences increased fitness as a result.

5.2.1 Dose–response relationship
Central to these criteria are the pharmacological concepts of dose–response, the therapeutic window and dose regulation. The dose–response relationship describes what PSMs do to the body and is generally referred to as pharmacodynamics (Gibaldi & Perrier, 1982; Tozer & Rowland, 2006). Systemic concentration (i.e. concentration of PSM in the body) is the true link to response. However, we assume linearity between the consumed dose and resultant systemic concentrations and, therefore, often use dose in terms of intake for simplicity. The therapeutic view is represented by a sigmoidal curve, wherein an increase in dose (and therefore systemic concentration) decreases external stress (benefit) through therapeutic properties of the PSM (Figure 5.1a). The toxic view of the dose–response relationship is often represented by the inverse of this curve, wherein an increase in dose (and therefore systemic concentration) elicits an adverse response (cost) through toxic properties of the PSM (Figure 5.1b). Efficacy is represented by the maximum response (percentage of maximum, positive or negative), and potency is represented by the slope of the curve and defines the size of the dose needed to produce a response. A steeper curve indicates a more potent, biologically active PSM, where small changes in dose have rapid effects. The curves together illustrate the concept of chemical hormesis (Calabrese, 2005; Hayes, 2007; Raubenheimer & Simpson, 2009), wherein small doses of a PSM have opposite effects (beneficial) than larger doses of the same PSM (costly).

5.2.2 Therapeutic window
The second concept that is critical for assessing self-medication in herbivores is the therapeutic window (TW). This window describes the relative safety of a potentially therapeutic PSM and is represented by the difference between
Figure 5.1 Hypothetical dose-response relationships demonstrating the chemical hormetic characteristics of a PSM, wherein increases in dose correspond to increases in systemic concentration, which elicit a therapeutic or toxic response. (a) The left hand, clear panel, dashed line, illustrates the therapeutic benefit of PSMs to reduce external stress at lower doses. (b) The right hand, shaded panel, solid line, illustrates the costs of toxicity at higher doses of a PSM. Efficacy is represented by the maximum response (% of maximum) for the therapeutic effect (bottom of curve in (a)) and toxic effect (top of curve in (b)). The slope of the curve represents therapeutic and toxic potency. Steep slopes indicate more potent therapeutic (a) or toxic (b) responses. The difference between the therapeutic dose and toxic dose is the therapeutic window. Adapted from Calabrese (2005), Hayes (2007) and Raubenheimer and Simpson (2009).

the dose (or corresponding systemic concentrations) alleviating an external stress and the dose resulting in some threshold of toxicity (Figure 5.1). Exceptionally wide TWs may indicate PSMs that are not potent for either reducing stress or increasing toxicity. In other words, they require large increases in dose and subsequent systemic concentration before having an effect. In medicine, the best drugs are those that are potent therapeutics with high efficacy (i.e. maximum response often less than 100%) in patients, but are not potent toxins and do not reach full toxic efficacy. For herbivores, the widest TWs will probably not facilitate self-medication, but may allow herbivores to consume large quantities of PSMs whilst meeting nutritional needs without toxic responses. The therapeutic window is influenced by the biological activity of the PSM and also the physiology of the herbivore of interest. For example, both vertebrate and invertebrate specialist herbivores with mechanisms that minimise PSM absorption and maximise PSM metabolism and elimination (Sorensen & Dearing, 2003; Li et al., 2004; Sorensen et al., 2004; Mao et al., 2006) may experience very low concentrations of systemic PSMs, which may reduce toxicity even when the intake dose is high. Physiological mechanisms
that limit systemic concentrations may also reduce efficacy and potency of a PSM to alleviate external stress. Thus, specialists may consume much higher amounts of PSMs to receive the same therapeutic benefit as their generalist counterparts. It is important that comparative studies between different PSMs in the same herbivore or the same PSMs in different herbivores are conducted to adequately investigate the mechanisms that influence the dose–response relationship across a range of doses (McLean & Duncan, 2006; Sorensen et al., 2006).

5.2.3 Dose regulation

Lastly, self-medication requires that animals are capable of regulating feeding to optimise the dose of PSMs. The optimal dose is dependent on what the body does to that dose to influence concentration over time (pharmacokinetics) and what that concentration does to the body (pharmacodynamics) (Figure 5.2). There are many factors that influence pharmacokinetics and pharmacodynamics, and these are described elsewhere (Gibaldi & Perrier, 1982; Tozer & Rowland, 2006). The key point is that there exists an optimal dose that results in a concentration above the therapeutic threshold but below the toxic threshold. In medicine, drugs are formulated and administered specifically to regulate systemic concentrations within the therapeutic window. For example, some drugs are administered intravenously to provide maximally allowing various stressors to coexist. Marsh et al. (1999) have suggested that the body’s ability to regulate stress levels is related to the presence of PSMs. Althou

Figure 5.2 The relationship between dose, systemic concentration and response to an ingested PSM. (a) The concentration–time course of an ingested PSM is called pharmacokinetics (PK). The curves represent two doses, one larger than the other, resulting in a larger maximum concentration, but both doses having a similar rate of elimination of PSM (terminal slope). (b) The concentration–response curve is called pharmacodynamics. It represents the effect of a given concentration on the body. Concentrations that are too low are suboptimal (sub) and are therefore not therapeutic, and concentrations that are too high become toxic. The concentration range between suboptimal and toxic is the therapeutic window. Herbivores can theoretically regulate the dose and frequency of doses to maintain an optimal range of concentrations to alleviate a stress response. See McLean and Duncan (2006) and Sorensen et al. (2006) for additional details.
maximal systemic concentrations immediately. Others are formulated to allow sustained release following oral administration or are prescribed over various time intervals (much like herbivores might consume plant meals) to sustain systemic concentrations over longer periods of time. The best dosing regime is that which provides the greatest benefit and lowest cost of PSMs in response to an external stress.

There is increasing evidence that herbivores can regulate doses of PSMs to maintain optimal concentrations (Torregrossa & Dearing, 2009). As concentrations of PSMs in the diet increase, many herbivores decrease meal size and increase the intervals between meals as a way to maximise daily intake, but minimise doses of PSMs per meal (Wiggins et al., 2003; Sorensen et al., 2005a; Marsh et al., 2007). Convincing evidence comes from brushtail possums, which, by regulating PSM dose through changes in meal size and frequency, regulate blood concentrations of PSMs below a threshold (approx. 10 μg mL⁻¹; Boyle et al., 2005; McLean et al., 2008). In these studies, PSM concentration in the blood was maintained even as concentrations of PSMs in the diet changed and as tolerance to them increased. An increase in tolerance due to an increase in activity of detoxification enzymes that reduces systemic concentration of PSMs allowed possums to consume larger doses, but did not increase average blood concentrations (i.e., area under the blood-concentration curve). In other words, tolerant possums eliminated PSMs faster from the circulation, but instead of reducing blood concentrations below some suboptimal level, those possums regulated the dose via increased intake to maintain concentrations of PSMs at a constant level. In this case, possums were not exposed to external stresses and voluntarily consumed the ‘optimal’ dose, most probably to gain the benefits of maximising nutrient intake while minimising the costs of toxins. Although the mechanisms for dose regulation remain unknown, these results offer the first documentation of self-regulation of PSM concentrations in vertebrate herbivores.

Several studies suggest that invertebrate herbivores also regulate PSM dose relative to its concentration in the diet (Glendinning & Gonzalez, 1995; Simpson & Raubenheimer, 2001; Singer et al., 2002). For example, the presence of PSMs in leaves results in reduced meal number and meal duration in weevils (Wright et al., 2003). Diet mixing may minimise the accumulation of any one PSM in some caterpillar species (Singer et al., 2002) but the selective advantage of diet mixing by insects remains enigmatic (Johns et al., 2009; Karban et al., 2010). Other studies show that PSMs influence feeding by insects in a dose-dependent manner (Senthil-Nathan et al., 2008; Cardinal-Aucoin et al., 2009). However, a critical component missing from these studies is whether changes in feeding behaviour influence systemic concentrations in the body. Additional studies are needed in both vertebrate and invertebrate species that directly measure the amount and frequency of feeding and
5.3 Evidence for self-medication with PSMs in herbivores

Is there any evidence that herbivores can regulate dose of PSMs to mitigate the negative consequences of external stress? We propose that the answer is yes. Herbivores meet many of the criteria established for self-medication. External stresses such as predators and parasites have direct and indirect fitness consequences for herbivores (Combes, 2001; Irvine et al., 2006). In addition, there is substantial evidence that PSMs are detrimental and costly to herbivores and are, therefore, generally avoided (Freeland, 1991; Rosenthal & Berenbaum, 1992; Foley & McArthur, 1994; Sorensen et al., 2005c). There is increasing evidence that herbivores choose particular plants when external stresses are elevated (see examples below). There is also evidence that herbivores can regulate intake (Bernays & Chapman, 2000; Wiggins et al., 2003; Sorensen et al., 2005a; Torregrossa & Dearing, 2009) and that doing so can maintain concentrations of PSMs in the body at specific thresholds (Boyle et al., 2005; McLean et al., 2007). Finally, there is evidence that the intentional intake of PSMs can alleviate fitness costs associated with external stresses (see examples below). We now describe some of the more relevant examples that illustrate most, if not all, of the criteria required for demonstrating self-medication in herbivores (see Forbey et al., 2009 for additional examples in vertebrates).

5.3.1 Predators

That PSMs can have both positive and negative effects on herbivores gained general acceptance with pioneering studies of PSM sequestration by insect herbivores as defence against vertebrate predators (Brower et al., 1975; Seiber et al., 1975). Clearly, predators represent a significant source of external stress to herbivores both by eliciting non-consumptive stress and causing mortality (Preisser et al., 2005). The high body concentrations of PSMs sequestered by some herbivores (Wink & Witte, 1991) inevitably impose metabolic costs (if they are metabolised) and storage costs (Rowell-Rahier & Pastels, 1986; Opitz & Muller, 2009). Sequestered PSMs or their metabolites may also impose toxicity costs and compromise the health of herbivores (Smilansich et al., 2009). However, the costs of sequestering PSMs can be offset if sequestered PSMs provide the benefit of reduced predation (Opitz et al., 2010). Diet mixing by herbivores may represent a foraging strategy to further offset toxicity and opportunity costs associated with sequestration of PSMs (Singer et al., 2004b), thus increasing the benefit-to-cost ratio of sequestration. Herbivores that sequester illustrate elegantly the costs and benefits of pharmacological exploitation of PSMs because there exists a broad range of mimetic insects, grading f...
grading from toxic to palatable, that represent points along a cost-benefit continuum based upon external conditions (predation pressure, frequency of unpalatable models etc.; Ritland & Brower, 1993). It has been proposed that sequestration may be less common in mobile vertebrate species, because other predator-avoidance strategies, such as flight or flight, are less costly than the costs required to process and sequester high concentrations of PSMs (Forbey et al., 2009). However, studies are needed to determine whether potential direct benefits of sequestration such as immobilisation of PSMs (rather than excretion) are indeed secondary to reduced predation. Future studies should investigate the mechanisms that dictate higher or lower accumulation of PSM concentrations in tissues (i.e. biodistribution), the costs of these mechanisms; and how various levels of sequestered PSMs influence the fitness of herbivores under varying levels of predation risk.

5.3.2 Endoparasites
More recently, ecologists and evolutionary biologists have come to recognise a much broader range of therapeutic exploitation of PSMs beyond those used to mitigate vertebrate predation (Cory & Hoover, 2006). PSMs are exploited for use against invertebrate predators, parasitoids, parasites and agents of disease (Hunter & Schultz, 1993; Huffman et al., 1998; Singer et al., 2004b). Underlying this body of work is the idea that some PSMs are generally ‘antibiotic’, with a range of negative effects against a diversity of organisms. For example, some phenolic and polyphenolic compounds in plants are active against bacteria, viruses, fungi, nematodes, insects and vertebrates (Schultz et al., 1992). For a herbivore to exploit a polyphenolic pharmacologically requires that the negative impact on the natural enemy more than balances any negative fitness effect of consuming PSMs on the herbivore itself (Foster et al., 1992). Here, we provide several examples of apparent therapeutic use of PSMs by insect and mammalian herbivores. The examples range from generalists to specialist herbivores with stresses ranging from viruses to parasites.

5.3.3 Gypsy moth–host-plant–virus interactions
The gypsy moth, Lymantria dispar (Lepidoptera: Lymantriidae), is a polyphagous defoliator of temperate forest trees in Europe, Asia and eastern North America (Doane & Mccamus, 1981). Despite its wide dietary breadth, the gypsy moth exhibits preferences among host-plant species, and those preferences appear related, in part, to the risk of viral infection (Rossiter, 1987). Plant polyphenols have been implicated as PSMs that may reduce the susceptibility of gypsy moth larvae to a nuclear polyhedrosis virus (NPV), the external stress (Keating et al., 1988). When larvae are dosed with virus on artificial diet, it requires only 800 virus particles to kill 50% of the larvae. In striking contrast, it takes closer to 60 000 virus particles to kill 50% of larvae when
Figure 5.3 Effects of defoliation-induced changes in red oak (Quercus rubra) foliage quality on the susceptibility of gypsy moth (Lymantria dispar) larvae to a nuclear polyhedrosis virus. Data are from Hunter and Schultz (1993).

The virus is administered on foliage containing PSMs. Moreover, virus-induced mortality of gypsy moth among host-plant species is negatively correlated with the polyphenol concentration of the foliage. Experimental application of polyphenols to artificial diet or viral inoculum reduces the susceptibility of larvae to viral infection (Keating et al., 1988, 1990).

In an interesting twist to this story, gypsy moth larvae are known to induce foliar polyphenolic concentrations when they feed on some host-plant species (Rossiter et al., 1988; Hunter & Schultz, 1995). As a consequence, larval susceptibility to virus may decrease as defoliation levels increase. An experimental test of this hypothesis demonstrates that defoliation-induced increases in foliar polyphenols are associated with subsequent inhibition of NPV and higher larval survivorship (Hunter & Schultz, 1993). Simply put, the more foliage that larvae eat, the more pharmaceutically active their diet becomes (Figure 5.3).

As we have stressed previously, we should expect PSMs to have both positive and negative impacts on herbivores, and this is the case with the gypsy moth. In the absence of NPV, foliar polyphenolics have negative impacts on both the growth and fecundity of gypsy moths (Rossiter et al., 1988). The consequences of consuming a polyphenolic diet are, therefore, a balance between negative effects on growth and fecundity at high doses and positive effects on viral inhibition at low doses, illustrating chemical hormesis. The costs and benefits have been modelled analytically (Foster et al., 1992), with results that define an optimal polyphenolic dose for gypsy moth larvae that live in the presence of NPV. Interestingly, that optimal polyphenolic dose is typical of upland oak communities (Kleiner & Montgomery, 1994) where gypsy moth outbreaks typically develop in eastern North America (Doane & McManus, 1981).
5.3.4 Monarch–milkweed–protozoan interactions

If the gypsy moth provides an example of pharmaceutical PSM use by a generalist herbivore, the monarch butterfly, Danaus plexippus (Lepidoptera: Danainae), provides an example of pharmaceutical PSM use by a dietary specialist. Monarch larvae feed on a subset of species in the genus Asclepias (the milkweeds) and closely related plants in the family Apocynaceae. Most milkweed species contain cardenolides, toxic steroids that compromise ion channels in cell membranes (Benson et al., 1977; Mebs et al., 2000). Specialist insects of milkweed, like the monarch, generally have mutations in the genes coding for ion channel proteins and often sequester cardenolides as defences against their own enemies (Seiber et al., 1975, 1980). Although monarchs suffer some fitness costs associated with cardenolide consumption (Zalucki et al., 2001a, 2001b), they are known to gain protection from vertebrate herbivores from sequestered cardenolides (Brower et al., 1975). There is some evidence that cardenolides are also active against parasitic flies in the family Tachinidae that attack monarch larvae (Hunter et al., 1996). More recently, it has been shown that monarchs also gain protection from a protozoan parasite by exploiting milkweeds (De Roode et al., 2008), and we focus on this example below.

Ophyrocystis elektrosvirha (McLaughlin & Myers, 1970) (phylum Apicomplexa) is a protozoan parasite that infects monarch populations worldwide (Leong et al., 1997). Transmission generally occurs when females that are laying eggs scatter parasite spores onto leaf surfaces or directly onto the eggs themselves (Altizer et al., 2004). Infection proceeds when caterpillars ingest parasite spores from contaminated eggs or host-plant leaves. Spores lyse in the larval gut, and parasites penetrate the intestinal wall to undergo asexual and sexual replication. Parasite infection imposes a significant fitness cost on hosts, and reduces monarch survival, body mass, ability to fly, mating success and fecundity (De Roode et al., 2007). Recent studies indicate that PSMs in milkweed, specifically cardenolides, can mitigate the costs of parasite infection in monarchs.

The pharmaceutical effects of milkweed on monarchs has been tested by exposing larvae to controlled parasite doses while they are feeding on milkweeds that vary in quality and quantity of cardenolides (De Roode et al., 2008). Larvae consuming parasites on a milkweed species (Asclepias curassavica) with higher total concentration and diversity of cardenolides are less likely to be infected than are larvae infected by feeding on a low cardenolide species (A. incarnata) (Figure 5.4a). Moreover, the spore load carried by monarchs is lower and adult longevity increases when larvae consume the high cardenolide species (Figure 5.4b, c). In other words, the high cardenolide milkweed, A. curassavica, appears to ameliorate some of the fitness costs associated with parasite infection. Consistent with the criteria for self-medication, infected female butterflies preferentially lay their eggs on high cardenolide milkweeds.
whereas uninfected females do not (Lefèvre et al., 2010). The precise biochemical mechanism underlying the effect of milkweed PSMs on monarch parasites is not yet established. Cardenolides are implicated as potential pharmacicals, in part because they are much more concentrated and diverse in *A. curassavica* than in *A. incarnata* (De Roode et al., 2008; Figure 5.4d). Currently, studies are investigating whether *Ophryocystis elektroscirrh* has membrane ion channels that are susceptible to cardenolides (Felibert et al., 1995) as a potential mechanism for parasite inhibition in monarchs.

### 5.3.5 Caterpillars-host-plant-parasitoid interactions

The 'holy grail' of pharm-ecology research is evidence of therapeutic self-medication - a change in PSM use by organisms in direct response to an external stressor. This phenomenon is observed in natural populations of caterpillars and plants, where stressful conditions can alter the chemical composition of plant tissues, potentially affecting the effectiveness of PSMs in repelling or eliminating parasites. In addition, the interactions between host plants and their herbivores can also be influenced by the presence of parasitoids, as seen in the example of the milkweed and monarch butterfly system. The presence of parasitoids may alter the chemical composition of milkweeds, affecting host selection and herbivore behavior. These interactions can be studied to understand the complex dynamics of host-plant-parasitoid systems and their role in the natural world.
external stressor that subsequently reduces the fitness costs imposed by that stressor. Although the stressors of interest in self-medication studies are traditionally agents of disease (Clayton & Wolfe, 1993), we consider therapeutic self-medication to include behavioural plasticity in PSM use that alleviates the fitness burden of any external stressor, including predators, parasites and the abiotic environment (Forbey et al., 2009). Studies of therapeutic self-medication in any wild animal are rare, and most provide only anecdotal and equivocal evidence (Clayton & Wolfe, 1993). In insects, most pharmaceutical use of PSMs appears to be prophylactic, in which PSM use is behaviourally fixed and unaffected by the presence or absence of the putative stressor (Keating et al., 1988; Chapsisat et al., 2007). However, in at least two systems (monarchs on milkweed, above, and arctiid caterpillars, below), insects change their use of PSMs depending upon the external conditions that they face, thus demonstrating a major criterion for true self-medication.

In addition to monarch butterflies (Lefèvre et al., 2010), caterpillars in the family Arctiidae provide excellent evidence for therapeutic self-medication in insects (Singer et al., 2004b, 2009). Some arctiid caterpillars are generalists and highly mobile. As a result, they encounter and exploit a variety of host-plant species, with variable quality and quantity of PSMs, during development. For example, the caterpillar Estigmene acrea includes both Senecio longifolius and Viguiera dentata in its diet, the former containing pyrrolizidine alkaloids and the latter lacking them. Laboratory studies indicate that E. acrea performs better on V. dentata than on S. longifolius in the absence of parasitoids, yet older larvae prefer S. longifolius under field conditions, where the mortality risk from parasitoids is about 28%. Do the alkaloids in S. longifolius help to protect larvae from their parasitoids? It turns out that a mixed diet that includes both plant species confers the greatest protection from parasitism. The combination of high growth (from V. dentata) and pharmaceutical defence (from S. longifolius) mediates the greatest reduction in parasitism (Singer et al., 2004b).

The pattern of incorporating PSM-rich host plants in their diet occurs in other arctiids also. For example, woolly bear caterpillars, Gramma incerta ( = genephrus), sacrifice superior growth on alkaloid-free host plants to include plants like S. longifolius that contain pyrrolizidine alkaloids in their mixed diets. Mixed diets that include PSM-rich plants provide G. incerta with some level of protection from at least three species of parasitoids across two insect orders, Braconid wasps and Tachinid flies (Singer et al., 2004a). However, a key question remains: do arctiid caterpillars increase the PSM content of their diets in response to parasitism? In other words, are they capable of therapeutic self-medication?
In at least *G. incorrupta*, the answer appears to be yes. In the absence of parasitoids, excessive ingestion of alkaloids causes a reduction in the survival of *G. incorrupta*, indicating a clear cost associated with alkaloid consumption. However, if larvae are already parasitised, larval survival is greater if caterpillars consume pyrrolizidine alkaloids. Critically, parasitised caterpillars are more likely than unparasitised caterpillars to consume large quantities of alkaloids, providing strong evidence for therapeutic self-medication in *G. incorrupta* (Singer et al., 2009). Future studies should investigate choice of diets in addition to regulation of dose by varying intake of a single diet under both stressed (parasitised) and unstressed (unparasitised) conditions.

### 5.3.6 Ungulates—tannins—parasite interactions

There are a number of vertebrate animals, including humans and domestic herbivores, that select particular plants and PSMs to mitigate the costs of parasites. Parasites negatively affect the body condition and fecundity of animals (Irvine et al., 2006; Pullan & Brooker, 2008) and therefore represent a large external stressor for vertebrates. Although the majority of evidence for exploiting PSMs to reduce parasite loads is found in the literature on humans (Sneader, 1996), humans and vertebrate herbivores often share the same parasites and, therefore, should both benefit from the antiparasitic properties of PSMs (Huffman et al., 1998).

Studies on grazing livestock provide the most thorough evidence for self-medication by vertebrates to combat parasites. Parasites compromise survival, reproduction and growth rates (Hutchings et al., 2003; Min et al., 2003) in ruminants. Given the economic role of ruminants in food production, substantial effort is aimed at identifying cost-effective methods to control parasite infections. One interesting hypothesis is that ruminants can learn to consume natural pharmaceuticals in plants to treat parasite infection (Lisonbee et al., 2009; Villalba et al., 2010). In support, parasitised lambs consume the optimal dose of tannins in artificial diet to reduce helminthoses (Lisonbee et al., 2009). In addition, sheep voluntarily consume more tannins when infected with parasites than when they are not infected, and this intake results in a decrease in faecal egg counts (Villalba et al., 2010). The voluntary intake of tannins to reduce parasite burdens (0.9 g kg\(^{-1}\); Villalba et al., 2010) is below toxic doses (<1.5 g kg\(^{-1}\)) in sheep (Hervas et al., 2003). These results suggest that sheep can detect parasite loads and can voluntarily regulate intake of tannins to reduce the costs of parasites, while minimising the toxicity of tannins.
5.4 Methodological advances in detecting self-medication behaviour

5.4.1 Controlled conditions

To fully understand the costs and benefits of PSM consumption by herbivores and reveal mechanisms that drive diet selection, the exploitation of PSMs for therapeutic use should be investigated under conditions of controlled variation of stress and PSM diversity. First, diet selection should be conducted under conditions that carefully control levels of stress such as predators or disease. Valid therapeutic and toxic endpoints are also needed to assess response. Endpoint measurements should be developed that are closely linked to fitness such as survival, mating success or fecundity (De Roode et al., 2007) and ultimately rates of population growth. Since PSMs often influence herbivores on an individual basis, lifetime reproductive success may be the most relevant fitness parameter to investigate. Pharmacology offers well-established assays for assessing toxicity (Boyd, 1968; Caldwell et al., 2009) and these should be integrated with biological measures of fitness. We recognise that such experimental protocols are more difficult to implement in studies of long-lived species, but we urge those studying vertebrate herbivores to move beyond measurements of food intake as the endpoint response to PSMs.

Researchers should also aim to establish a true dose–response relationship by administering herbivores with a range of doses of PSMs, rather than relying on voluntary consumption, and they should also measure the link between dose, concentrations in the body and various responses. Once dose–response relationships have been established, studies should be conducted that allow herbivores to select diets from a range of food that varies in PSM quality and quantity available in their natural habitat. Because parasites can evolve resistance to a single PSM, and herbivores are likely to be exposed to multiple external stressors simultaneously, the best self-medication practice may be a mixture of PSMs with different biological activities (Villalba & Provenza, 2007). The maximum intake dose in the field of any single PSM should rarely exceed thresholds for toxicity established in controlled dose–response studies. However, dose of any PSM may be higher in the field than that established in laboratory studies, owing to complementary interactions between PSMs, wherein one PSM can increase tolerance to another (Marsh et al., 2006; Mody et al., 2007). Moreover, the dose for PSMs consumed in the field is expected to increase in natural conditions above typical levels when herbivores are exposed to external stress that can be mitigated by PSMs. Field approaches that simultaneously measure shifts in foraging behaviour (i.e. dose regulation and mixed diets) and stress levels may provide further insight into the advantages of mixed diets beyond the traditional hypotheses of nutrient balance and
detoxification limitation (Bernays et al., 1994; Dearing et al., 2000; Raubenheimer & Jones, 2006; Mody et al., 2007)

5.4.2 Field conditions
The ultimate goal should be to apply pharm-ecology to understanding host-plant use in the field. This requires developing biomarkers that can be used to assess natural variation in external stressors in herbivores, measuring PSM quality and quantity, and tracking the responses of herbivores to changes in stress and PSMs. This is a major challenge. However, several techniques are currently available and are ripe for broader use in ecological studies. For example, faecal cortisol metabolites (FCMs) are being increasingly used as an effective biomarker of stress in vertebrates (Sheriff et al., 2010). Metabolomics is an emerging pharmacological approach used to monitor and detect physiological responses to both external stress from diseases and PSM consumption (Griffin, 2003; Lindon et al., 2004). Infrared thermography provides a non-invasive technique that has been used to detect viral infection in animals (Schaef er et al., 2004; Dunbar et al., 2009) and has the potential to be used to detect parasites in wild animals. Any biomarker for stress should first be validated in the laboratory to ensure that it reflects an accurate assessment of the effects of the external stressor of interest.

Near-infrared reflectance spectrometry (NIRS) offers an emerging tool for assessing the quality and quantity of PSM within and among landscapes (Foley et al., 1998; Wallis & Foley, 2003; Stolter et al., 2006; Wiedower et al., 2009; Moore et al., 2010). For example, it was recently used to describe spatial variation in nitrogen and PSMs in eucalyptus foliage within a habitat and to establish a link between PSM variation and tree use by koalas (Moore et al., 2010). Once NIRS calibrations are established for a given PSM, quantities of PSM can then be determined in the field using hand-held devices (Walsh et al., 2000). Such technology is currently used in the agricultural sector to determine PSMs in fruits (Sinelli et al., 2008). Visible and near-infrared reflectance techniques have also been used to detect stress in plants from pesticides and infection (Delalieux et al., 2007; Luedeling et al., 2009). NIRS is expected to work best for specialist herbivores that have narrow feeding niches because mapping PSM concentration in the habitat can be transcribed directly to the quality of food consumed. However, spectral techniques applied to a variety of plants could be coupled with telemetry technology that tracks animal location and movement to measure the spatial and temporal shifts in foraging in both specialist and generalist herbivores in response to changes in parasite loads or other environmental stressors.

Finally, future work should explore the relationship between specialist and generalist diets and the evolution of self-medication (Singer et al., 2004a, 2004b). In part, the breadth we explore medical choices for self-tolerance (Mackenzie et al., 1988) in medicating self-tolerance (For...
There is little doubt that escape from stressors can drive, in part, the evolution of host choice in herbivores (Bernays & Graham, 1988; Lill et al., 2002). However, an open question is the degree to which current breadth of diet should influence the prevalence of self-medication. Should we expect dietary generalists to exhibit prophylactic or therapeutic self-medication? How will this compare with dietary specialists? It is tempting to assume that specialist herbivores may have responded evolutionarily to pharmaceutical needs but, as a result, have little flexibility in their current choices among plants. However, even specialists such as the monarch butterfly may exhibit preferences among congeneric plants based on their pharmaceutical properties (Lefèvre et al., 2010). Some specialists with narrow tolerance to plants beyond the preferred host (Putuyma & Moreno, 1988; Mackenzie, 1996; Sorensen et al., 2005b) may have diminished opportunities to self-medicate. It has been suggested that prophylactic self-medication should evolve when parasite risk is predictably high in space or time and that therapeutic self-medication is more beneficial when parasite risk is unpredictable and low (Carrai et al., 2003; Hart, 2005; Castella et al., 2008). However, we cannot assume that dietary specialisation reflects evolution for prophylaxis (Schultz, 1988) nor that generalists are more likely to engage in therapeutic self-medication. Both empirical and theoretical studies of the ecology and evolution of self-medicating behaviours that continue to draw on advances in pharmacology (Forbey & Foley, 2009) are required to answer these questions.

5.5 Conclusions and future directions
Although the notion of pharmaceutical use of PSMs by herbivores has broad appeal, there is increasing recognition that the discipline needs to develop firm criteria for establishing that a behaviour is indeed ‘self-medication’ (Clayton & Wolfe, 1993; Hart, 2005; Singer et al., 2009). We suggest that the establishment and use of such criteria is of fundamental importance in future research. Just as the term ‘co-evolution’ came to be used without sufficient care or evidence (Janzen, 1980), so too can the term self-medication be applied without appropriate rigour (Sapolsky, 1994). For a behaviour to be considered self-medication requires that (a) the consumer exhibits increased fitness as a result of the behaviour when the stressor is present; (b) the consumer exhibits decreased fitness as a result of the behaviour when the stressor is absent; and (c) the stressor induces the behaviour (Singer et al., 2009). To this list, we should add that the behaviour should not increase the fitness of any stressor organism (parasite, predator, agent of disease), otherwise it might represent parasite manipulation of host behaviour (Lefèvre et al., 2009). In other words, future studies of self-medication should take a hypothesis-driven, experimental approach based on a priori criteria.
We urge ecologists to use pharmacological insight and tools to establish necessary dose–response relationships. They should test the costs and benefits of stressors, and the intake and systemic concentrations of PSMs, in both laboratory and field conditions. Pharmacological knowledge should also be integrated with advances in metabolomics, remote sensing and telemetry in field studies to fully understand the balance between the positive and negative effects of consuming PSMs under varying environmental conditions. These novel approaches will shed new light on the factors that shape evolutionary trends in host-plant use by herbivores in natural systems.

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