A Specialist Herbivore (*Neotoma stephensi*) Absorbs Fewer Plant Toxins than Does a Generalist (*Neotoma albigula*)

J. S. Sorensen[1,2,*]
C. A. Turnbull[1]
M. D. Dearing[1]
[1]Department of Biology, University of Utah, Salt Lake City, Utah 84112; [2]Department of Botany and Zoology, Australian National University, Canberra 0200, Australia

Accepted 6/9/03

ABSTRACT

Detoxification capacity of enzymes in the liver is thought to be the primary factor governing dietary toxin intake by mammalian herbivores. Recently, toxin absorption in the gut was proposed as an alternative process that also influences toxin intake. We examined the role of the gut in regulating toxin absorption by quantifying excretion of a plant secondary compound in the feces. We hypothesized that specialists have a greater capacity to reduce intestinal absorption of toxins than do generalists. To test this hypothesis, we compared fecal excretion of alpha-pinene in specialist (*Neotoma stephensi*) and generalist (*Neotoma albigula)* woodrats. Alpha-pinene is the most abundant monoterpene in *Juniperus monosperma*, which occurs in the natural diet of both woodrat species. Woodrats were fed alpha-pinene in diets containing juniper foliage for 3 wk and, in a separate experiment, were given a single oral dose of alpha-pinene. Feces were collected from animals at the end of each experiment and analyzed for alpha-pinene concentration using gas chromatography. Both woodrat species excreted unchanged alpha-pinene in the feces. However, specialist woodrats excreted 40% more alpha-pinene per unit ingested from a juniper diet and excreted nearly four times a greater percentage of an oral dose of alpha-pinene compared with generalists.

Introduction

A fundamental objective in the study of plant-herbivore interactions has been to identify factors that limit dietary specialization in mammalian herbivores. The consumption of one plant species is rare in mammals because only a handful of mammalian herbivores are dietary specialists (Freeland 1991). Conventional wisdom presumes that the foraging ecology of herbivorous mammals is limited by the detoxification capacity of the liver (Freeland and Janzen 1974; Freeland 1991; Foley et al. 1999). The detoxification limitations hypothesis predicts that the few specialist mammalian herbivores that exist use rapid detoxification and elimination of plant secondary compounds, or toxins, to reduce toxin concentrations in the blood. In support of this hypothesis, a recent study found that a specialist mammalian herbivore had lower blood levels of toxins than did a generalist when both were given the same dose of a toxin. However, lower toxin levels were not a result of differences in the rate of toxin elimination from the blood (Sorensen and Dearing 2003). These results suggested that the rate of distribution and/or the rate of liver detoxification played a limited role in differences in toxin levels between specialists and generalists. Instead, disparities in toxin absorption appeared to be responsible for lower blood concentration of toxins in specialists compared with generalists.

The ability of mammals to reduce absorption of toxins in the gut has received little attention from scientists studying plant-mammal interactions but is well known to pharmacologists (Bellamy 1996; Wang et al. 2001; Washington et al. 2001). Several mechanisms have been identified that enhanced excretion of drugs in the feces, thereby decreasing concentrations of drugs in the blood (Hunter and Hirst 1997). First, numerous efflux systems transport drugs out of gut cells and back into intestinal lumen against a concentration gradient (Kartner et al. 1983; Gleeson 1992; Muller et al. 1994; Zaman et al. 1994; Zhang et al. 1998). For example, P-glycoproteins are transmembrane proteins in gut epithelial cells that actively transport exogenous and endogenous compounds to the outside of the gut cell and reduce delivery to circulation (Sparreboom et al. 1997; Watkins 1997; Silverman 1999; Wang et al. 2001). Wild-type mice with P-glycoproteins have sixfold lower blood concentrations and greater fecal excretion of taxol, an anticancer drug and plant secondary compound derivative, compared with mice without P-glycoproteins (Sparreboom et al. 1997). Second, detoxification enzymes in the gut may also reduce blood concentrations by metabolizing drugs into water-soluble compounds before entry into the circulation (Hartiala 1973; Ilett et al. 1993). It is possible that P-glycoproteins and detoxification enzymes operate jointly to minimize blood concentrations of toxins.

* Corresponding author; e-mail: jennifer.sorensen@anu.edu.au.

Physiological and Biochemical Zoology 77(1):139–148. 2004. 1522-2152/2004/7701-3034$15.00

We tested whether mammalian herbivores use mechanisms that reduce the absorption of plant toxins present in their natural diet. We investigated absorption of alpha-pinene in two closely related species of woodrats. Alpha-pinene is the predominant monoterpene in one-seeded juniper, *Juniperus monosperma* (2% dry weight [DW]; Dearing et al. 2000). Both species of woodrats naturally consume juniper, and thus alpha-pinene, daily (Dial 1988). Woodrats voluntarily consume large quantities of alpha-pinene in their natural diets in concentrations (Dearing et al. 2000) that can cause neurotoxicity, mucous membrane irritation, diuresis, and nephritis in mammals (Sperling et al. 1967; Savolainen and Pfaffli 1978; Hedenstierna et al. 1983; Falk et al. 1990*a*; Dearing et al. 2000). The regular and considerable ingestion of alpha-pinene by woodrats suggests that they have mechanisms that reduce blood concentrations of alpha-pinene. We quantified the concentration of alpha-pinene excreted in the feces of woodrats as an indicator of alpha-pinene absorption. We predicted that woodrats would excrete significant quantities of alpha-pinene unchanged in the feces.

The dietary specialist *Neotoma stephensi* (Goldman) and generalist *Neotoma albigula* (Hartley) are a model study system to initially compare toxin absorption between specialist and generalist mammalian herbivores. The species are in the same genus (Edwards and Bradley 2002), occur sympatrically (Dial 1988), are similar in body size (Dearing et al. 2000), and naturally consume juniper and thus alpha-pinene (Dial 1988; Dearing et al. 2000). The foraging behavior and the effects of alpha-pinene in these woodrat species have been evaluated (Vaughan 1982; Dial 1988; Dearing et al. 2000, 2002). *Neotoma stephensi* specializes on juniper (80%–95% of the diet) across its range, whereas the generalist *N. albigula* consumes far less juniper (15%–33%). In the laboratory, *N. stephensi* voluntarily consumes twice as much juniper, and thus alpha-pinene, as does *N. albigula* (Dearing et al. 2000). On the basis of foraging differences, *N. stephensi* will be referred to as a "specialist," and *N. albigula* will be referred to as a "generalist" for simplicity. The disparate foraging behavior of the two woodrat species is attributed to the plant secondary compounds in juniper, particularly alpha-pinene (Dearing et al. 2000). Recent work suggests that alpha-pinene absorption, not rates of elimination from the blood, explains greater alpha-pinene intake by specialist woodrats compared with generalists (Sorensen and Dearing 2003). We predicted that specialists would excrete proportionally more alpha-pinene than would generalists.

Material and Methods

Specialist (*Neotoma stephensi*) and generalist (*Neotoma albigula*) woodrats were trapped outside the south border of Wupatki National Park, 45 km northeast of Flagstaff, Arizona (35°30′N, 111°27′W), and transported to the University of Utah Animal Facility. Animals were housed individually in shoebox cages (48 cm × 27 cm × 20 cm) with bedding and cotton batting. Animals were placed on a 12L : 12D photoperiod and kept at 22°C. All animals were fed Harland Teklad ground rabbit chow (formula 2031) and water ad lib. before the study. All experiments on woodrats conform to Institutional Animal Care and Use Committee protocol 0102002. Juniper foliage was collected from trees at the study site where animals were trapped. Juniper was stored on dry ice immediately and until arrival at the University of Utah, where it was stored at −20°C.

The absorption model was evaluated using two complementary feeding experiments. First, to simulate the natural situation of alpha-pinene ingestion, woodrats were fed alpha-pinene in a diet containing juniper foliage. The juniper diet contained nutrients and a suite of other toxins that are similar to a natural diet of juniper. The second feeding experiment consisted of dosing woodrats with a single bolus of alpha-pinene. The single-dose experiment allowed more precise control of the dose of alpha-pinene ingested by woodrats compared with the juniper diet. In addition, the single-dose experiment allowed us to evaluate the constitutive capacity of woodrats to absorb alpha-pinene and eliminate possible effects of other juniper toxins.

Juniper Diet

Eight specialist and eight generalist woodrats were maintained on a control diet for 10 d. The control diet was a formulation designed to simulate the water, nutrient, and fiber content of juniper but without plant secondary compounds (Dearing et al. 2000). Woodrats maintained a constant intake level and body mass while consuming the control diet. Woodrats were then fed an acclimation diet containing a homogenous mix of 75% control and 25% ground juniper for 3 d. Immediately following the acclimation period, woodrats were fed a juniper diet containing 50% control and 50% juniper for 22 d. Although specialists readily consume 50% juniper in their diet and even gain body mass, this concentration was selected because it was the maximum concentration that generalists could tolerate without rapid and excessive loss of body mass. For all juniper diet treatments, juniper was homogenized with water using a Polytron homogenizer (Brinkman Polytron) to produce a slurry that was added to the control diet. It was necessary to homogenize juniper to eliminate selective foraging. Juniper was kept on ice during diet preparation to minimize volatilization of toxins. All diets were made fresh daily and were provided in excess of intake requirements to maintain body mass. Freshly prepared diets were presented daily to woodrats at approximately 1600 hours, which was just before major periods of activity and feeding (J. S. Sorensen, personal observation). Water was provided ad lib. throughout the experiment. Body mass was monitored every 3 d.

The quantity of alpha-pinene each animal ingested from the juniper diet was estimated from alpha-pinene concentrations

in the diet after accounting for losses as a result of volatilization. Woodrats consume the majority of the diet within the first 15 h after administration of diet (J. S. Sorensen, personal observation). Minimum alpha-pinene intake was estimated by multiplying alpha-pinene concentration in the juniper diet at $t = 15$ h (6.83 mg/g) by daily intake of juniper diet (g/d). Because woodrats forage repeatedly within the 15-h period (M. D. Dearing, unpublished data), our estimates of alpha-pinene intake on the basis of the levels in the diet at 15 h are conservative and represent the minimum amount of alpha-pinene consumed.

We quantified alpha-pinene volatilization from the diet by determining the concentration of alpha-pinene in the juniper diet over three time periods after administration to woodrats. Subsamples of diet from a single specialist and generalist woodrat were collected at time 0, 15, and 24 h postoffering. Samples were stored at −20°C before analysis. The DW of each diet sample was determined by drying a subsample of each collection at 45°C for 2 d. To extract alpha-pinene from the diet, we placed approximately 3 g (wet weight [WW]) of frozen diet into a 15-mL plastic centrifuge tube with 4 mL of ethanol (100%) and capped it. Extraction occurred for 24 h at room temperature (Boyle et al. 2000). Gas chromatography (GC) was used to quantify the concentration of alpha-pinene in each diet sample by using a Hewlett-Packard (HP) 5890 gas chromatograph and a 30-m HP-5 capillary column (0.32-mm inner diameter coated with cross-linked 5% PhMe Silicone, HP 19091J-413). Operating conditions were as follows: helium flow 2 mL/min; splitless injection; oven temperature 60°C for 2 min, increasing by 15°C/min to a final temperature of 260°C; injector 250°C; flame ionization detector 260°C. Samples were run in duplicate and averaged to determine concentration values. Standard curves for the diet analysis ($y = 2.84x + 0.07$; $R^2 = 0.97$) were prepared by spiking the control diet with increasing concentrations of analytical-grade alpha-pinene (Aldrich cat. no. 14,752-4) and 20 μL of 5 mg borneol/mL ethanol as the internal standard. Concentrations of alpha-pinene ranged from 0 to 10 mg alpha-pinene/g DW diet. Alpha-pinene was identified from chromatograms by retention time (~5.32 min) as confirmed from the standard curve.

Single Dose

In a separate experiment, four specialist and nine generalist woodrats were orally gavaged with a single dose of alpha-pinene (128.7 μg/g body mass) following the procedures of Sorensen and Dearing (2003). Because body mass did not differ between specialist (181 ± 14.23 g) and generalist (192.0 ± 4.13 g) woodrats ($F_{(1, 12)} = 0.823$, $P = 0.38$), both species received the same oral dose of alpha-pinene. Doses of alpha-pinene represented approximately twice the amount that generalists consume per meal and half the amount that specialists consume per meal (Dearing et al. 2000). None of the animals used in this experiment consumed any dietary toxin within 3 mo before experimentation. Water and control diet were provided ad lib. immediately following gavage.

Fecal Collection and Analysis

At the end of each experiment (juniper diet and single dose), animals were moved from shoebox cages to metabolic chambers (Lab Products 2100R) for 48 h. Cages permitted separate collection of feces and urine. Animals were given Harland Teklad ground rabbit chow throughout the 48-h collection period to eliminate any contribution of alpha-pinene in the diet to feces. Feces were collected on ice every 24 h for 48 h. Fecal collections were pooled for each animal and stored at −20°C before analysis. Alpha-pinene is completely excreted in the feces within 48 h following ingestion of alpha-pinene as determined in a preliminary study (J. S. Sorensen, unpublished data). We found no difference in water content of feces between specialists (89.9% ± 0.003% DW) and generalists (89.6% ± 0.002% DW; $F_{(1, 12)} = 1.02$, $P = 0.33$) as determined gravimetrically from a subsample of feces collected from each animal. We therefore report fecal output and alpha-pinene concentration in feces in terms of WW.

Fecal samples were prepared for extraction by homogenizing fecal pellets to an even consistency by mashing feces within a closed plastic bag to reduce volatilization. A known quantity (~1.5 g WW) of each fecal sample was placed in 15-mL glass screw-top test tube. To each test tube, 4 mL hexane solvent (high-performance liquid chromatography grade) and 20 μL of an internal standard (5 mg borneol/mL ethanol) were added. Feces, solvent, and internal standard were homogenized and vortexed for 30 s. An additional 1 mL of hexane solvent was used to rinse feces from the sides of each test tube. Samples were kept on ice to minimize volatilization throughout the preparation process. Once feces and solvent were mixed, test tubes were tightly capped and kept at room temperature for 24 h. Samples were then centrifuged with lids at 1,000 rpm for 3 min. The supernatant was drawn off into glass vials, capped, and sealed with parafilm. Samples were stored at −20°C before GC analysis. GC conditions were the same as described for analysis of alpha-pinene in diet extracts.

An accurate standard curve for concentrations of alpha-pinene in the feces requires adding known quantities of alpha-pinene to feces free of alpha-pinene ("control feces"). To produce such a curve, we collected feces from woodrats fed control diet (i.e., no alpha-pinene) for 10 d. Animals were placed in metabolic cages for 24 h on day 9 of the control diet. Feces were collected on ice and stored at −20°C. We verified that alpha-pinene was not present in feces of animals on control diet by using GC. Control feces from all animals were mashed to an even consistency, combined, and used to prepare alpha-pinene standards. Standard curves were prepared by spiking control feces with alpha-pinene. Standard curves ($y =$

$1.33x - 0.02$; $R^2 = 0.97$) were determined using the same extraction procedure, internal standard, and GC conditions as described for analysis of alpha-pinene in feces. Concentrations of alpha-pinene ranged from 0.05 to 3.5 mg alpha-pinene/g WW feces.

Statistics

Juniper Diet. Food intake (g DW/d) and body mass (g) were compared between specialist and generalist woodrats on juniper diets using independent one-way ANOVAs, with species (specialist vs. generalist) as the independent variable. Percent change in food intake and body mass from the last day of the control diet to the last day of the juniper diet were calculated for specialist and generalist woodrats. Percent change in food intake and body mass were compared between specialist and generalist woodrats with separate one-way ANOVAs, with species as the independent variable.

Because specialist woodrats consumed more juniper diet than did generalists, analyses of excretion were performed using ANCOVAs, with species (specialists vs. generalist) as the main effect and food intake (g/d) as the covariate. Fecal output (g WW/d), milligrams of alpha-pinene per grams of WW feces, milligrams of alpha-pinene excreted per day, and percentage of ingested alpha-pinene excreted were analyzed with separate ANCOVAs. Interactions between species and food intake were not significant for any of the analyses and were therefore removed from the model.

Single Oral Dose. Body mass (g) and alpha-pinene dose (mg/d) were analyzed with separate one-way ANOVAs, with species as the main effect. To control for slight differences in body mass, separate ANCOVAs were used to analyze fecal output (g WW/d), milligrams of alpha-pinene excreted per grams of WW feces, milligrams of alpha-pinene excreted per day, and percentage of alpha-pinene dose excreted, with species as the main effect and body mass (g) as the covariate. Interactions between species and body mass were not significant for any of the analyses and were therefore removed from the model.

Results

Juniper Diet

The concentration of alpha-pinene in the juniper diet at 0, 15, and 24 h after diet administration was 7.08, 6.83, and 5.81 mg alpha-pinene/g DW diet, respectively. Only 3.5% of the alpha-pinene was lost from the diet during the 15-h time period when woodrats consume the majority of daily food intake.

Specialist woodrats consumed 14% more juniper diet than did generalists ($F_{1,14} = 6.06$, $P = 0.03$; Table 1). In addition, specialists increased intake by 22% on a juniper diet compared with control, whereas generalists decreased intake by 9% ($F_{1,14} = 24.73$, $P = 0.0003$; Table 1). Specialists increased body mass by 5.5% on a juniper diet compared with control diet, whereas generalists lost 10% body mass ($F_{1,14} = 4.51$, $P = 0.05$; Table 1). Body mass of specialists was 10% greater than that of generalists at the end of the juniper diet treatment ($F_{1,14} = 63.62$, $P < 0.0001$; Table 1).

Table 1: Food intake (dry weight) and body mass of specialist and generalist woodrats at the end of the control diet (10 d) and at the end of the juniper diet (22 d)

	Control	Juniper
Intake (g/d):		
Specialist	11.6 (.72)	14.9 (.49)
Generalist	14.0 (.51)	12.8 (.72)
Body mass (g):		
Specialist	189.6 (6.02)	200.7 (5.88)
Generalist	200.2 (7.32)	180.1 (7.72)

Note. SEs are given in parentheses. $N = 8$ for each species.

Specialist woodrats excreted a larger quantity of unchanged alpha-pinene in the feces than did generalists, primarily because of greater alpha-pinene per gram of feces excreted, not because of higher fecal output. Specialists and generalist did not differ in the amount of feces excreted each day when food intake was controlled (species: $F_{1,13} = 12.58$, $P = 0.13$; intake: $F_{1,13} = 2.46$, $P = 0.14$; Table 2). Specialists excreted nearly twice the amount of alpha-pinene per unit fecal matter (mg alpha-pinene/g WW feces) when food intake was controlled (species: $F_{1,13} = 12.63$, $P = 0.004$; intake: $F_{1,13} = 2.52$, $P = 0.14$; Table 2). Specialists excreted 54% more alpha-pinene per day than did generalists, and this difference was significant even after juniper intake was controlled (species: $F_{1,13} = 13.09$, $P = 0.003$; intake: $F_{1,13} = 1.75$, $P = 0.21$; Table 2). Specialists also excreted a higher percentage of the ingested alpha-pinene from the juniper diet in the feces than did generalists (species: $F_{1,13} = 7.85$, $P = 0.02$; intake: $F_{1,13} = 2.37$, $P = 0.15$; Table 2; Fig. 1). Specialists excreted an estimated 20% of the total ingested alpha-pinene in the feces compared with 12% by generalists.

Single Oral Dose

Body mass and alpha-pinene intake did not differ between specialist and generalist woodrats given a single oral dose of alpha-pinene (body mass: $F_{1,12} = 0.82$, $P = 0.38$; alpha-pinene intake: $F_{1,12} = 0.82$, $P = 0.38$; Table 3). Fecal output also did not differ between specialist and generalist woodrats given a single oral dose of alpha-pinene when body mass was controlled (species: $F_{1,9} = 0.55$, $P = 0.48$; body mass: $F_{1,9} = 4.07$, $P = 0.07$; Table 3). Specialists excreted more alpha-pinene than did generalists, both on the basis of the alpha-pinene excreted per unit fecal matter (mg alpha-pinene/g WW feces) as well as absolute alpha-pinene excreted daily (mg alpha-pinene/d) when

Table 2: Mean alpha-pinene intake, fecal output (g wet weight [WW]/d), alpha-pinene excreted per gram of feces (WW), daily excretion of alpha-pinene, and percentage of ingested alpha-pinene that is excreted in specialist and generalist woodrats fed a juniper diet

	Specialist	Generalist
Intake (mg alpha-pinene/d)	102.1 (3.33)[a]	87.5 (4.92)
Fecal output (g WW/d)	7.94 (.20)	6.81 (.35)
Excretion (mg alpha-pinene/g feces)	2.98 (.43)[a]	1.55 (.20)
Excretion (mg alpha-pinene/d)	23.5 (3.39)[a]	10.8 (1.63)
Percentage excreted	20.2 (2.51)[a]	12.7 (2.26)

Note. Alpha-pinene intake was estimated assuming the entire diet is consumed within 15 h following administration of diet. SEs are given in parentheses. $N = 8$ for each species.

[a] Specialists are significantly different from generalists using an ANCOVA, with species as the main effect and intake as the covariate ($P < 0.05$). Data are shown in Figure 1.

body mass was controlled. Specialists excreted four times more alpha-pinene per gram of feces than did generalists (species: $F_{1,9} = 9.78$, $P = 0.01$; body mass: $F_{1,9} = 0.29$, $P = 0.60$; Table 3). In addition, specialists excreted 3.3 times more alpha-pinene per day than did generalists (species: $F_{1,9} = 11.61$, $P = 0.008$; body mass: $F_{1,9} = 1.11$, $P = 0.32$; Table 3). Specialists also excreted 3.9 times the percentage of the ingested dose of alpha-pinene than did generalists (species: $F_{1,9} = 14.50$, $P = 0.004$; body mass: $F_{1,9} = 0.81$, $P = 0.39$; Table 3, Fig. 1).

Discussion

We tested the hypothesis that specialist woodrats would excrete larger quantities of a plant secondary compound than would generalist woodrats. We found that two species of woodrats are capable of excreting alpha-pinene, a juniper toxin, unchanged in the feces. Moreover, specialist woodrats excreted more alpha-pinene in the feces per unit consumed than did generalist woodrats fed alpha-pinene either in juniper foliage or by oral gavage. In the subsequent paragraphs, we propose several mechanisms that may explain excretion of alpha-pinene in the feces of mammalian herbivores. We also offer several possible physiological and morphological differences between specialist and generalist woodrats to explain the greater alpha-pinene excretion in specialist woodrats compared with generalists.

Potential Mechanisms for Fecal Excretion of Alpha-Pinene

This study demonstrated that fecal excretion might be a critical route of alpha-pinene elimination in herbivorous woodrats. Woodrats excreted alpha-pinene in feces regardless of mode of intake (juniper diet vs. gavage) or length of acclimation to alpha-pinene (22 d of juniper diet vs. single dose). Toxin excretion is an essentially unexplored mechanism that, along with rapid detoxification, can lower the concentration of toxins in the blood and thereby reduce toxicity. The amount of alpha-pinene excreted in the feces, and thus not absorbed, could be biologically relevant to a woodrat in keeping toxin levels below lethal concentrations. This point is exemplified by a comparison between the blood concentration of alpha-pinene when a fraction of the alpha-pinene is excreted unchanged in the feces (as documented in this study) versus the blood concentration if the alpha-pinene excreted in the feces were instead delivered to the bloodstream. These concentrations were estimated from blood concentrations of alpha-pinene (4.2 μg/mL at 3 min

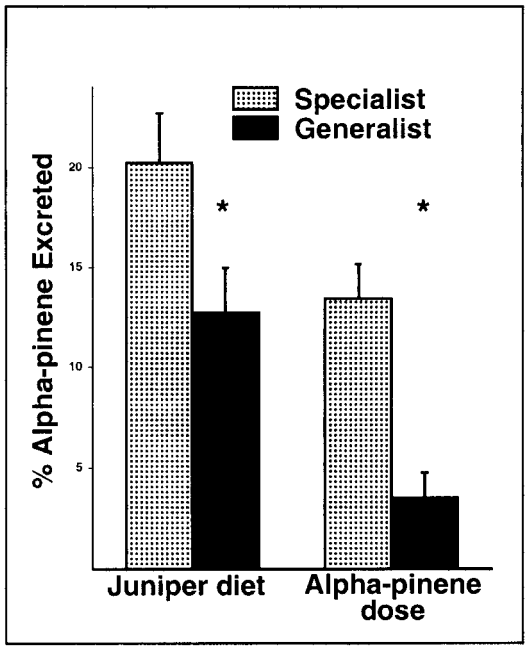

Figure 1. Percentage of ingested alpha-pinene excreted in the feces of specialist (*Neotoma stephensi*) and generalist (*Neotoma albigula*) woodrats fed a juniper diet or given a single oral dose of alpha-pinene. Specialists excreted a higher percentage of ingested alpha-pinene in the feces than did generalists when fed a juniper diet and a single dose of alpha-pinene. Bars represent $\pm$SE. An asterisk indicates a significant difference ($P < 0.05$) between specialist and generalist woodrats within an experiment (Tables 2, 3).

Table 3: Mean body mass, alpha-pinene dose, fecal output (g wet weight [WW]/d), alpha-pinene excreted per gram of feces (WW), daily excretion of alpha-pinene, and percentage of alpha-pinene dose that is excreted in specialist and generalist woodrats given a single oral dose of alpha-pinene (128.7 μg/g body mass)

	Specialist	Generalist
Body mass (g)	181.4 (4.13)	192.0 (7.72)
Dose (mg alpha-pinene/d)	23.3 (1.83)	24.7 (.53)
Fecal output (g/d)	3.8 (.47)	4.8 (.68)
Excretion (mg alpha-pinene/g feces)	.79 (.10)[a]	.20 (.08)
Excretion (mg alpha-pinene/d)	2.92 (.42)[a]	.89 (.31)
Percentage excreted	13.5 (1.70)[a]	3.5 (1.22)

Note. SEs are given in parentheses. $N = 4$ specialists; $N = 8$ generalists.

[a] Specialists are significantly different from generalists using an ANCOVA, with species as the main effect and body mass as the covariate ($P < 0.05$). Data are shown in Figure 1.

postgavage) and rate of alpha-pinene elimination (0.037 slope of logarithmic concentration-time curve) in woodrats from previous work (Sorensen and Dearing 2003), fecal excretion (2.92 mg/d) from this study, and mass-specific estimates of blood volume (14.5 mL; Davies and Morris 1993). In addition, concentrations were estimated assuming that alpha-pinene blood concentrations, rates of elimination, and quantity excreted in feces are similar between specialists used in previous studies (Sorensen and Dearing 2003) and those used in this study. We estimated that normal excretion of unchanged alpha-pinene in the feces results in maximum blood concentrations of alpha-pinene (5.4 μg/mL) that are four times lower than the LC_{50} (20 μg/mL, concentration at which mortality occurs in 50% of animals) of alpha-pinene for laboratory rats (Sperling et al. 1967). If the amount of alpha-pinene detected in the feces of specialist woodrats (2.92 mg/d) were instead delivered into the bloodstream, the resulting blood concentrations (25.5 μg/mL) would be higher than the LC_{50} of alpha-pinene for laboratory rats.

The chemical structure and absorption kinetics of monoterpenes suggest that alpha-pinene should be nearly completely absorbed across the epithelial cells of the intestine. Alpha-pinene and other monoterpenes are lipophilic and are highly soluble in blood (Falk et al. 1990*b*). In support, inhaled monoterpenes are absorbed almost entirely in the lungs and then delivered to the liver, where they are completely metabolized by detoxification enzymes. Furthermore, studies investigating the fate of either inhaled or orally ingested monoterpenes found less than 5% of the dose unchanged in the feces (Igimi and Nishimura 1974; Foley et al. 1987; Boyle et al. 1999, 2000). Specifically, a single study on the distribution of orally administered limonene, a monoterpene similar to alpha-pinene, suggests that monoterpenes are highly absorbed in the intestine. Less than 2% of orally ingested limonene was detected in the large intestine of rats during any time period, suggesting that limonene is not shunted through the intestine unchanged (Igimi and Nishimura 1974). That specialist woodrats excrete up to 20% of alpha-pinene unchanged in the feces suggests that woodrats possess mechanisms that either actively prevent absorption or enhance the fecal excretion of alpha-pinene. The extensive evolutionary history between woodrats and alpha-pinene may have selected for such mechanisms to reduce the toxicity of plant secondary compounds in the diet.

There are essentially two physiological processes that might contribute alone or in concert to minimize absorption of plant toxins in woodrats. First, toxins can be transformed into compounds that are not readily absorbed (Hartiala 1973; Ilett et al. 1993). Second, toxins can be actively transported out of the gut epithelium and into the gut lumen by various transporter proteins (Kartner et al. 1983; Gleeson 1992; Muller et al. 1994; Zaman et al. 1994; Zhang et al. 1998). In the following paragraphs, we describe which of these two mechanisms is most likely responsible for the excretion of unchanged alpha-pinene in specialist and generalist woodrats.

Absorption of toxins may be reduced if ingested toxins are bound to other substrates or are biotransformed in the gut before absorption. For example, several species of mammals produce salivary proteins that bind to tannins, a group of plant secondary compounds (Mehansho et al. 1987; Austin et al. 1989; Robbins et al. 1991). The tannin-protein complexes are large, water-soluble moieties that are not readily absorbed. Animals producing tannin-binding proteins excrete proportionally more tannins in the feces than do animals lacking these proteins (Robbins et al. 1991). Lipophilic compounds can also be transformed by gastrointestinal microbes into water-soluble compounds, thus reducing absorption (Cotler et al. 1983; Kiessling et al. 1984; Bhat et al. 1998). For example, nivalenol, a mycotoxin, is detoxified by microorganisms in the gastrointestinal tract, resulting in excretion of nivalenol metabolites in the feces of cows, pigs, and chickens (Hedman and Pettersson 1997). Mammalian detoxification enzymes located in enterocyte cytoplasm can also metabolize toxins into water-soluble com-

pounds before delivery into the general circulation (George 1981; Hall et al. 1999). However, these two classes of mechanisms, toxin-substrate binding and gastrointestinal biotransformation, can result in excretion of unchanged toxin in the feces only if the toxin-protein complexes and metabolites are reconverted into parent compounds. Although it is possible that intestinal enzymes or microbes in the large intestine can dissociate toxin-protein complexes (O'Brien et al. 1986; Osawa 1990; McArthur and Sanson 1991), it is unlikely that the converted compounds are identical to ingested parent compounds.

We propose that epithelial transporter proteins are the most likely candidates responsible for the incomplete absorption of alpha-pinene by woodrats. There is substantial evidence in mammals that absorbed toxins are actively transported out of the gut epithelium and into the gut lumen across a concentration gradient by various proteins. Transporter proteins such as P-glycoproteins and other multidrug resistance–associated proteins that actively regulate absorption of toxins in the gut have been identified (Muller et al. 1994; Zaman et al. 1994; Hunter and Hirst 1997; Watkins 1997; Washington et al. 2001). Several plant secondary compounds potentially consumed by mammalian herbivores are acted on by gut transporters (Hwang et al. 1999; Kim et al. 1999; Chavez et al. 2002). For example, vinblastine, an antitumor drug, is a plant secondary compound from *Vinca rosea* (periwinkle) that is transported by P-glycoproteins (Burns 1972; Sharom 1997). Although it is not known whether alpha-pinene is a substrate, alpha-pinene shares physical and chemical properties with known substrates (Zamora et al. 1988; Falk et al. 1990*b*; Penzotti et al. 2002; Schinkel and Jonker 2003). For example, the diterpene extracts from *Euphorbia* (spurges) contain cyclic hydrocarbons that are lipophilic, interact with P-glycoprotein, and are similar in structure to alpha-pinene (Hohmann et al. 2002; Appendino et al. 2003). Further investigation of transporter proteins and their binding affinity with plant secondary compounds may contribute to understanding the general role of toxin transporters on the foraging behavior of herbivores.

Neotoma stephensi *versus* Neotoma albigula

Although specific mechanisms are yet unknown, our data indicate that the specialist *Neotoma stephensi* excretes more alpha-pinene unchanged in the feces than does the generalist *Neotoma albigula*. Specialist woodrats excreted more alpha-pinene per unit consumed than did generalists both when consuming alpha-pinene in juniper foliage as well as when orally gavaged with a single dose of alpha-pinene. These data suggest that specialist woodrats absorbed less alpha-pinene, and therefore received a lower dose of alpha-pinene per unit ingested in the general circulation, than did generalists. Reduced toxin absorption is consistent with previous findings that specialist woodrats have lower initial blood concentrations of alpha-pinene than do generalists, despite being given similar doses and having similar rates of alpha-pinene elimination from the blood (Sorensen and Dearing 2003). Furthermore, greater excretion of toxin by the specialist woodrat supports ecological hypotheses that the intake of a single plant by mammalian herbivores is mediated by physiological mechanisms that minimize concentration of plant toxins in the blood (Freeland and Janzen 1974; Freeland 1991; Foley et al. 1999).

The hypothesis that foraging behavior is related to the fecal excretion capacity of plant secondary compounds and that excretion capacity is enhanced in dietary specialists has received mixed support in other herbivore study systems. The *Eucalyptus* specialist, the koala (*Phascolarctos cinereus*), excretes a maximum of 15% of ingested oil from *Eucalyptus punctata* leaves unchanged in feces (Eberhard et al. 1975), whereas the brushtail possum (*Trichosurus vulpecula*), a generalist forager, excreted only 3% of *Eucalyptus* oils ingested (Foley et al. 1987). *Eucalyptus* oils contain high levels of cineole and *p*-cymene, monoterpenes that have similar molecular weights, lipophilicity, solubility, and toxicity as alpha-pinene (Adams 1994; Lawler et al. 1998; Lawler et al. 1999). In contrast, others have detected only trace amounts of ingested eucalyptus terpenes in the feces of both koalas and brushtail possums (Boyle et al. 1999, 2000). Although support in the eucalyptus-marsupial model is ambiguous, the hypothesis that toxin absorption may be a general mechanism used by mammalian herbivores, and specialists in particular, deserves further attention.

Our data are consistent with the prediction that decreased absorption of toxins may facilitate greater toxin intake. The specialist woodrat examined in this study excreted more alpha-pinene per unit consumed than did the generalist woodrat and ingested more alpha-pinene in juniper foliage. Moreover, specialist woodrats were constitutively better at excreting alpha-pinene than were generalists, as demonstrated from the results of the single-dose experiment in which woodrats were not acclimated to alpha-pinene. This ability was maintained after chronic intake of alpha-pinene as demonstrated from results of woodrats fed a juniper diet for 22 d. Although these results are true for only one plant toxin and are limited in the extent to which they apply to the specialist-generalist paradigm in general, they are the first to demonstrate that toxin intake is partially related to fecal excretion of unmetabolized plant toxins.

We propose that, if present, specialist woodrats might possess larger quantities of transporter proteins and/or have proteins that are more specific to alpha-pinene than might generalist woodrats. For example, drug or toxin resistance is positively correlated with quantities of P-glycoprotein, a drug transporter in mammalian cells (Juliano and Ling 1976; Debenham et al. 1982). In addition, P-glycoprotein can be selective for certain drugs (Kartner et al. 1983). We are currently investigating the presence and substrate selectivity of transporter proteins in specialist and generalist woodrats.

Ecologists have proposed for decades that the foraging be-

haviors of mammalian herbivores were governed by the efficacy by which plant secondary compounds are eliminated (Freeland and Janzen 1974). Our results suggest that in addition to detoxification, toxin absorption may also play a critical role in the elimination of plant toxins and ultimately influence plant intake. Furthermore, we demonstrated that specialist woodrats excreted more of the plant secondary compound, alpha-pinene, unchanged in the feces than did generalist woodrats. This is the first study to suggest that toxin absorption may be important in regulating the intake of plant secondary compounds in mammalian herbivores. Although we acknowledge the limitations of a two-species comparison, our study is in agreement with ecological hypotheses (Freeland and Janzen 1974; Freeland 1991; Foley et al. 1999) and provides a testable mechanism that can be broadly applied to a variety of plant-herbivore study systems. We are currently testing the absorption model in additional species of mammalian herbivores to better understand how results apply to the specialists-generalist paradigm in general.

Acknowledgments

We thank F. Goller, S. Haley, G. S. Yost, and two anonymous reviewers for comments on the manuscript. We thank B. Hudson and employees of Wupatki National Monument Visitor Center for assistance and accommodations during our trapping sessions. H. Baldwin, R. Boyle, D. Green, C. Heilderberger, and S. O'Grady assisted in collecting woodrats in the field. We thank R. Boyle for assistance with chemical analyses. E. van Dijk, S. Haley, and L. Birdsall assisted in experimental procedures and animal husbandry. This project was supported by National Science Foundation IBN-0079865, American Museum of Natural History Theodore Roosevelt Memorial Fund, Sigma Xi Grant-in-Aid of Research, American Society of Mammalogists Grant-in-Aid, and the University of Utah Biology Undergraduate Research Program.

Literature Cited

Adams R.P. 1994. Geographic variation in the volatile terpenoids of *Juniperus monosperma* and *J. osteosperma*. Biochem Syst Ecol 22:65–71.

Appendino G., C. Porta, G. Conseil, O. Sterner, E. Mercalli, C. Dumontet, and A. Di Peitro. 2003. A new P-glycoprotein inhibitor from the caper spurge (*Euphorbia lathyris*). J Nat Prod 66:140–142.

Austin P.J., L.A. Suchar, C.T. Robbins, and A.E. Hagerman. 1989. Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. J Chem Ecol 15:1335–1347.

Bellamy W.T. 1996. P-glycoproteins and multidrug resistance. Annu Rev Pharmacol Toxicol 36:161–183.

Bhat T.K., B. Singh, and O.P. Sharma. 1998. Microbial degradation of tannins: a current perspective. Biodegradation 9: 343–357.

Boyle R., S. McLean, and N.W. Davies. 2000. Biotransformation of 1,8-cineole in the brushtail possum (*Trichosurus vulpecula*). Xenobiotica 30:915–932.

Boyle R., S. McLean, W.J. Foley, and N.W. Davies. 1999. Comparative metabolism of dietary terpene, *p*-cymene, in generalist and specialist folivorous marsupials. J Chem Ecol 25: 2109–2127.

Burns J.H., ed. 1972. Analytical Profiles of Drug Substances. Academic Press, New York.

Chavez D., B. Cui, H. Chai, R. Garcia, M. Mejia, N.R. Farnsworth, G.A. Cordell, J.M. Pezzuto, and A.D. Kinghorn. 2002. Reversal of multidrug resistance by tropane alkaloids from the stems of *Erythroxylum rotundifolium*. J Nat Prod 64:606–610.

Cotler S., C.J. Bugge, and W.A. Colburn. 1983. Role of gut contents, intestinal wall, and liver on the first pass metabolism and absolute bioavailability of isotretinoin in the dog. Drug Metab Dispos 11:458–462.

Davies B. and T. Morris. 1993. Physiological parameters in laboratory animals and humans. Pharm Res 10:1093–1095.

Dearing M.D., A.M. Mangione, and W.H. Karasov. 2000. Diet breadth of mammalian herbivores: tests of the nutrient constraints and detoxification-limitations hypotheses. Oecologia 123:397–405.

———. 2002. Ingestion of plant secondary compounds causes diuresis in desert woodrats. Oecologia 130:576–584.

Debenham P., N. Kartner, L. Siminovitch, J. Riordan, and V. Ling. 1982. DNA mediated transfer of multiple drug resistance and plasma membrane glycoprotein expression. Mol Cell Biol 2:881–889.

Dial K.P. 1988. Three sympatric species of *Neotoma*: dietary specialization and coexistence. Oecologia 76:531–537.

Eberhard I.H., J. McNamara, R.J. Pearse, and I.A. Southwell. 1975. Ingestion and excretion of *Eucalyptus puntata* D.C. and its essential oil by the koala *Phascolarctos cinereus*. Aust J Zool 23:169–179.

Edwards C.W. and R.D. Bradley. 2002. Molecular systematics of the genus *Neotoma*. Mol Phylogenet Evol 25:489–500.

Falk A.A., E. Gullstrand, A. Lof, and E. Wigaeus-Hjelm. 1990*a*. Liquid/air partition coefficients of four terpenes. Br J Ind Med 47:62–64.

Falk A.A., M.T. Hagberg, A.E. Lof, E.M. Wigaeus-Hjelm, and W. Zhiping. 1990*b*. Uptake, distribution and elimination of alpha-pinene in man after exposure by inhalation. Scand J Work Environ Health 16:372–378.

Foley J.W., G.R. Iason, and C. McArthur. 1999. Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? Pp. 130–209 in H.G. Jung and G.C. Fahey, eds. Nutritional Ecol-

ogy of Herbivores. American Society of Animal Science, Savoy, Ill.

Foley W.J., E.V. Lassak, and J. Brophy. 1987. Digestion and absorption of *Eucalyptus* essential oils in greater glider (*Petauroides volans*) and brushtail possums (*Trichosurus vulpecula*). J Chem Ecol 13:2115–2130.

Freeland W.J. 1991. Plant secondary metabolites. Biochemical evolution with herbivores. Pp. 61–82 in R. Palo and C. T. Robbins, eds. Plant Defenses Against Mammalian Herbivory. CRC, Boca Raton, Fla.

Freeland W.J. and D.H. Janzen. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. Am Nat 108:269–289.

George C.F. 1981. Drug metabolism by the gastrointestinal mucosa. Clin Pharmacokinet 6:259–274.

Gleeson D. 1992. Acid-base transport systems in gastrointestinal epithelia. Gut 33:1134–1145.

Hall S., K. Thummel, P. Watkins, K. Lown, L. Benet, M. Paine, R. Mayo, et al. 1999. Molecular and physical mechanisms of first-pass extraction. Drug Metab Dispos 27:161–166.

Hartiala K. 1973. Metabolism of hormones, drugs, and other substances by the gut. Physiol Rev 53:496–534.

Hedenstierna G., R. Alexanderson, K. Wimander, and G. Rosen. 1983. Exposure to terpenes: effects on pulmonary function. Int Arch Occup Environ Health 51:191–198.

Hedman R. and H. Pettersson. 1997. Transformation of nivalenol by gastrointestinal microbes. Arch Anim Nutr 50: 321–329.

Hohmann J., J. Molnar, D. Redei, F. Evanics, P. Forgo, A. Kalman, G. Argay, and P. Szabo. 2002. Discovery and biological evaluation of a new family of potent modulators of multidrug resistance: reversal of multidrug resistance of mouse lymphoma cells by new natural jatrophane diterpenoids isolated from *Euphorbia* species. J Med Chem 45:2425–2431.

Hunter J. and B. Hirst. 1997. Intestinal secretion of drugs: the role of P-glycoprotein and related drug efflux systems in limiting oral drug absorption. Adv Drug Delivery Rev 25: 129–157.

Hwang B.Y., S.E. Kim, Y.O. Kim, Y. Hong, J.S. Ro, K.S. Lee, and J.J. Lee. 1999. Pregnane glycoside multidrug-resistance modulators from *Cynanchum wilfodii*. J Nat Prod 62:640–643.

Igimi H. and M. Nishimura. 1974. Studies on the metabolism of d-limonene (p-Mentha-1,8-diene). I. The absorption, distribution, and excretion of d-limonene in rats. Xenobiotica 4:77–84.

Ilett K., L. Tee, P. Reeves, and R. Minchin. 1993. Metabolism of drugs and other xenobiotics in the gut lumen and wall. Pharmacol Ther 46:67–93.

Juliano R. and V. Ling. 1976. A surface glycoprotein modulating drug permeability in chinese hamster ovary cell mutants. Biochim Biophys Acta 455:152–162.

Kartner N., J. Fiordan, and V. Ling. 1983. Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. Science 221:1285–1288.

Kiessling K.H., H. Pettersson, K. Sandholm, and M. Olsen. 1984. Metabolism of aflatoxin, ochratoxin, zearalenone, and three trichothecenes by intact rumen fluid, rumen protozoa, and rumen bacteria. Appl Environ Microbiol 47:1070–1073.

Kim S.E., H.S. Kim, Y.S. Hong, Y.C. Kim, and J.J. Lee. 1999. Sesquiterpene esters from *Celastrus orbiculatus* and their structure-activity relationship on the modulation of multidrug-resistance. J Nat Prod 62:697–700.

Lawler I.R., W.J. Foley, B.M. Eschler, D.M. Pass, and K. Handasyde. 1998. Intraspecific variation in *Eucalyptus* secondary metabolites determines food intake by folivorous marsupials. Oecologia 116:160–169.

Lawler I.R., D.M. Schliebs, B.M. Eschler, and W.J. Foley. 1999. Relationship between chemical functional groups on *Eucalyptus* secondary metabolites and their effectiveness as marsupial antifeedants. J Chem Ecol 25:2561–2573.

McArthur C. and G.D. Sanson. 1991. Effects of tannins on digestion in the common ringtail possum (*Pseudocheirus peregrinus*), a specialized marsupial folivore. J Zool (Lond) 225: 233–252.

Mehansho H., L.G. Butler, and D.M. Carlson. 1987. Dietary tannins and salivary proline-rich proteins: interactions, induction and defense mechanisms. Annu Rev Nutr 7:423–440.

Muller M., C. Meijer, G. Zaman, P. Borst, R. Scheper, N. Mulder, E. De Vries, and P. Jansen. 1994. Overexpression of the gene encoding the multidrug resistance-associated protein results in increased ATP-dependent glutathione S-conjugate transport. Proc Natl Acad Sci USA 91:13033–13037.

O'Brien T., A. Lomdahl, and G. Sanson. 1986. Preliminary microscopic investigations of the digesta derived from foliage of *Eucalyptus ovata* (Labill.) in the digestive tract of the common ringtail possum, *Pseudocheirus peregrinus* (Marsupialia). Aust J Zool 34:157–176.

Osawa R. 1990. Formation of a clear zone on tannin-treated brain heart infusion agar by a *Streptococcus* sp. isolated from the feces of koalas. Appl Environ Microbiol 56:829–831.

Penzotti J.E., M.L. Lamb, E. Evensen, and P.D.J. Grootenhuis. 2002. A computational ensemble pharmacophore model for identifying substrates of P-glycoprotein. J Med Chem 45: 1737–1740.

Robbins C.T., A.E. Hagerman, P.J. Austin, C. McArthur, and T.A. Hanley. 1991. Variation in mammalian physiological responses to a condensed tannin and its ecological implications. J Mammal 72:480–486.

Savolainen H. and P. Pfaffli. 1978. Effects of longterm turpentine inhalation on rat brain protein metabolism. Chem-Biol Interact 21:271–276.

Schinkel A.H. and J.W. Jonker. 2003. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. Adv Drug Delivery Rev 55:3–29.

Sharom F. 1997. The P-glycoprotein efflux pump: how does it transport drugs? J Membr Biol 160:161–175.

Silverman J.A. 1999. Multidrug-resistance transporters. Pharm Biotechnol 12:353–386.

Sorensen J.S. and M.D. Dearing. 2003. Elimination of plant toxins: an explanation for dietary specialization in mammalian herbivores. Oecologia 134:88–94.

Sparreboom A., J. Van Asperen, U. Mayer, A.H. Schinkel, J.W. Smit, D.K.F. Meijer, P. Borst, W.J. Nooijen, J.H. Beijnen, and O. van Tellingen. 1997. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci USA 94:2031–2035.

Sperling F., W.L. Marcus, and C. Collins. 1967. Acute effects of turpentine vapor on rats and mice. Toxicol Appl Pharmacol 10:8–20.

Vaughan T.A. 1982. Stephen's woodrat, a dietary specialist. J Mammal 63:53–62.

Wang E., K. Lew, M. Barecki, C.N. Casciano, R.P. Clement, and W.W. Johnson. 2001. Quantitative distinctions of active site molecular recognition by P-glycoprotein and cytochrome P450 3A4. Chem Res Toxicol 14:1596–1603.

Washington N., C. Washington, and C.G. Wilson. 2001. Physiological Pharmaceutics: Barriers to Drug Absorption. Taylor & Francis, New York.

Watkins P.B. 1997. The barrier function of CYP3A4 and P-glycoprotein in the small bowel. Adv Drug Delivery Rev 27: 161–170.

Zaman G., M. Flens, M. van Leusden, M. De Haas, H. Mulder, J. Lankelma, H. Pinedo, et al. 1994. The human multidrug resistance-associated protein MRP is a plasma membrane drug-efflux pump. Proc Natl Acad Sci USA 91:8822–8826.

Zamora J.M., H.L. Pearce, and W.T. Beck. 1988. Physical-chemical properties shared by compounds that modulate multidrug resistance in human leukemic cells. Mol Pharmacol 33:454–462.

Zhang L., C. Brett, and K. Giacomini. 1998. Role of organic cation transporters in drug absorption and elimination. Annu Rev Pharmacol Toxicol 38:431–460.