

M. Denise Dearing · Antonio M. Mangione
William H. Karasov

Diet breadth of mammalian herbivores: nutrient versus detoxification constraints

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Abstract Two hypotheses, nutrient constraints and detoxification limitation, have been proposed to explain the lack of specialists among mammalian herbivores. The nutrient constraint hypothesis proposes that dietary specialization in mammalian herbivores is rare because no one plant can provide all requisite nutrients. The detoxification limitation hypothesis suggests that the mammalian detoxification system is incapable of detoxifying high doses of similar secondary compounds present in a diet of a single plant species. We experimentally tested these hypotheses by comparing the performance of specialist and generalist woodrats (*Neotoma*) on a variety of dietary challenges. *Neotoma stephensi* is a narrow dietary specialist with a single species, one-seeded juniper, *Juniperus monosperma*, comprising 85–95% of its diet. Compared with other plants available in the habitat, juniper is low in nitrogen and high in fiber, phenolics, and monoterpenes. The generalist woodrat, *N. albigula*, also consumes one-seeded juniper, but to a lesser degree. The nutrient constraint hypothesis was examined by feeding both species of woodrats a low-nitrogen, high-fiber diet similar to that found in juniper. We found no differences in body mass change, or apparent digestibility of dry matter or nitrogen between the two species of woodrats after 35 days on this diet. Moreover, both species were in positive nitrogen balance. We tested the detoxification limitation hypothesis by comparing the performance of the generalist and specialist on diets with and without juniper leaves, the preferred foliage of the specialist, as well as on diets with and without α -pinene, the predominant monoterpene in juniper. We found that on the juniper diet, compared with the specialist, the generalist consumed less juniper and lost more

mass. Urine pH, a general indicator of overall detoxification processes, declined in both groups on the juniper diet. The generalist consumed half the toxin load of the specialist yet its urine pH was slightly lower. Moreover, the generalist consumed significantly less of the treatment with high concentrations of α -pinene compared to the control treatment, while the specialist consumed the same amount of food regardless of α -pinene concentration. For both groups, urine pH declined as levels of α -pinene in the diet increased. The generalist produced a significantly more acidic urine than the specialist on the treatment with the highest α -pinene concentration. Our results suggest that in this system, specialists detoxify plant secondary compounds differently than generalists and plant secondary compounds may be more important than low nutrient levels in maintaining dietary diversity in generalist herbivores.

Key words Detoxification · Digestion · *Juniperus* · *Neotoma* · Nutrients

Introduction

Of the thousand or so mammalian herbivores, only approximately 1% are considered dietary specialists (Freeland 1991). This pattern is in stark contrast to insect herbivores, in which dietary specialization is the rule not the exception (Bernays and Chapman 1994). Two hypotheses, nutrient constraints (Westoby 1978) and detoxification limitations (Freeland and Janzen 1974), have been proposed to explain the preponderance of dietary generalism among mammalian herbivores. Although numerous investigations have used correlational approaches to test these two hypotheses (Cooper et al. 1988; Dearing and Schall 1992; Ganzhorn 1988; Goldberg et al. 1980; Randolph et al. 1991; Snyder 1992; Tahvanainen et al. 1985; Willig and Lacher 1991; Wrangham and Waterman 1981), few experimental studies have been undertaken.

The nutrient constraint hypothesis proposes that no one species of plant can satisfy the nutritional demands

M.D. Dearing¹ (✉) · A.M. Mangione · W.H. Karasov
Department of Wildlife Ecology, University of Wisconsin,
Madison, WI 53706, USA

Present address:

¹ Department of Biology, University of Utah,
Salt Lake City,
UT 84112, USA,
e-mail: dearing@biology.utah.edu
Fax: +1-801-5812174

of a mammalian herbivore (Westoby 1978). Vegetative tissues of plants are low in essential nutrients such as nitrogen and digestible energy compared to foods such as seeds or animal tissues (Robbins 1993). They also vary greatly in their nutritional contents both intra- and interspecifically. Moreover, typically, the ratios of nutrients are not correlated (Dearing and Schall 1992). For example, one species may be high in digestible energy but low in nitrogen, whereas another species may be high in sodium but low in digestible energy (Belovksy 1978, 1981). Thus, to obtain a nutritionally adequate diet, mammalian herbivores are predicted to forage on a variety of plant species.

Surprisingly, the majority of mammalian specialists consume plants that are relatively low in nutrients, particularly nitrogen, and are high in fiber. For example, the koala (*Phascolarctos cinereus*) specializes on *Eucalyptus punctata* foliage, with a nitrogen content of 1–1.5% and a 60% crude fiber content (Cork 1986). The nitrogen levels of the food plants of specialists are low compared to those found in other available plant species and approach the minimum nitrogen level considered necessary for mammalian growth and maintenance (Robbins 1993). That some mammals are able to specialize on plants low in nitrogen and high in fiber suggests that they may possess unique physiological traits that permit them to utilize these nutrients more efficiently than their generalist counterparts.

In contrast to nutrient constraints, plant secondary compounds may govern the foraging habits of mammalian herbivores. Freeland and Janzen (1974) proposed that the detoxification system of generalist mammalian herbivores is unable to process large amounts of secondary compounds that are chemically similar, i.e., those from a single plant species. Rather, generalist herbivores were predicted to detoxify high toxin loads only when toxins originated from a variety of plant sources. The heightened detoxification efficiency realized by herbivores consuming mixed-species diets was attributed to an increase in detoxification pathways that could be recruited to process the chemical miscellany. Equivalent quantities of secondary compounds from a diet of a single plant species, being less chemically diverse and therefore detoxified via fewer pathways, would surpass the capacity of any one detoxification pathway.

In addition to detoxification abilities *sensu stricto*, the excretion of detoxification products may affect plant secondary compound intake (Foley 1992; Foley et al. 1995; Freeland and Janzen 1974). Recent work suggests that excretion of detoxification metabolites, the majority being strong organic acids, challenge an animal's acid-base homeostasis (Foley 1992; Foley et al. 1995; Freeland and Janzen 1974; McLean et al. 1993). The mammalian kidney and associated acid-base-regulating organs have finite tolerances for buffering and/or excreting organic acids. Excretion of acidic detoxification products may restrict the extent to which detoxification can occur (Foley 1992; Foley and McArthur 1994; Foley et al. 1995). The various mammalian detoxification pathways

produce different organic acids during detoxification, and predominance of one pathway over another may affect an organism's ability to regulate its acid-base balance. Interspecific variability in detoxification products and their subsequent elimination may contribute to dietary preferences among species.

Natural history of the study system

To test these hypotheses, we used a specialist and generalist species of woodrat: *Neotoma stephensi* (Stephen's woodrat) and *N. albigula* (whitethroat woodrat). Two independent studies have documented that *N. stephensi* is a dietary specialist on one-seeded juniper, *Juniperus monosperma* (range in diet: 60–80% Dial 1988; 83–96% Vaughn 1982). Each of these studies was conducted for 2 or more years with fecal samples collected during every month of the year. Sample sizes were large in both instances: 362 (Vaughn 1982) and 114 (Dial 1988). In certain habitats, *N. stephensi* occurs sympatrically with a generalist species, *N. albigula*, which also consumes some one-seeded juniper. However, its reliance on this food source is much less, varying during the year from 18 to 35% of its diet ($n=128$; Dial 1988).

One-seeded juniper is an abundant but low-quality food compared with other plant species available in the habitat where the specialist and generalist co-occur (Dial 1984). It is low in nitrogen (1.00% by dry weight; Dial 1988) and high in fiber (24% cellulose and lignin, this study). Juniper produces considerable quantities of secondary compounds, particularly monoterpenes and phenolics (Adams et al. 1981; Holchek et al. 1990). The predominant monoterpene, α -pinene, has numerous deleterious effects on mammals including neurotoxicity, irritation of mucous membranes, and nephritis (Hedenstierna et al. 1983; Koppel et al. 1981; Levin et al. 1992). Using this study system, we experimentally tested whether specialist herbivores were more efficient at extracting nutrients from a low-nutrient diet than generalists as predicted by the nutrient constraint hypothesis. We also tested whether specialist herbivores were able to consume and detoxify greater quantities of secondary compounds from one plant species than generalists as predicted by the detoxification limitation hypothesis.

Materials and methods

Study sites

N. stephensi and *N. albigula* were trapped at Woodhouse Mesa, Ariz. (35°30' N 111°27' W). We trapped at the same site and at many of the exact locations as Dial (1988) as indicated by trap markers. Additional *N. albigula* were trapped in Castle Valley, Utah (38°38' N 109°18' W). Vegetation in both trapping areas was similar (for a detailed description see Dearing et al. 1998). To determine whether differences between *N. stephensi* and *N. albigula* resulted from using *N. albigula* from two populations, for each experiment, we compared the performance of *N. albigula* from both sites. No significant differences were found and they are therefore

treated as one group in the analyses. We confirmed that *N. stephensi* was consuming more juniper than *N. albigula* by analyzing feces from trapped woodrats for juniper fragments.

Nutrient constraint hypothesis

To determine whether the specialist had a greater net gain of N, and better fiber digestibility than the generalist on a juniper diet, we formulated a diet with nitrogen and fiber levels similar to that found in *J. monosperma*. The juniper samples collected from the Woodhouse Mesa study site contained 1% N (Kjeldahl assay) and 24% fiber [acid detergent fiber (ADF); Goering and Van Soest 1970]. The nitrogen content of our artificial diet was 1.25% N (Kjeldahl assay) and fiber was 23% ADF. The artificial diet ingredients and their proportions were Harland Teklad ground rabbit chow formula 8630 (50%), Amersham Life Science Cellulose (12%), corn oil (3%), vitamin mix (0.5%), Teklad mineral mix TD 79055 (1.75%), cornstarch (13%), pectin (7%), methionine (0.055%), and sucrose (13%). We developed this formulation in consultation with Dr. Ron Rose, an animal nutritionist from the Teklad Corporation. Ingredients were homogenized in an industrial mixer (Hobart Instruments) and pelleted (5 mm in diameter). Seven *N. stephensi* and ten *N. albigula* were fed the artificial diet for 35 days. We measured body mass and food intake (dry mass) daily. Dry mass of the artificial diet offered to woodrats was determined gravimetrically. Leftovers were removed and dried; fresh food was replaced daily. During the last 3 days of the experiment, we monitored daily food intake and collected all feces produced. Feces were dried at 45°C prior to weighing. During the last 24 h of the experiment, animals were restricted to a portion of their cage that allowed for separate collection of urine and feces. The urine and feces collected during this period were assayed for nitrogen (Kjeldahl assay).

Digestibility of dry matter and nitrogen was calculated as:

$$100 \times (\text{g ingested} - \text{g excreted in feces}) / \text{g ingested.}$$

Nitrogen balance was calculated as:

$$\text{g N ingested} - \text{g N excreted in urine} + \text{feces.}$$

Change in body mass was calculated as:

$$100 \times (\text{body mass on day 35} - \text{body mass on day 0}) / \text{body mass on day 0.}$$

Dry matter digestibility, nitrogen digestibility, change in body mass, and nitrogen balance were compared between the generalist and specialist with one-way ANOVAs with species as the main effect.

Detoxification limitation hypothesis

Juniper experiments

To determine whether the specialist was better able than the generalist to ingest juniper toxins, we fed nine *N. stephensi* and nine *N. albigula* juniper foliage. Prior to this treatment with juniper, woodrats were given a control treatment consisting of the chow used in the nutrient constraint experiment (described above) for 3 days. Food was provided ad libitum. The control was followed by a 3-day acclimation period where control chow and fresh juniper were provided ad libitum. Following the acclimation period, woodrats were provided with a juniper treatment that consisted of fresh juniper (*J. monosperma*) ad libitum, and a reduced amount of control chow equal to 15% of each animal's food intake on the control diet. It was necessary to provide this minimal amount of chow in the juniper treatment because in preliminary trials, some woodrats that were offered only juniper lost too much mass (>15% of initial body mass) to remain in the experiment for 3 days, the amount of time required for induction of detoxification enzymes (Sipes and Gandolfi 1986). During all treatments, food was replaced daily and leftovers were dried at 45°C. To control for slight

differences in body weights between the two species, food intake was analyzed with a repeated-measures ANCOVA with body mass as the covariate. Species was the between-subjects factor and diet treatment the within-subjects factor. Body mass was monitored daily. In the two treatments where woodrats had ad libitum access to juniper (acclimation and juniper), juniper intake was used as the covariate. Initial body masses on day 0 of the experiment were compared with an ANOVA to determine if there were body mass differences between the two species. Change in body mass was calculated for the acclimation and juniper treatments by:

$$100 \times (\text{body mass on day 3 of treatment} - \text{body mass on day 3 of control}) / \text{body mass on day 3 of control}$$

The change in body mass was compared using repeated-measures ANOVA.

Juniper used in this experiment was collected from the Woodhouse Mesa study site and kept at -20°C until use. Foliage was collected from approximately ten trees and homogenized in a large plastic bag. Woody stems were removed from juniper branches and woodrats were presented with the terminal tips (distal 10 cm of the branch). We provided 70 g of freshly thawed juniper daily during the acclimation and juniper treatments. This amount is approximately threefold the amount woodrats require to maintain weight (Vaughn 1982).

Urine acidity is an indicator of detoxification metabolism (Foley 1992; Foley et al. 1995). During the last 24 h of the control, acclimation, and juniper treatments, we collected urine samples in 50-ml tubes surrounded by an ice pack. Urine pH was measured at 24°C with an Acumet (Model 15) pH probe. Urine pH values were analyzed for each of the three diet treatments using independent ANCOVAs with food intake as the covariate. In the two treatments where woodrats had ad libitum access to juniper (acclimation and juniper), intake of juniper was used as the covariate.

α-Pinene experiments

We conducted a similar experiment using an artificial diet to which the primary monoterpene in *J. monosperma*, α-pinene, was added. The artificial diet was the same formulation described in the nutrient constraint experiment with microencapsulated α-pinene added. Microencapsulation was necessary to reduce volatilization of α-pinene during the feeding trials. α-Pinene was microencapsulated in gelatin-gum arabic capsules by complex coacervation (Clancy et al. 1992; Usher et al. 1989). Coacervation consists of the formation of gelatin and gum arabic microcapsules around oil droplets when subjected to temperature and pH changes (in this case either corn oil or α-pinene). Capsules range in diameter from about 30 to 80 μm. Capsules were hardened using a 25% glutaraldehyde solution as a cross-linking catalyst; the residual glutaraldehyde was removed by rinsing capsules with deionized water. Capsules were suspended in water (1:2.3 capsule:H₂O v:v). Preliminary feeding trials with corn oil that had been microencapsulated verified that a diet containing approximately 12% microcapsules had no effect on food intake. To further control for any effect of microcapsules in the treatments, the control treatment contained microencapsulated corn oil. The only difference between the microencapsulated corn oil and microencapsulated α-pinene was the oil used, i.e., corn oil versus α-pinene; otherwise, the coacervation process and reagents used were identical.

Nine *N. stephensi* and nine *N. albigula* were fed the following treatments for 3 days each: control [0% α-pinene/g dry weight (DW)], low (2.3 μl α-pinene/g DW), intermediate (18.5 μl α-pinene/g DW), and high (23.3 μl α-pinene/g DW). α-Pinene concentrations were determined by measuring the α-pinene concentrations in each of the diet treatments using gas chromatography (Hewlett Packard 5860). Dietary concentrations of the treatments correspond to the amount of α-pinene that would be consumed in diets containing 5, 80, and 100% *J. monosperma*. These predicted concentrations of α-pinene in the diet of *J. monosperma* are based on the total oil yield from steam distillation of samples

Table 1 Performance parameters of specialist and generalist woodrats consuming an artificial diet with nitrogen and fiber contents similar to juniper. Dry matter (DMD) and nitrogen digestibilities (ND) were calculated as $100 \times (\text{g ingested} - \text{g excreted in feces}) / \text{g ingested}$.

	Intake(g/day)	DMD(%)	ND(%)	NB(g N/day)	Mass change (%)
Specialist	12.1 (0.8)	69.5 (1.8)	68.4 (2.0)	0.044 (0.009)	0.3 (1.7)
Generalist	12.7 (0.6)	69.3 (1.1)	68.5 (2.0)	0.036 (0.009)	1.9 (1.3)

of juniper from the study site: 3.73% oil by dry weight and measurements of α -pinene in juniper oil by gas chromatography (63%). Diet treatments were prepared and leftovers collected daily. There was no apparent sorting of food. Depending on the treatment, which includes the control, the microcapsule slurry comprised 2.9–7.4% of the wet mass of the diet. We monitored body mass and food intake on a daily basis. Data were analyzed as described for the juniper experiment.

Results

Nutrient constraints

There was no difference in initial body mass of the generalist and specialists ($F_{1,15}=0.073$, $P=0.79$). The specialist was on average 189.4 ± 17.5 g (mean \pm SE, $n=7$), whereas the generalist was 190.1 ± 15.8 g ($n=10$).

There was no evidence that the specialist, *N. stephensi*, performed better on a low-nutrient diet than the generalist, *N. albigula*. Food intake rates of the two species did not differ ($F_{1,15}=0.023$, $P=0.88$; Table 1). There was no apparent sorting of food: woodrats consumed entire portions of pellets. After 35 days on a low-nutrient diet, there were no differences in digestibilities of either dry matter ($F_{1,11}=0.0113$, $P=0.92$) or nitrogen ($F_{1,13}=0.001$, $P=0.97$; Table 1) between the specialist and the generalist. Both the generalist and specialist were in positive nitrogen balance when consuming a low-nitrogen diet. We found no significant difference between the two species with respect to nitrogen balance ($F_{1,9}=0.24$, $P=0.63$; Table 1) or body mass from day 0 to day 35 in either species ($F_{1,14}=0.60$, $P=0.45$; Table 1).

Detoxification limitations

Juniper foliage

Average body mass differed significantly between the specialist and generalist at the start of the experiment (Table 2). The specialist was on average 188.4 ± 11.0 g (mean \pm SE, $n=9$) and the generalist was 204.4 ± 11.0 g ($n=9$).

The specialist performed better than the generalist when given diets high in juniper toxins. Specialist woodrats consumed significantly more juniper leaves than the generalist (Table 2, Fig. 1). This result was consistent in both the acclimation and juniper treatments (Tukey's HSD; Fig. 1). During the acclimation treatment, there was no difference in percent change in body mass be-

tween the specialist and generalist, yet the specialist incorporated more than twice as much juniper in its diet as the generalist (Figs. 1, 2). On the juniper treatment, the specialist consumed nearly twice as much juniper as the generalist and generalist woodrats lost three times as much weight on the juniper diet compared with the specialists (Table 2, Fig. 2).

Table 2 Results of analyses of variance for tests of the detoxification limitation hypothesis. *F*-values for diet and interaction effects are based on Wilks' lambda. Significant results are *italicized*. In food intake experiments, initial body mass was used as a covariate to correct for differences in body mass between the two species. The analysis was a repeated-measures ANCOVA with "species" as the between-subjects factor, "diet treatment" as the within-subjects factor and body mass as the covariate. Repeated-measures ANOVA was used to analyze changes in body mass. ANCOVA was used to analyze urine pH for each diet treatment; only significant results are presented in this table. Food intake was the covariate and species the main effect. Fixed-effects models were used for the analyses

	<i>F</i>	<i>df</i>	<i>P</i>
Juniper experiment			
Food intake			
Species	9.7	1,15	<i>0.007</i>
Body mass (covariate)	5.3	1,15	<i>0.036</i>
Diet Treatment	2.1	2,14	0.14
Interaction	6.7	2,14	<i>0.009</i>
Percent change in body mass			
Species	3.2	1,16	0.10
Diet treatment	179.9	1,16	<i>0.0001</i>
Interaction	9.7	1,16	<i>0.007</i>
Urine pH: acclimation treatment			
Model	32.2	1,11	<i>0.0001</i>
Species	30.7	1,11	<i>0.0002</i>
Intake	0.34	1,11	0.53
α -Pinene experiment			
Food intake			
Species	4.4	1,16	<i>0.05</i>
Body mass (covariate)	11.9	1,16	<i>0.003</i>
Diet treatment	0.65	3,14	0.59
Interaction	11.5	3,14	<i>0.0005</i>
Percent change in body mass			
Species	0.05	1,17	0.82
Diet treatment	0.87	2,16	0.45
Interaction	5.1	2,16	<i>0.02</i>
Urine pH: high α -pinene treatment			
Model	6.1	2,16	<i>0.01</i>
Species	11.7	1,16	<i>0.004</i>
Intake	7.0	1,16	0.42

tween the specialist and generalist, yet the specialist incorporated more than twice as much juniper in its diet as the generalist (Figs. 1, 2). On the juniper treatment, the specialist consumed nearly twice as much juniper as the generalist and generalist woodrats lost three times as much weight on the juniper diet compared with the specialists (Table 2, Fig. 2).

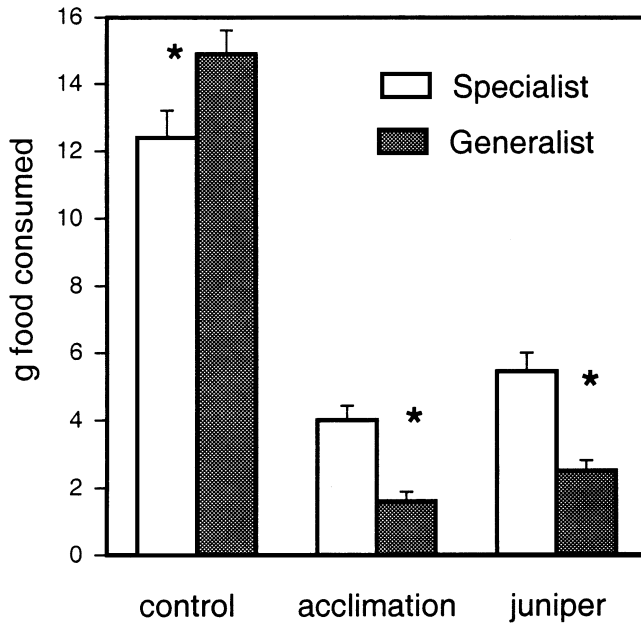


Fig. 1 Food intake by specialist and generalist woodrats on control, acclimation, and juniper treatments. For the control treatment, the amount of chow consumed is plotted as a reference for the dry matter necessary to maintain body mass. In the acclimation and juniper treatments, only the amount of juniper consumed is plotted. Asterisks indicate significant differences (Tukey's HSD) between the specialist and generalist within a treatment. Error bars are 1 SE

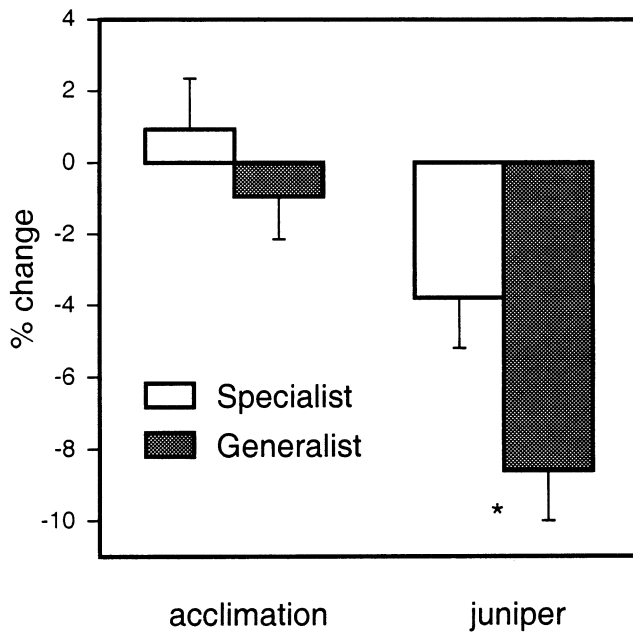


Fig. 2 Percent change in body mass on the acclimation and juniper diet treatments. Asterisks indicate significant differences between the specialist and generalist within a treatment. The generalists lost significantly more mass than the specialist on the juniper treatment (Tukey's HSD.) Error bars are 1 SE

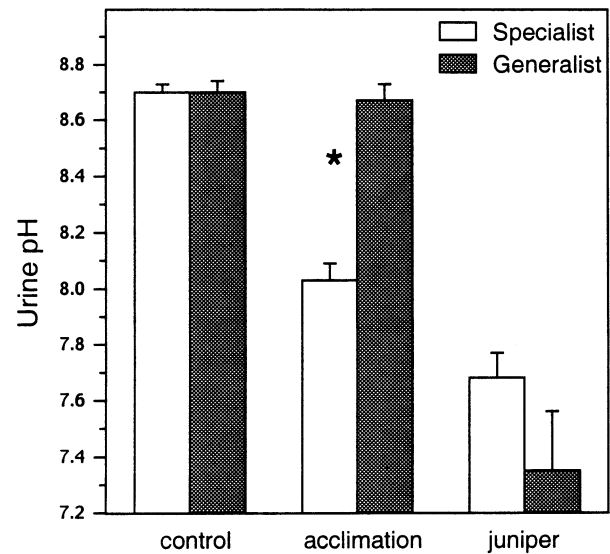


Fig. 3 Urine pH on control, acclimation, and juniper treatments. Asterisks indicate significant differences between the specialist and generalist within a treatment. The specialist produced urine with significantly lower pH on the acclimation diet and almost significantly higher pH on the juniper diet compared to the generalist (Tukey's HSD). Error bars are 1 SE

We could not measure urine pH for three woodrats (two generalists, one specialist) which produced <5 ml of urine, the limit for accurate measurement by our pH probe. One specialist was an extreme outlier (JMP outlier analysis) with respect to urine pH. These four woodrats were removed from the final analysis of urine pH reducing the sample sizes to seven for each species.

There was a significant change in urine pH with diet treatment (Table 2). Although there was no difference in urine pH of woodrats on the control diet (Tukey's HSD, Fig. 6), urine pH decreased with the addition of juniper to the diet (Fig. 3). There was a significant effect of species on urine pH (Table 2, Fig. 3). The urine pH of the specialist was significantly lower on the acclimation diet and was higher (although not statistically) on the juniper treatment (Tukey's HSD; Fig. 3) than that of the generalist.

α-Pinene diet

There was a significant difference in initial body weights between the specialist and generalist [specialist=189.4±11.2 g (mean±SE), generalist 202.3±10.5 g; Table 2]. The generalist had higher food intakes than the specialist on the control and low- α -pinene treatments, but the generalist decreased its food intake on the intermediate and high- α -pinene concentrations such that its food intake did not differ from the specialist (Fig. 4). On the high- α -pinene treatment, the generalist consumed 22.0% less food than on the control treatment (paired $t=7.64$, $P=0.0001$, $df=8$). The specialist maintained constant food intake regardless of levels of α -pinene (Fig. 4).

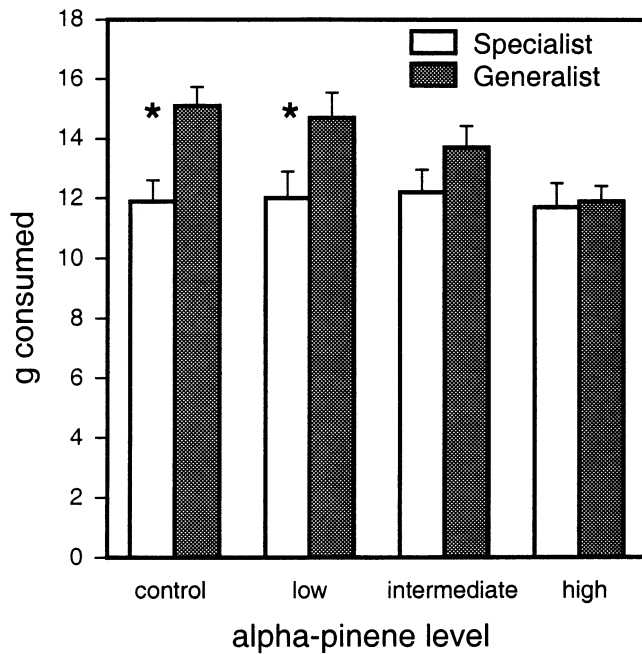


Fig. 4 Food intake of woodrats on diets containing α -pinene. Asterisks indicate significant differences (Tukey's HSD) between the specialist and generalist within a treatment. The generalist decreased its food intake with increasing concentrations of α -pinene whereas the intake of the specialist was unchanged across α -pinene concentrations. Error bars are 1 SE

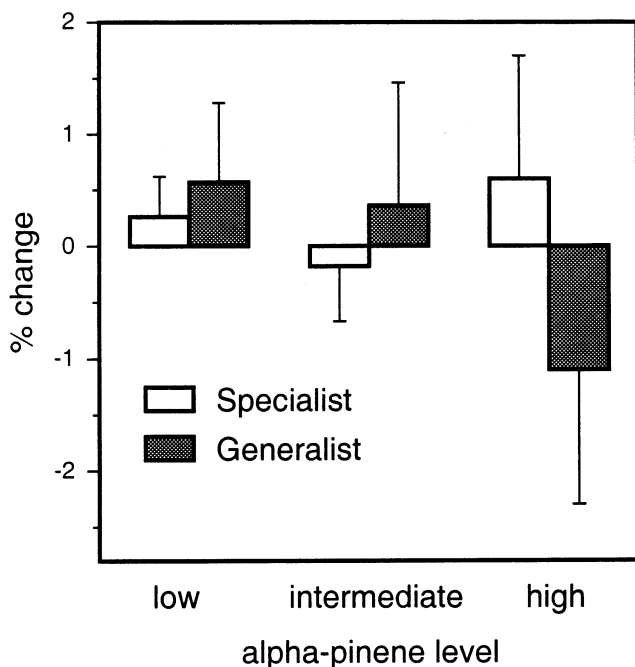


Fig. 5 Percent change in body mass on the control and α -pinene-treated diets. There were no significant differences in the percent change in body mass between the specialist and generalist within any of the treatments. Error bars are 1 SE

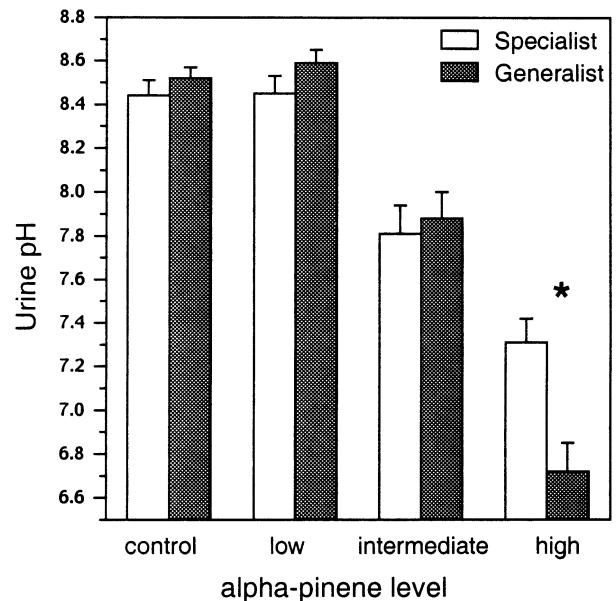


Fig. 6 Urine pH of the specialist and generalist on the control and α -pinene-treated diets. The generalist produced urine with a significantly lower pH than that of the specialist on the high- α -pinene treatment (asterisk Tukey's HSD). Error bars are 1 SE

There was no significant change in body mass between the specialist and generalists during the experiment (Fig. 5). There was no significant difference in urine pH between the specialist and generalist on the control, low-, or intermediate- α -pinene diet (Table 2, Fig. 6). However, on the high- α -pinene treatment, the generalist produced urine with a significantly lower pH than the specialist (Fig. 6).

Discussion

A central goal in the study of the ecology of mammalian herbivores has been to identify factors limiting dietary specialization. We experimentally tested predictions of the two principal hypotheses, nutrient constraints and detoxification limitations, that have been proposed to explain dietary diversity in herbivores. We found no evidence for the nutrient constraint hypothesis with respect to digestibility of two important diet components, nitrogen and dry matter. Moreover, there was no difference between the specialist and generalist in nitrogen balance. There was substantial support for the detoxification limitation hypothesis. In both the juniper foliage and α -pinene experiments, the generalist consumed significantly lower concentrations of plant toxins than the specialist. The generalist lost more mass than the specialist on the juniper foliage treatment. The specialist maintained a higher urine pH than the generalist in both the juniper and α -pinene experiments. These results suggest that in this system, plant secondary compounds play a larger role than nutrient availability in maintaining the broad diet of generalist herbivores.

Data from the literature on another pair of woodrats (Atsatt and Ingram 1983) further corroborate that plant secondary compounds are important determinants of diet breadth. *N. fuscipes*, specializes on oak leaves (*Quercus agrifolia*), which are high in tannins. It is sympatric with a congener, *N. lepida*. In feeding trials in the laboratory, *N. fuscipes* ingested twice the amount of oak leaves as *N. lepida* and maintained body mass whereas *N. lepida* lost body mass on a diet of oak leaves. Although parameters of detoxification were not investigated in this study, the data are consistent with the hypothesis that *N. fuscipes* is better able to detoxify oak tannins than its sympatric congener, *N. lepida*.

We recognize that our results are from one specialist and one generalist and therefore our ability to generalize about the evolution of dietary specialization is limited. However, we feel that the experiments presented here combined with those from other investigations represent the initial step in an empirical comparison of the nutrient constraint versus detoxification hypotheses. By using closely related species we have controlled for large differences in physiology and morphology. Moreover, the specialist and generalist occur sympatrically and thus both species have an evolutionary and ecological history with the same plant toxins. That both species in the specialist-generalist pair have historical experience with similar plant secondary chemistry is critical to tests of the proposed hypotheses. Our work and that of others suggests that individuals are locally adapted to detoxify secondary compounds in their habitat and that specialist woodrats can consume higher levels of toxins than sympatric generalist counterparts (Atsatt and Ingram 1983, Mangione et al., in press; but see Justice 1985). Comparisons of a specialist to a generalist with no prior experience with the toxin of the specialist would likely be biased in favor of the specialist. In the future, we plan to examine other pairs of closely related and sympatric generalist and specialist herbivores.

It was striking that the generalist was comparable to the specialist in its ability to maintain a positive nitrogen balance on the low-nitrogen diet. The nitrogen levels in the artificial diet used in this study approached the minimum value required for nitrogen balance in nonruminating mammals (Robbins 1993). That the generalist was able to sustain a positive nitrogen balance on a low-nitrogen diet was especially surprising because the diet it selects in nature is nearly fourfold higher in nitrogen than that given in this study (Dial 1984, 1988).

The generalist digested dry matter as well as the specialist did, even though the diet was almost 25% cellulose. This result suggests that the cellulose content of juniper does not prevent the generalist from specializing on it. We were unable to measure digestibility of dry matter in the juniper experiment because the animals were not in steady state, a requirement for digestibility estimates (Smith 1995). A potential criticism in comparing the digestibility of fiber in our artificial diet, predominantly cellulose from wood pulp, to that in juniper is that juniper leaves also contain lignin, which is thought

to be completely indigestible in the gut even by microbes (Van Soest 1994). Lignification of cellulose and other cell wall carbohydrates decreases their digestibility. Thus, if the cellulose in juniper is heavily lignified and if the specialist possesses a unique adaptation for processing lignified cellulose that the generalist lacks, then it is conceivable that lignification could reduce cellulose digestibility thus prohibiting the generalist from specializing on juniper.

We think this scenario is highly improbable for several reasons. First, we know of no physiological mechanism for enhanced digestion of lignified cellulose (Van Soest 1994). It seems more likely that animals differ in their ability to digest fiber through possessing, or not, mechanisms that allow them to efficiently utilize cellulose, such as enlarged guts, large body size, longer retention times, or fermenting bacteria (Smith 1995; Van Soest 1994). Second, the lignin concentration and lignin to cellulose ratio of juniper is equivalent to or lower than that of other plants included in the generalist's diet (Dial 1984, 1988; Holcsek et al. 1990; Van Soest 1994).

The specialist exhibited a much stronger preference than the generalist for juniper foliage. On the acclimation treatment, where both juniper foliage and chow were provided ad libitum, the specialist selected a diet that was 27% juniper, whereas the generalist diet contained only 10% juniper. That the specialist would voluntarily select a diet with such a large juniper component, especially in the presence of other abundant, non-toxic food (chow) suggests that the cost of detoxification of juniper may be relatively low at these intake levels. Alternatively, the specialist may have an innate preference for its host plant, as seen in many insects (Bernays and Chapman 1994).

In nature, juniper comprises up to 95% of the specialist's diet (Vaughn 1982), yet the specialist was unable to maintain its body mass on the juniper offered in this experiment as it consumed only approximately 50% of its maintenance intake on the juniper treatment. This result was probably not due to a captivity effect, as *N. stephensi* has been maintained in the laboratory on a diet of juniper (Vaughn 1982; Vaughn and Czaplewski 1985). It is possible that in our case, even though we provided juniper in threefold excess of maintenance requirements, not all of the juniper provided may have been acceptable. In the field, *N. stephensi* is highly selective and forages on only a few individual trees in its territory (Vaughn 1982). The basis for this selectivity has not yet been demonstrated, and we are currently investigating it. Because the juniper fed in this experiment was randomly collected from a number of individuals, it is possible that part of the foliage offered was from less preferred or unacceptable trees. Other mammalian specialists such as the koala and ringtail possum are highly selective at the level of individual trees and are incapable of maintaining body mass when fed foliage of certain individuals within the host plant species (Lawler et al., in press).

α -Pinene, the predominant terpene in juniper appears to play a significant role in reducing food intake in gen-

eralist woodrats. Levels of α -pinene in the diet comparable to a diet of 100% juniper had no effect on the specialist's intake, whereas the generalist decreased food intake by 22% compared to the control diet. In two respects these results are consistent with those of other studies on the deterrent effect of secondary compounds. First, the effects of α -pinene in the diet acted in a dose-dependent manner (Lawler et al., in press). As α -pinene levels increased, food intake of the generalist decreased. Second, at concentrations comparable to those found in plants, α -pinene decreased intake but did not completely deter feeding. Individual compounds that deter mammalian herbivores are rarely entirely responsible for the deterrent quality found at the whole-plant level (Iason and Palo 1991; Meyer and Karasov 1989; Reichardt et al. 1990a, 1990b; but see Lawler et al., in press). Typically, several secondary compounds within an individual plant affect palatability. Given the difference in food intake of animals on the juniper diet versus the α -pinene diet, α -pinene appears to be only one of the compounds responsible for juniper deterrence.

The reduction in food intake was not large enough to produce a significant change in body mass in the generalist during the 3 days of the high- α -pinene treatment. In other such short-term experiments, we have documented that woodrats fed control chow (no toxins) at 80% of maintenance are capable of maintaining normal body mass in the short term, presumably through decreased activity. It should be noted that animals used in this experiment were captive in a temperature-controlled environment. Under natural conditions, a 20% reduction in food intake could have far more significant consequences than in the laboratory.

The strong organic acids produced during detoxification of plant secondary compounds are excreted in the urine (Foley 1992; Foley et al. 1995; McLean et al. 1993). Thus, urine pH is an indicator of an animal's acid-base status and also its capacity for detoxifying and eliminating metabolites. Given that the specialist consumed equal or greater quantities of plant secondary compounds compared to the generalist, it was surprising that the specialist maintained an equal or higher urine pH. In the experiment where juniper foliage was offered, compared to the generalist, the specialist consumed twice the quantity of juniper toxins but its urine pH was the same or slightly higher. In the experiment where the predominant monoterpene in juniper, α -pinene, was added to an artificial diet, the specialist and generalist consumed equal quantities of α -pinene; however, the specialist produced urine with a significantly higher pH than the generalist's. That the specialist was able to maintain urine pH equal to that of the generalist while consuming greater toxin loads suggests that the former is the more competent at processing secondary compounds.

The specialist could be maintaining a higher urine pH through three mechanisms related to detoxification and elimination. During detoxification, the specialist may produce metabolites that are less acidic than those produced by the generalist, resulting in a higher urine pH.

Alternatively, the specialist may generate the same acid load during detoxification as the generalist, but may eliminate hydrogen ions generated from this process by combining them with bicarbonate, yielding water and CO_2 that are eliminated in urine and lungs, respectively. Finally, the specialist and generalist may generate the same acid load per unit of secondary compound consumed; however, the specialist may buffer urine to a higher pH. We are currently testing these hypotheses to illuminate general mechanisms by which specialist herbivores are able to consume higher concentrations of plant secondary compounds than generalists.

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