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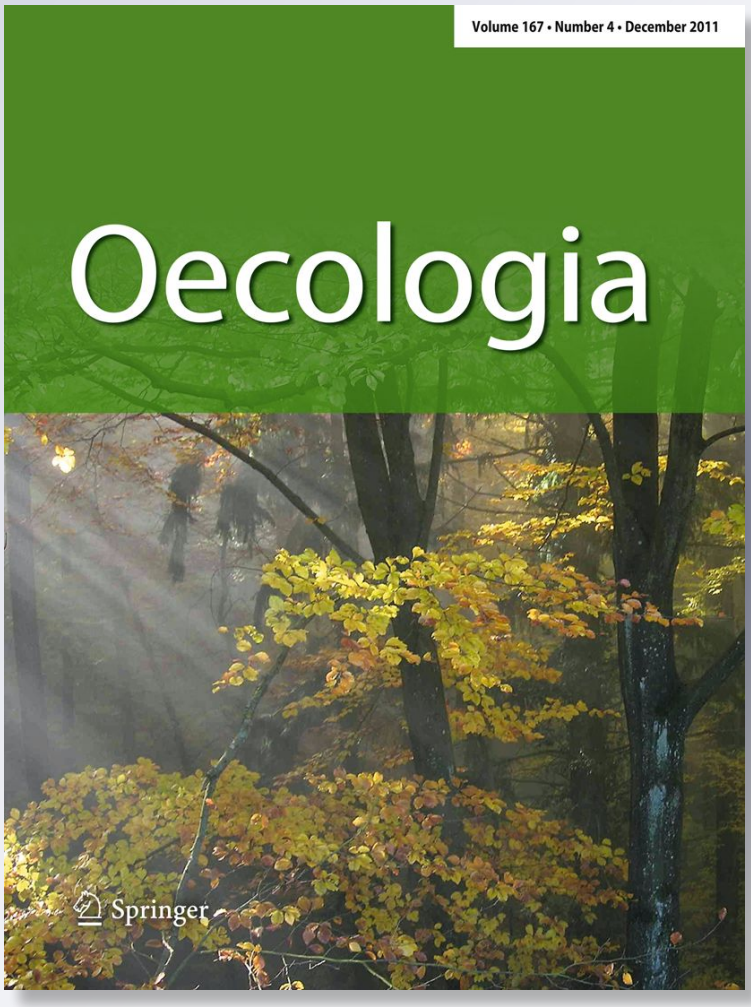

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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Testing the diet-breadth trade-off hypothesis: differential regulation of novel plant secondary compounds by a specialist and a generalist herbivore

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Abstract Specialist herbivores are predicted to have evolved biotransformation pathways that can process large doses of secondary compounds from the plant species on which they specialize**.** It is hypothesized that this physiological specialization results in a trade-off such that specialists may be limited in ability to ingest novel plant secondary compounds (PSCs). In contrast, the generalist foraging strategy requires that herbivores alternate consumption of plant species and PSC types to reduce the possibility of over-ingestion of any particular PSC. The ability to behaviorally regulate is a key component of this strategy. These ideas underpin the prediction that in the face of novel PSCs, generalists should be better able to maintain body mass and avoid toxic consequences compared to specialists. We explored these predictions by comparing the feeding behavior of two herbivorous rodents: a juniper specialist, *Neotoma stephensi*, and a generalist, *Neotoma albigula,* fed diets with increasing concentrations of phenolic resin extracted from the creosote bush (*Larrea tridentata*), which produces a suite of PSCs novel to both species. The specialist lost more mass than the generalist during the 15-day trial. In addition, although the specialist and generalist both regulated phenolic resin intake by reducing meal size while on the highest resin concentration (4%), the generalist began to regulate intake on the 2% diet. The ability of the generalist to regulate intake at a lower PSC concentration may be the source of the generalist's performance advantage over the specialist. These data provide evidence for the hypothesis that the specialist's foraging strategy may result in behavioral as well as physiological trade-offs in the ability to consume novel PSCs.

Keywords Neotoma · Meal size · Plant–animal interactions · Biotransformation · Dietary toxin

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Introduction

Mammalian herbivores are repeatedly faced with toxic challenges during food consumption. Plants have evolved many classes of chemicals, known as plant secondary compounds (PSCs) in part as a defense against herbivory. These chemicals are often toxic to herbivores (Spearling et al. 1967; Savolainen and Pfaffli 1978; Bernays et al. 1989). PSCs are energetically costly to detoxify and some may cause extreme physiological effects such as neurotoxicity, while others may hinder growth or disrupt nutrient uptake (Spearling et al. 1967; Savolainen and Pfaffli 1978; Bernays et al. 1989; Sorensen et al. 2005a). However, as plants evolved defenses against herbivores, herbivores, in turn evolved mechanisms to cope with plant toxins.

Most herbivorous mammals are considered generalists with respect to their dietary habits (Freeland and Janzen 1974; Dearing et al. 2000; Shipley et al. 2006). Generalists consume many different species of plants on a daily basis and therefore tend to ingest small doses of diverse PSCs (Dial 1988; Dearing 1996; Randolph and Cameron 2001).

It has been hypothesized that this foraging strategy is a mechanism to cope with PSCs by decreasing the cost or risk associated with ingesting large doses of any single compound or suite of related compounds (Freeland and Janzen 1974). This hypothesis, however, presupposes that the generalist is capable of halting the ingestion of individual plant species before suffering ill effects from the PSCs in that species. Thus, generalists should have a behavioral mechanism in place to minimize PSC intake below the threshold for toxicity (Torregrossa and Dearing 2009).

In contrast to the generalist strategy, a few mammalian herbivores have evolved the ability to specialize almost exclusively on a single species or genus of plant (Shipley et al. 2006). It is thought that specialists have evolved biotransformation pathways and capacities specific to the chemistry of a single plant species (Freeland and Janzen 1974). Recent studies using pharmacological approaches support this idea (Ngo et al. 2003, 2006; Haley et al. 2007a, b).

Both strategies, generalization and specialization, incur trade-offs (Freeland and Janzen 1974). Generalists are predicted to have liver enzymes that can act on a broad range of substrates to facilitate the biotransformation of a wide variety of PSCs. However, limitations in the capacity of any particular type of biotransformation enzyme is thought to prevent the generalist from metabolizing large quantities of single or similar PSCs (Freeland and Janzen 1974; Mangione et al. 2000; Boyle and McLean 2004; Dziba and Provenza 2008; Sorensen et al. 2005a; Wiggins et al. 2006a). In contrast, specialists are thought to have evolved liver enzymes with greater specificity and capacity to PSCs, often in high concentrations, in a single species of plant (Mangione et al. 2000; McLister et al. 2004; Haley et al. 2007a, b; Skopec et al. 2007). Such honing of the detoxification system is thought to result in a trade-off such that specialists will have reduced abilities to process novel PSCs (Sorensen et al. 2005b). Together, these hypotheses predict that the generalist will perform (e.g., maintain body mass) better than the specialist on novel toxins. We refer to the pairing of these hypotheses as the diet-breadth trade-off.

Although these trade-offs were originally put forth based on hypothetical differences in biotransformation capacity, we propose that such trade-offs also represent a concomitant shift in behavioral regulation. Regulating the intake of PSCs involves controlling PSC intake below a level that the animals can sufficiently biotransform. Herbivores often reduce total intake when concentrations of PSCs increase in the diet (Mangione et al. 2000; Boyle and McLean 2004; McLister et al. 2004; Dziba and Provenza 2008; Sorensen et al. 2005a; Wiggins et al. 2006a). Because total intake is made up of discrete feeding events, intake can be reduced by restricting the number of meals and/or reducing the size of each meal. In the case of behavioral regulation of PSC intake, meal size reduction should take precedence over meal number because it effectively controls the dose of PSCs ingested and subsequently processed by the liver (Foley et al. 1999; Torregrossa and Dearing 2009).

Generalists are capable of limiting the dose of familiar PSCs at the level of the meal (Wiggins et al. 2003; Boyle et al. 2005; Sorensen et al. 2005a; Torregrossa et al. 2011). Specialists also appear to regulate intake of PSCs. Specialists forage selectively when offered foliage from different individuals of their preferred plant species and reduce meal size when feeding on individual plants with high concentrations of PSCs (Lawler et al. 1998; Marsh et al. 2006; Wiggins et al. 2006b). However, when specialists were offered experimental diets containing <100% of their preferred plant species; they did not demonstrate behavioral regulation. This result suggests that regulation may be limited to situations when specialists approach or exceed their detoxification capacity (Torregrossa et al. 2011).

Specialists are assumed to have evolved the capacity to biotransform the PSCs of their preferred plant species (Freeland and Janzen 1974; Ngo et al. 2003; Shipley et al. 2006; Ngo et al. 2006; Haley et al. 2007a, b), and therefore may not require behavioral regulation at PSC concentrations below the doses typically ingested. Specialists consuming novel PSCs, i.e., unlike those found in their preferred plant species, should not have a detoxification advantage and, thus, are expected to behaviorally regulate to the same extent or to a greater extent as generalists. However, if specialists are usually exempt from regulation at low doses of their preferred plant species, they may not have the machinery to regulate low doses of novel PSCs. This hypothesis predicts that specialists do not have the ability to detect and behaviorally regulate PSCs at low concentrations; therefore, they might be expected to eventually consume doses of novel compounds too high for their biotransformation capacity and thereby incur toxicosis. We define toxicosis as a negative condition (e.g., body mass loss) that can be attributed to ingestion of PSCs.

Demonstrations of the trade-offs with respect to diet-breadth are rare. Mammalian specialists forage differently on novel PSCs compared with generalists (Sorensen et al. 2005b). For example, when specialist and generalist woodrats were fed a diet containing novel PSCs for 10 days, the specialist consumed less and lost a greater percentage of body mass than the generalist. This study did not, however, address whether or not the specialist and generalist differ in meal size regulation.

To address the diet-breadth trade-off hypothesis, we examined the feeding behavior of a specialist and a

generalist herbivore in the genus *Neotoma*. We fed both woodrat species a diet containing a phenolic resin extracted from creosote bush (*Larrea tridentata*). Neither species has ecological or evolutionary experience with creosote bush (Dial and Czaplewski 1990). The PSCs in creosote represent a novel collection of compounds, particularly with respect to the predominant phenolic compound, nordihydroguaiaretic acid (NDGA; Arteaga et al. 2005). These PSCs do not occur in the specialist's preferred plant, juniper (*Juniperus monosperma*; Mabry and Gill 1979; Adams et al. 1981; Adams et al. 1983; Dial 1988). We predicted that the generalist herbivore would perform better than the specialist herbivore (i.e., maintain body weight longer) on the novel diet due to differences in behavioral regulation.

Materials and methods

Animal collection and maintenance

The generalist herbivore, *Neotoma albigula* ($n = 11$; 5 males and 6 females) was collected from Castle Valley, UT, and the specialist herbivore, *Neotoma stephensi* ($n = 10$; 5 males and 5 females) was collected from outside the Waputki National Monument, AZ. All animals were maintained in quarantine until they tested negative for Sin Nombre virus (Dearing et al. 1998). Animals were maintained prior to testing on Harland Teklad rabbit chow pellets (2031) and tap water ad libitum for a minimum of 2 weeks. Woodrats readily consume food and water in captivity. Animals were acclimated to a 12-h light/dark cycle. All experimental protocols were approved by the University of Utah Institutional Animal Care and Health Committee (protocol number 07-02015).

Diet collection

Creosote was collected in the Mojave Desert outside of Lytle Ranch (Washington Co.), Utah. Foliage was collected from >10 bushes, stored on dry ice and transported to the University of Utah. Foliage was stored in airtight bags at −20°C until use. Creosote resin was extracted by soaking the leaf tissue in acetone for 45 min (1:6 wet leaf weight:acetone volume). The extract was filtered (Whatman No. 4 paper) and evaporated under low pressure until the resin was highly viscous. Remaining solvent was removed by drying the extract to constant mass under high vacuum (10^{-3} Torr) for ~48 h. The extraction yielded 18.9% powdered creosote resin by dry weight of creosote leaves. The resin was stored in the dark at −20°C for <3 months before use.

Feeding trials

In feeding trials, the specialist and generalist were progressively presented with increasing levels of resin in the diet to permit induction of detoxification enzymes (Alvares and Pratt 1990). The concentrations used and the number of days offered were 0.5% creosote resin for three consecutive days, 1.0% resin diet, 2.0% resin diet and 4.0% resin diet each for four consecutive days. To prepare all "creosote diets" the phenolic resin was dissolved in acetone, using a volume equal to 25% of the dry weight of the total food and added to ground rabbit chow (Harlan Teklad formula 2031). Acetone was evaporated from diet treatments in a fume hood. The diet was then placed under high vacuum (10^{-3} Torr) for 1 h to remove the remaining solvent. Complete evaporation was confirmed gravimetrically and dry diets were stored at −20°C until presentation. Subsamples of diet were collected at presentation to verify water concentrations. Diets were 98% dry mass.

Body mass, food intake, water intake, and feeding behaviors (meal size, inter-meal interval and the number of meals) were monitored daily. Animals that lost more than 10% of starting body mass were removed from the trial because further mass loss typically results in mortality. Water intake was measured as the difference in the water bottle (g) at the time of presentation versus 24 h later.

To measure meal patterns, animals were housed in shoebox cages (48 × 27 × 20 cm) with a feeder hood (8 × 9 × 13 cm) attached to each cage. A hole at the bottom of the feeder (4.5 cm) allowed access to a spill resistant food bowl (Lab Products, Seaford, DE, USA) mounted on an electronic balance (EW 300; A&D, Tokyo, Japan; ±0.1 g). The balance reported changes in mass (10× per second) to a computer (Dell Dimension 1100, Round Rock, TX, USA). Changes in the mass of the food bowl were acquired by the Scale Monitor Program (Nervestaple, Easthampton, MA, USA) and written into Excel files (Microsoft, Seattle, WA, USA). These files were uploaded into MatLab (The MathWorks, Natic, MA, USA) for analysis in MealReader (TimeScience; Innovative Timelapse Solutions, Salt Lake City, UT, USA). All intake measures are reported as wet weight.

Data analysis

For purposes of analysis, meals were defined as food intake of ≥0.1 g where no consecutive changes were >5 min apart. Consequently, the conclusion of a meal was defined by 5 min of no activity. PSC intake per meal was calculated as the mg per gram of food consumed.

Each animal's feeding measures were averaged across each diet. Animals were only included in the feeding analysis if they completed the trial. Water intake was also

averaged for each treatment, but body mass was analyzed as a daily value. Data were analyzed by two-way repeated measure ANOVA using Systat 12 (Systat Software, Chicago, IL, USA) with species and diet as factors. Bonferroni-corrected post hoc tests were used to explore significant interaction terms and species differences, i.e., comparing the species on each diet using a two-sample *t* test or pair-wise comparisons of diets within species. To compare the outcome of behavioral changes on toxin intake to a null model of no behavioral change, a predicted toxin intake was calculated based on the average meal size from the control diet (0.05%) and compared to actual toxin intake using an ANOVA for each species independently. Fisher's exact test was conducted to compare persistence of each species in the trial as determined by body mass maintenance. Lastly, a series of Cox proportional hazards models (R Foundation for Statistical Computing, Vienna, Austria) were used to determine what factors were significant predictors of a specialist's ability to remain in the trial.

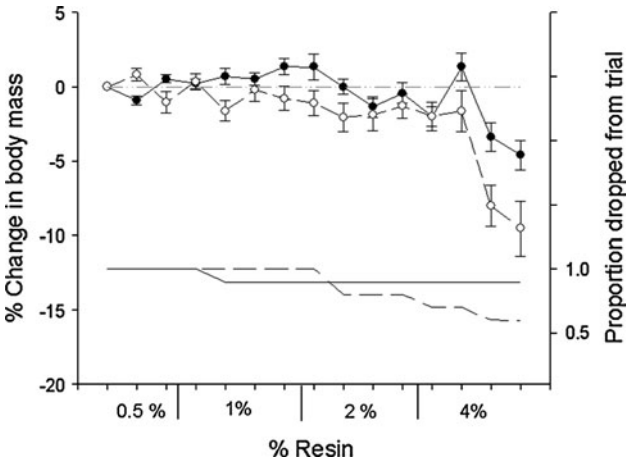

Fig. 1 *Lines with circles* are scaled on the *left y-axis* and represent the percent body mass change across the days of the experiment for the generalist, *N. albigula* (*solid circles* and *solid line*) and the specialist, *N. stephensi* (*open circles* and *dashed line*). *Light gray dashed line* represents 0% change in body mass. *Lines without circles* are scaled on the *right y-axis* and represent the survival curves across the days of the experiment for the generalist, *N. albigula* (*solid line*) and the specialist, *N. stephensi* (*dashed line*). Persistence in the trial was defined as maintenance of at least 90% of initial body mass

Results

The specialist and generalist did not significantly differ in body size at the initiation of the experiment ($F_{1,21} = 0.182$, $P = 0.674$), but the specialist lost more than twice as much mass as the generalist ($F_{1,21} = 12.2$, $P = 0.002$; Fig. 1) during the trial. Although the weight loss did not significantly alter the specialist's ability to persist in the trial ($P = 0.13$; Fig. 1), 4 of 10 specialists were removed from the trial due to excessive mass loss (2 on 2% and 2 on 4% resin diets), whereas only 1 generalist out of 11 was removed from the trial (on the 1% resin diet).

Individuals of both species that completed the trial decreased total intake by approximately 50% on the highest resin diet (4%, $F_{3,13} = 108.9$, $P < 0.001$; Table 1). There was a significant species effect as well as a significant interaction ($F_{1,15} = 9.97$, $P = 0.006$; $F_{3,13} = 8.35$, $P = 0.002$, respectively). Two factors seem to contribute to the significant interaction effect. First, the species differed only on the 2% creosote diet, where the specialist ate more than the generalist. Second, the generalist decreased total intake with each increase in PSC concentration, while the specialist did not decrease total intake until the 4% diet.

Meal size decreased in both the specialist and the generalist by as much as ~40% with increasing resin concentrations ($F_{3,13} = 21.45$, $P < 0.001$; Table 1). Although there was no effect of species ($F_{1,15} = 0.001$, $P = 0.97$), there was a significant interaction effect ($F_{3,13} = 5.1$, $P = 0.02$). The interaction can be attributed to the decrease in meal size of the generalist on the 2% diet, whereas the specialist did not decrease meal size until the 4% diet.

Toxin intake per meal increased in both the specialist and the generalist ($F_{3,13} = 140.99$, $P < 0.01$). There was no significant species effect ($F_{1,15} = 1.03$, $P = 0.43$); however, there was a significant interaction effect ($F_{3,13} = 5.93$, $P < 0.01$). This interaction was likely due to differences in the rate of increase of toxin intake between the species. In both cases, observed toxin intake was significantly lower than that predicted based on intake of the control diet (0.5% creosote; Fig. 2a, b; generalists: $F_{1,39} = 42.2$, $P < 0.001$; interaction between concentration and difference from predicted value: $F_{3,39} = 18.8$, $P < 0.001$; specialists: $F_{1,31} = 56.79$, $P < 0.001$; interaction between concentration and difference from predicted value: $F_{3,31} = 36.89$, $P < 0.001$). The generalist and specialist diverge in behavior at different concentrations of resin. The generalist significantly decreased toxin intake on the 2% diet, but the specialist did not reduce toxin intake until the 4% diet (Fig. 2).

Time between meals was altered by resin concentration. Inter-meal interval increased with resin concentration in both the specialist and the generalist ($F_{3,13} = 9.6$, $P < 0.01$; Table 1). There was no effect of species and no interaction effect ($F_{1,15} = 1.18$, $P = 0.29$; $F_{3,13} = 0.7$, $P = 0.57$).

The total number of meals was affected by resin concentration. Meal number decreased as resin concentration increased ($F_{3,13} = 23.5$, $P < 0.01$; Table 1). There was also a species effect ($F_{1,15} = 5.9$, $P = 0.028$) and significant interaction ($F_{3,13} = 3.8$, $P = 0.04$). However, post hoc testing did not reveal any obvious cause of the

Table 1 Behavioral feeding measures

Behavioral measure	Species	Diet treatments			
		0.5% resin	1% resin	2% resin	4% resin
Food intake	*N. albigula*	12.3 ± 0.3 a	10.7 ± 0.4 b	7.7 ± 0.5 c*	5.0 ± 0.4 d
	N. stephensi	12.4 ± 0.6 a	12.2 ± 0.5 a	10.6 ± 0.8 a	5.8 ± 0.4 b
Meal size	*N. albigula*	0.89 ± 0.1 a	0.79 ± 0.1 ab	0.65 ± 0.1 bc	0.47 ± 0.1 c
	N. stephensi	0.82 ± 0.1 a	0.80 ± 0.1 a	0.72 ± 0.1 a	0.50 ± 0.1 b
IMI	*N. albigula*	94.65 ± 8.1	84.78 ± 6.1	93.32 ± 7.5	111.22 ± 9.8
	N. stephensi	79.12 ± 6.5	78.69 ± 9.6	87.01 ± 6.1	111.31 ± 15.9
Meal number	*N. albigula*	14.3 ± 0.9 a	13.84 ± 0.7 ab	12.35 ± 0.7 ab	10.7 ± 0.6 b
	N. stephensi	15.68 ± 1.1 ab	16.46 ± 1.4 a	14.60 ± 0.9 ab	12.02 ± 1.1 b
Water intake	*N. albigula*	17.34 ± 0.6 a*	20.25 ± 0.6 ab*	23.51 ± 0.9 b*	31.35 ± 2.5 c*
	N. stephensi	43.6 ± 4.4 a	50.13 ± 4.9 a	54.04 ± 3.7 a	52.18 ± 4.4 a

24-h food intake (g), average meal size (g), average inter-meal interval (IMI, min), the average number of meals, and 24-h water intake (g) across increasing concentration of creosote resin in the diet for a generalist (*N. albigula*) and a specialist (*N. stephensi*) herbivore

Letters represent within species differences

* Represents between species differences (Bonferroni-corrected $P < 0.05$)

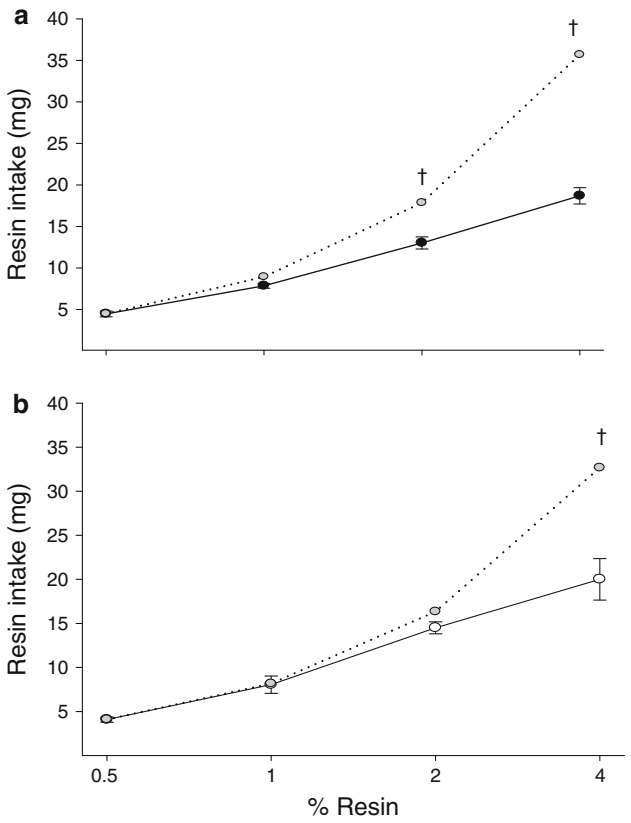

Fig. 2 **a** Predicted resin intake (*dashed lines*) based on control intakes compared to actual resin intake (*solid lines*) for the generalist, *N. albigula* (*black circles*). **b** Predicted resin intake (*dashed lines*) based on control intakes compared to actual resin intake (*solid lines*) for the specialist, *N. stephensi* (**b**, *open circles*). †represents a significant difference from predicted (Bonferoni-corrected $P < 0.05$)

interaction, both species significantly reduced meal number on the 4% resin diet compared to control diet.

Lastly, there was a significant increase in water intake with increasing resin concentration in the generalist ($F_{3,13} = 11.88$, $P < 0.001$: Table 1). The specialist drank twice as much water as the generalist across resin concentrations ($F_{1,15} = 12.34$, $P < 0.001$). There was a significant interaction effect ($F_{3,13} = 5.09$, $P = 0.014$), as the specialist did not significantly alter its water intake whereas the generalist increased water intake with resin intake. Additionally, water intake was the only factor that significantly predicted an animal's ability to remain in the trial (Table 2). Animals able to complete the trial drank 5–10% more water than those unable to complete the trial.

Discussion

Theory predicts that generalists are capable of eating a diverse spectrum of PSCs while specialists are restricted in their ability to consume novel PSCs (Freeland and Janzen 1974; Dearing and Cork 1999; Marsh et al. 2006). We proposed that the specialist and generalist would differ not only in their ability to maintain body mass on a novel diet but also in their ability to behaviorally regulate novel PSCs. Since specialists do not require behavioral modification at low to intermediate doses of their preferred diets (Torregrossa et al. 2011), we suggested that they may not be able to regulate or possibly detect low doses of PSCs.

Although the specialist ingested as much food or more than the generalist across dietary treatments, its ability to

Table 2 Water intake significantly predicted feeding trial completion

Predictor	Regression coefficient	SE (coeff)	z	P
Water intake at time of failure	−0.128	0.06	−2.13	0.03
Average water intake	0.024	0.02	1.22	0.22
Body mass at start of trial	0.179	0.02	1.12	0.26
Total intake at time of failure	−0.155	0.42	−0.367	0.71
Meal size at time of failure	1.84	2.57	0.72	0.47

Cox proportional hazards models were used to test behavioral parameters for predictive value for the ability of *Neotoma stephensi* to complete a 15-day feeding trial while consuming increasing concentrations of creosote bush (*Larrea tridentata*) resin. Persistence in the trial was measured as the ability to maintain greater than 90% of initial body mass

maintain body mass was considerably less than that of the generalist. Indeed, the generalist maintained body mass across three increases in resin concentration. In contrast, the specialist began to lose body mass within days of the initial resin treatment followed by a precipitous drop on the 4% resin diet (Fig. 1). Interestingly, total intake and meal size were not significant predictors of the ability of the specialist to maintain body mass. That is, the animals that were removed from the trial were not ingesting food in different amounts or at different rates compared to animals that remained in the trial. Furthermore, total intakes and meal sizes of the two specialists that were removed from the trial while consuming the 2% diet were indistinguishable from those that continued (average total intake of drop outs: 10.1 vs. 10.5 g for animals that completed the trial, meal size: 1.0 vs. 0.7 g). The excessive loss of mass of these individuals despite adequate intake is indicative of toxicosis. The same was true for the two specialists who were removed from the trial while feeding on the 4% resin diet (total intake: 5.7 vs. 5.8 g, and meal size: 0.47 vs. 0.50 g). Thus, the behavior of the animals included in the analysis of feeding behavior was equivalent to the behavior of animals removed from the trial.

The generalist began to show behavioral regulation on an intermediate concentration of resin by reducing meal size. The generalist demonstrated a significant reduction in meal size on 2% creosote resin and this reduction was not compensated for by increasing meal number or altering inter-meal interval and therefore translated into a reduction in total intake. The specialist, however, did not adjust its behavior until the 4% diet and therefore did not decrease total intake until consuming the 4% diet.

The specialist and generalist not only differed in behavioral regulation but also exhibited disparate physiological responses to diet. While on the 2% resin diet, the generalist ate approximately 40% less than the control diet but lost >1% body mass. In contrast, the specialist lost a similar amount (~1.5%) of starting body mass on the 2% diet without significantly reducing intake. We offer two possible explanations for the discrepancy in intake and body mass loss between the specialist and generalist. First, the generalist may have adjusted energy expenditure by reducing activity whereas the specialist did not. Although we did not measure activity, a previous study documented a differential pattern of activity in response to dietary toxins between these species (Sorensen et al. 2005b). Specifically, the generalist reduced time spent in voluntary wheel running by >50% when consuming a diet with creosote resin compared to a control diet. However, the specialist did not change its activity with respect to a creosote diet. Second, differential energy costs in the biotransformation of creosote between these species could also explain why the specialist lost mass despite similar or greater intakes than the generalist. That is, the specialist may incur a greater metabolic cost associated with biotransformation of novel compounds than the generalist. Either or both of these factors could contribute to the different patterns of food intake and change in mass in these species.

An idiosyncratic pattern of mass loss similar to the one in this study has been documented in populations of the desert woodrat *Neotoma lepida*, a dietary generalist. Populations that naturally feed on creosote maintained body mass at creosote concentrations that resulted in mass loss in populations that had never fed on creosote despite similar food intakes (Mangione et al. 2000). The results of Mangione et al. (2000) combined with those here imply that generalist woodrats or populations with previous experience to PSCs are able to better protect against mass loss than specialists or naïve populations. More work is necessary to determine the underlying mechanisms behind this pattern.

The decrease in body mass of specialist on high resin diets could be due to water loss. Water was a significant predictor of the specialist's ability to remain in the trial, i.e., animals that consumed more water were better able to maintain body mass. Consumption of creosote resin increases the minimum water requirement of another species of woodrat (*N. lepida)* by 18–30% (Mangione et al. 2004). In a previous study, animals could compensate for increases in fecal water loss on resin diets through increases in water intake (Mangione et al. 2004). In our study, the generalist increased water intake by 180% at the highest resin concentration whereas the specialist modestly increased water intake by 20%. However, the specialist

drank 1.7–2.5 times more water constitutively, than the generalist. The high baseline water intake of the specialist may limit its ability to further increase water consumption. If resin causes an increase in fecal water loss in the specialist on high resin diets, but the specialist is unable to offset this lost through increased water intake then the specialist could be in negative water balance. The resulting dehydration could be the source of the mass loss and may have larger system effects.

In addition to impeding numerous other biological functions, water limitation may make herbivores more sensitive to PSCs. The biotransformation enzyme glutathione-s-transferase is important in the metabolism of PSCs (Haley et al. 2007a, b; Skopec et al. 2007). The hepatic activity of this enzyme is reduced during periods of water restriction (Kim et al. 2001). Furthermore, dehydration results in more water reabsorption from the kidneys and may inadvertently allow for reabsorption of biotransformed metabolites of the PSC (Shitara et al. 2006).

In summary, the specialist did not perform as well as the generalist while consuming a diet containing novel PSCs. Furthermore, although the specialist was capable of detecting novel PSCs and regulating intake at high concentrations, it did not modulate its intake at lower toxin concentrations that imparted physiological consequences. This result implies that the specialist has a decreased sensitivity for detection and regulation of novel PSCs. Under natural circumstances, the foraging strategy of a generalist herbivore requires that the animal is cognizant of its physiological limits for PSCs and that it initiates a behavioral response to regulate PSC intake. In contrast, a specialist herbivore is subjected to different selective pressures. It is predicted to have evolved the capacity to biotransform the large doses of PSCs ingested in a diet of a single plant species or genus (Mangione et al. 2000; McLister et al. 2004; Haley et al. 2007a, b; Skopec et al. 2007). Therefore, the specialist may not require fine-tuned detection for low PSC concentrations. The inability to behaviorally regulate dose on novel PSCs may be an additional component of a trade-off with respect to dietary specialization and may limit a specialist's ability to expand its diet breadth to include novel secondary compounds.

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