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Differential regulation of plant secondary compounds by herbivorous rodents

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Summary

1. Theory predicts that herbivores will regulate doses of potentially toxic plant secondary compounds (PSCs) by adjusting meal size, the interval between meals and water intake. Furthermore, the PSC concentrations at which these behavioural modifications are employed are predicted to be species dependent because of interspecific variation in biotransformation capacities.

2. To investigate these hypotheses, we examined ingestive behaviour of two species of rodents that differ in diet breadth and biotransformation capacity. We compared the spontaneous feeding behaviour of *Neotoma stephensi*, a juniper specialist, and *Neotoma albigula*, a generalist, fed a series of diets with increasing concentrations of one-seeded juniper (*Juniperus monosperma*). Juniper contains significant quantities of PSCs, particularly alpha-pinene, a diuretic. We fed each species a series of diets with increasing concentrations of juniper.

3. The specialist, *N. stephensi*, did not regulate PSC intake at any juniper concentration nor did it alter its drinking behaviour. The generalist, however, showed PSC regulation by decreasing meal size in a dose-dependent manner, by increasing the interval between meals, and by substantially increasing water intake.

4. Water consumption was the only factor that could significantly predict an individual's ability to maintain body mass, which suggests that water consumption may be exceptionally important as the PSC content of an individual's diet varies.

5. These data provide support for the hypotheses that generalist herbivores can and do behaviourally regulate PSC intake and suggest that the differences in foraging behaviour may be correlated with diet content and biotransformation capacity.

Key-words: biotransformation, bitter, herbivore, meal size, *Neotoma*

Introduction

Foraging is often described as an effort in optimization. Traditionally, the roles of competition, predation and the size and quality of a patch have been considered the primary drivers of food selection (Newman 2007). Herbivores face the additional challenge of minimizing the negative consequences of plant toxins while optimizing intake of calories and beneficial nutrients (Sorensen & Dearing 2003; Sorensen, Turnbull & Dearing 2004; Marsh *et al.* 2006). Plants protect themselves against herbivory in part by producing a suite of chemicals referred to as plant secondary compounds (PSCs). Many PSCs can be toxic and have been documented to have a wide variety of dose-dependent effects such as neurotoxicity, nutrient absorption disruption, diuresis and malaise (Savolainen & Pfaffli 1978; Bernays, Driver & Bilgener 1989; Dearing, Mangione & Karasov 2002).

To avoid toxicosis while feeding, herbivores must have suitable behavioural responses that complement their physiological response. Two major feeding strategies are thought to have evolved as mechanisms for herbivores to cope with PSCs (Shipley, Forbey & Moore 2009). A few mammalian herbivores have evolved the detoxification capacity necessary to biotransform PSCs in high doses; these 'specialists' are capable of eating, almost exclusively, a single species with high concentrations of PSCs. Most herbivorous mammals, however, consume many different plants and therefore small doses of diverse PSCs (Wiggins *et al.* 2003). It has been hypothesized that this 'generalist' strategy is a mechanism to

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cope with PSCs by decreasing the challenge or risk associated with ingesting large quantities of similar compounds (Freeland & Janzen 1974). In support of this hypothesis, it has been demonstrated that generalist herbivores increase total food intake when combining two plants with different PSC profiles, which are detoxified by separate pathways (Dearing & Cork 1999; Marsh *et al*. 2006).

While much attention has been focused on understanding the specialist's physiological ability to process high concentrations of PSCs, less attention has been directed at the behavioural responses of the generalist, which are as complex and requisite. The generalist strategy for managing toxins presupposes that the generalist is capable of detecting PSC concentrations, halting a meal and switching plant species before suffering ill effects from any particular plant. To accomplish this, herbivores are predicted to have a detection system to assess their physiological status as PSCs are ingested (Torregrossa & Dearing 2009a). When the PSC concentration of a diet is within a range that the herbivore can safely biotransform, the animal should ingest the appropriate caloric/nutrient intake. However, when an animal is faced with a high concentration of PSCs in the diet such that the intake required to maintain body mass would result in PSC intake greater than its biotransformation capacity, the herbivore is predicted to reduce intake. This strategy maintains PSC concentrations in the body within the biotransformation capacity thereby preventing toxicosis. We predict that differences in biotransformation capacity drive regulation of PSC intake such that generalists should regulate intake at lower doses of PSCs compared to a specialist feeding on its preferred plant.

The regulation model predicts not only the regulation of total daily intake but, more specifically, two controls to prevent toxicosis, i.e. the regulation of meal size and inter-meal interval (IMI) (Torregrossa & Dearing 2009a). The first prediction is that herbivores should decrease the amount consumed in a meal as the PSC concentration increases in the diet (Wiggins *et al*. 2003; Boyle *et al*. 2005; Sorensen, McLister & Dearing 2005a). This mechanism is fundamental because the amount ingested in a meal represents the immediate dose of PSCs and is the scale which an overdose takes place. The second prediction of the regulation model is that if an herbivore increases its dose of PSCs, it should also extend the time between meals, referred to as the IMI. The increase in IMI would increase the time for PSCs to be biotransformed before a new dose of PSCs (i.e. a new meal) is introduced. This prediction assumes that larger doses of PSCs require more time to biotransform and clear from the blood stream than smaller doses; evidence exists to support this contention (Boyle *et al*. 2005).

We tested the regulation model by comparing the spontaneous feeding behaviour of two herbivorous woodrats, *Neotoma stephensi* and *Neotoma albigula*. These species have different diet breadths and biotransformation capacities (Vaughan 1982; Dial 1988; Sorensen & Dearing 2003; Sorensen, McLister & Dearing 2005a; Haley *et al*. 2007; Skopec, Haley & Dearing 2007). Juniper (*Juniperus monosperma*) occurs in the diets of both woodrats and contains marked concentrations of numerous terpenes (Vasek & Scora 1967; Adams *et al*. 1981). The most abundant terpene in juniper is alpha-pinene, which can constitute up to 2% of the dry weight of the foliage (Adams *et al*. 1981; Dearing, Mangione & Karasov 2000) and has demonstrable neurotoxicity in mammals (Spearling, Marcus & Collins 1967; Savolainen & Pfaffli 1978). Additionally, low levels of phenolics (~2–5% phenolic equivalents) have also been measured in juniper foliage using colorimetric techniques, but these compounds have not molecularly characterized (Holchek *et al*. 1990; Sorensen, Heward & Dearing 2005b). *Neotoma stephensi* is a juniper specialist, up to 90% of its natural diet is juniper foliage (Vaughan 1982), whereas *N. albigula* is a generalist that consumes ~30% of its natural diet as juniper (Dial 1988). *Neotoma stephensi's* higher tolerance of juniper compared to *N. albigula* is correlated with differences in their biotransformation capacities and excretion of PSCs (Sorensen & Dearing 2003; Green *et al*. 2004; Sorensen, Turnbull & Dearing 2004; Sorensen, McLister & Dearing 2005a; Haley *et al*. 2007; Skopec, Haley & Dearing 2007).

In addition to altering feeding behaviour, ingestion of PSCs may cause diuresis and therefore alter water balance and drinking behaviour (Dearing, Mangione & Karasov 2001, 2002). Generalists are more prone to water loss after consuming PSCs than specialists (Dearing, Mangione & Karasov 2002). This finding suggests that differences in biotransformation capacity interact with water balance during the processing of PSCs. Alterations in water balance could lead to shifts in foraging strategy akin to the shifts documented with variable water availability (e.g. Molokwu *et al*. 2007).

We hypothesized that these two species would differentially regulate intake of both PSCs and water. We predicted that the specialist, *N. stephensi,* would not exhibit modification in meal size, IMI, or water intake with increasing concentrations of juniper in the diet as it naturally consumes large doses of juniper and PSCs. Conversely, we hypothesized that *N. albigula,* the generalist, would show modification of meal size and IMI at high doses of juniper. Likewise, we predicted that because of the diuretic quality of juniper, the generalist would modify its drinking behaviour by increasing water consumption with increasing concentrations of PSCs.

Materials and methods

ANIMAL COLLECTION AND MAINTENANCE

The specialist, *N. stephensi* ($N = 7$, four female, three male), was collected at Woodhouse Mesa, adjacent to Wupatki National Monument in AZ. Sufficient numbers of the generalist, *N. albigula,* were not available at Woodhouse Mesa during the collection of *N. stephensi*; therefore, we collected *N. albigula* ($N = 11$, five female, six male) from Castle Valley, UT. We have previously used this population for comparative studies between the specialist and generalist. There is no difference between the two *N. albigula* populations with respect to their intake of juniper collected at the Arizona site (Dearing, Mangione & Karasov 2000). All animals were maintained in quarantine until they tested negative for Sin Nombre virus using an ELISA

assay as described in Dearing *et al.* (1998). Animals were acclimated to a 12-h light/dark cycle and maintained on Harlan Teklad high fibre rabbit chow pellets (2031) and tap water *ad lib*. All experimental protocols were approved by the University of Utah Institutional Animal Care and Health Committee (protocol number 07-02015).

DIET TREATMENTS

Juniper (*J. monosperma*) was collected from the Arizona site, although it also occurred at the Utah site. Juniper foliage was collected from >10 trees, stored on dry ice and transported back to the University of Utah. Foliage was stripped from branches and ground using a Waring blender until it passed through a 1-mm mesh. Juniper was handled on dry ice from the time it was collected until it was presented to the animals to minimize volatilization of the PSCs, including during the grinding and stripping processes. Ground foliage was then stored in airtight bags at −20 °C. The plant material presented in animal diets represented a homogenous mixture of the foliage collected.

We chose to present the animals with ground juniper foliage rather than amending the diet with individual compounds for two reasons. First, we wanted to mimic as best as possible the assortment of compounds that woodrats may encounter in the nature because we do not know what cues are used to make feeding decisions. Second, the volatilization of terpenes amended to diets is greater than that of terpenes in diets made of ground juniper (Torregrossa & Dearing 2009b). While it is possible to microencapsulate volatile compounds, this process adds considerable dietary bulk and fundamentally alters the sensory cues (odour, texture) of the diet compared to that of plant material, even ground plant material. Such cues may be critical to ingestive behaviour. Therefore, to provide as natural a diet as possible while also presenting uniform diet treatments across all animals, we chose to use ground juniper in the treatments.

Juniper diet treatments were prepared by mixing a known amount of ground juniper with ground rabbit chow to produce diets that were 25%, 50%, 75%, 90% juniper on a dry weight basis. Because there were differences in the water content of ground juniper and rabbit chow, water was added as needed to all diets (including the control) to standardize water content at 50% water. The addition of water retarded the volatilization of terpenes from the chow (pers. obs.) and simulated that found in juniper foliage. It is unlikely that the additional water radically changed the chemical nature of the hydrophobic terpenes in juniper. It is possible that water may have interacted with phenolics compounds. However, given that the total food intakes of juniper in our experiment are comparable to that observed for woodrats consuming juniper in nature (Vaughan 1982; Dial 1988), it is unlikely that the diet preparations considerably altered the nutritional or PSC profiles of juniper. Additional nutritional information on the diets is presented in Table 1. Woodrats were sequentially presented for 3 days each with the following treatments: control diet, 25% juniper diet, 50% juniper diet, 75% juniper diet and 90% juniper diet. Three days is an adequate dietary exposure for full induction of detoxification and digestive enzymes (Alvares & Pratt 1990; Parkinson 1996; Karasov & Hume 1997; Karasov & Martınez del Rio 2007; Karasov, Martınez del Rio & Caviedes-Vidal 2011). Food was presented daily at dark onset because woodrats are nocturnal; leftovers from the previous day were collected and dried to estimate dry matter intake.

Body mass, food intake, spontaneous feeding behaviour (described below) and 24-h water intake were monitored daily. Animals that lost more than 10% of starting body mass were removed from the trial, as further loss was considered life threatening. Water intake was calculated as the water ingested from the water bottle plus that ingested in food.

Table 1. Nutritional contents of diet treatments

	Control	25% Juniper	50% Juniper	75% Juniper	90% Juniper
Fibre (%)	32·5	31·7	31·05	30·3	29·9
Crude protein (%)	14·5	12·3	10·2	7·9	6·7
Energy (kJ g^{-1})	16·5	17·7	19·0	20·3	21·0
Alpha-pinene (mg g^{-1})	0	3·0	6·0	8·9	10·7

Fibre represents the % cellulose and lignin as determined by the acid detergent fibre method. Fibre and crude protein were measured by Dairy One forage testing laboratory (Ithaca, NY, USA). Energy and alpha-pinene were measured in house. Energy content of juniper is greater than that of the control diet because the essential oils like alpha-pinene contribute to measurements of energy contents; however, the energy contained in essential oils is unavailable to woodrats.

MEAL ANALYSIS

To measure spontaneous feeding behaviour and meal patterns, animals were housed in shoebox cages (48 × 27 × 20 cm) with a feeder (8 × 9 × 13 cm) extending from each cage. An opening 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 to a computer (Dell Dimension 1100, Round Rock, TX, USA) 10× per second. All data were processed through the Scale Monitor Program (Nervestaple, Easthampton, MA, USA) and written to Excel files (Microsoft, Seattle, WA, USA). These files were then uploaded into MatLab (The MathWorks Inc., Natic, MA, USA) for analysis in MealReader 2.0 (TimeScience, Innovative Timelapse Solutions, Salt Lake City, UT, USA).

Meals were defined as food intake of ≥0·1 g where no consecutive changes in the food mass were >5 min apart. Consequently, the conclusion of a meal was defined by 5 min of no subsequent activity. Bout parameters differ across species; therefore, to define minimum meal size and IMI for the woodrat, we conducted a multi-step, iterative process of varying the bout parameters to determine the bout length that best described the animals' feeding behaviour on control diets. The parameters described here were chosen because they explained the highest percentage of the animals' total intake (>95%, Torregrossa 2009) and were comparable to previously published work in woodrats (Sorensen, Heward & Dearing 2005b). IMIs were calculated as the average time between each meal ingested during the dark cycle. The light period was excluded for two reasons: first, this is the inactive period and therefore represents substantially longer intervals than nocturnal IMIs. Second, the animals were disturbed by the experimenter during this time period to measure body mass and replace diet.

MEASUREMENT OF TOXIN INTAKE

We could not prevent volatilization of terpenes during diet presentation; however, we accounted for this issue by measuring the rate of alpha-pinene volatilization that took place during diet presentation

and incorporated this rate in estimation of total alpha-pinene ingestion. We could not measure the volatilization of all terpenes in juniper and selected alpha-pinene because it is the most abundant terpene in juniper and one of the most volatile (Adams *et al.* 1981). We used it as a proxy to account for changes in overall terpene content over time. Bowls containing the 25% and 90% juniper diets were placed in the animal room, and subsamples were collected at 0, 4, 8, 12 and 24 h. Alpha-pinene was immediately extracted from the diet subsamples, and the amount of alpha-pinene was quantified using gas chromatography using the methods described in Sorensen, Turnbull & Dearing (2004). Volatilization of alpha-pinene followed an exponential time course, such that alpha-pinene content in mg at time T(min) = (initial content) e$-0{\cdot}001T$, in samples of 25% and 90% juniper. This equation was used to determine the actual alpha-pinene content during a meal given the volatilization rate.

STATISTICAL ANALYSIS

Body mass, total intake, alpha-pinene and water intake were compared by multivariate repeated measures general linear model with species and time as factors. Behaviours that contribute to differences in total intake (meal size, number of meals and IMI) were analysed by univariate repeated measures general linear models. In all cases, statistically significant differences were explored using Bonferroni corrected pair wise comparisons. Comparisons were made using SYSTAT 12 (Systat Software, Chicago, IL, USA). All behavioural data were averaged across the 3 days of diet treatment for each animal.

Survival analyses were conducted in SYSTAT 12 to compare each species' ability to stay in the trial as determined by body mass maintenance. Animals that maintained body mass within 10% of their initial mass from the start through the presentation of the last diet treatment were classified as survivors. Cox proportional hazard models were coded in R (R Foundation for Statistical Computing, Vienna, Austria) to test behavioural parameters (water intake, total food intake and meal size during the previous day, as well as, average water intake during the trial and starting body mass) for predictive value of completion of the trial.

Results

BODY MASS AND SURVIVAL ANALYSIS

The specialist was significantly more likely to maintain body mass and remain in the trial than the generalist ($\chi^2_1 = 5{\cdot}8$, $P = 0{\cdot}02$). All seven specialists who began the trial completed the trial. However, more than half the generalists were unable to maintain body mass on the 90% juniper diet. Four of the 11 generalists were removed from the trial for losing $>10\%$ of their starting body mass during the first 2 days of the 90% juniper diet. Of the generalists that completed the trial, an additional two lost $>10\%$ of their starting body mass on the final day of testing.

There was no significant difference between species in body mass of woodrats that persisted through the feeding trial ($F_{1,12} = 0{\cdot}03$, $P = 0{\cdot}8$); there was an effect of juniper concentration ($F_{4,48} = 25{\cdot}7$, $P < 0{\cdot}01$) as well as an interaction between the two ($F_{4,48} = 5{\cdot}4$, $P < 0{\cdot}01$). The specialist increased body mass on diets with intermediate juniper concentrations ($F_{4,3} = 6{\cdot}5$, $P = 0{\cdot}08$, Fig. 1), whereas the generalist lost body mass as the concentration of juniper increased in the diet ($F_{4,3} = 11{\cdot}5$, $P = 0{\cdot}03$, Fig. 1).

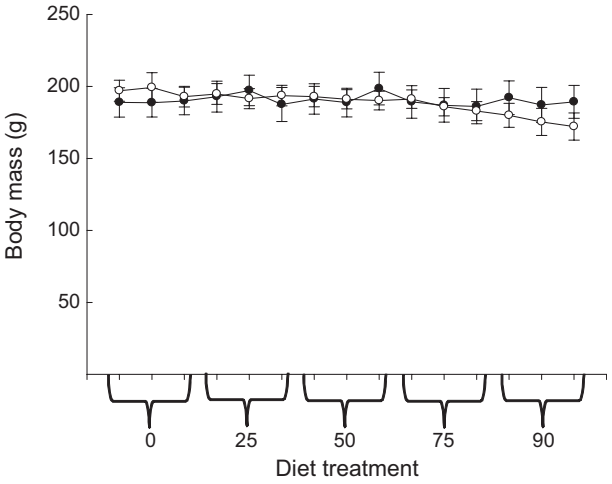

Fig. 1. Body mass (mean ± SE) for the generalist *Neotoma albigula* ($N = 7$, white circles) and the specialist, *Neotoma stephensi* ($N = 7$, black circles). The diet treatment represents the per cent of the diet that was juniper (by dry weight). Only animals that completed the trial were included in the analysis.

TOTAL INTAKE

The specialist ate more than the generalist ($F_{1,12} = 5{\cdot}12$, $P = 0{\cdot}04$). There was a significant effect of diet as well as a significant interaction between diet and species ($F_{4,48} = 19{\cdot}36$, $P < 0{\cdot}01$, $F_{4,48} = 23{\cdot}9$, $P < 0{\cdot}01$). The specialist did not significantly change intake with increasing juniper concentration (Fig. 2a). However, the generalist significantly decreased total intake by 30% between the control diet and the 75% juniper diet and further reduced total intake another 40% on the 90% juniper diet (Fig. 2b).

MEAL SIZE

As with the total intake, the specialist showed no modification of meal size in response to increasing amounts of juniper in the diet ($F_{4,3} = 2{\cdot}5$, $P = 0{\cdot}24$, Fig. 3a), whereas the generalist decreased meal size by *c.* 30% between the control diet and the 75% juniper diet and further reduced meal size an additional 30% on the 90% juniper diet ($F_{4,3} = 12{\cdot}8$, $P = 0{\cdot}03$, Fig. 3b).

INTER-MEAL INTERVAL

The specialist and generalist also differed in the IMI response to increasing concentrations of juniper. While the specialist did not show a significant change in IMI ($F_{4,3} = 0{\cdot}4$, $P = 0{\cdot}82$, Fig. 4a) across juniper concentrations, the generalist significantly increased IMI from the control diet to the juniper containing diets by nearly 10% ($F_{4,3} = 62{\cdot}5$, $P = 0{\cdot}003$, Fig. 4b).

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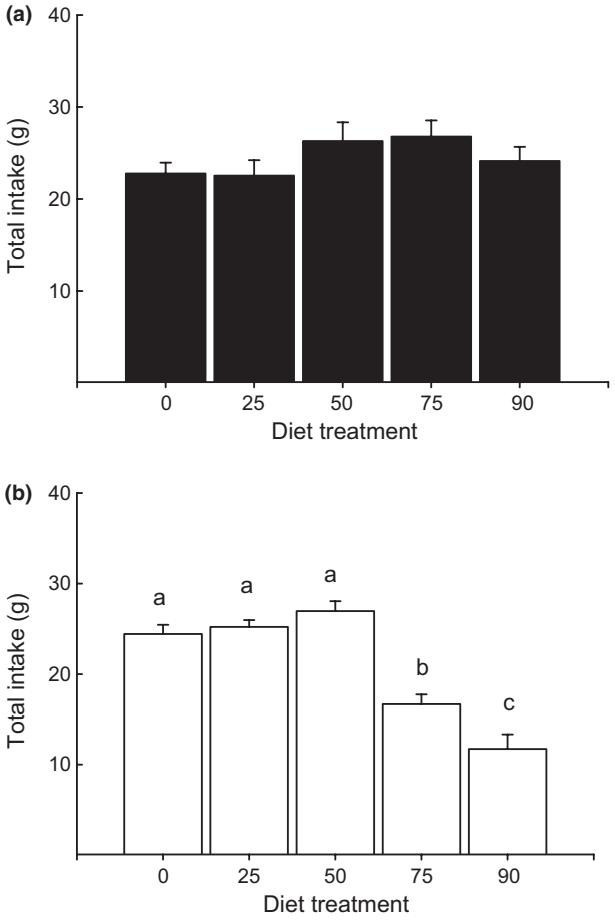

Fig. 2. Total intake (mean ± SE) with increasing concentrations of juniper for the specialist, *Neotoma stephensi* (a, black bars) and the generalist, *Neotoma albigula* (b, white bars). The diet treatment represents the per cent of the diet that was juniper (by dry weight). Different letters represent significant differences (Bonferroni corrected $P < 0{\cdot}05$).

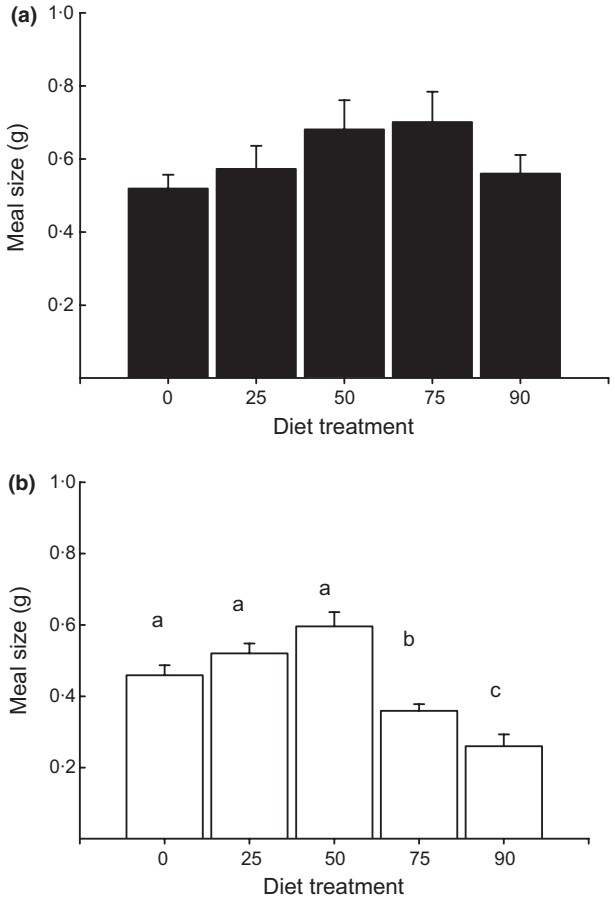

Fig. 3. Meal size (mean ± SE) with increasing concentrations of juniper for the specialist, *Neotoma stephensi* (a, black bars) and the generalist, *Neotoma albigula* (b, white bars). The diet treatment represents the per cent of the diet that was juniper (by dry weight). Different letters represent significant differences (Bonferroni corrected $P < 0{\cdot}05$).

NUMBER OF MEALS

Only the generalist regulated the number of meals consumed in 24 h in response to juniper diets. The specialist did not change the number of meals it consumed across increasing concentrations of juniper maintaining a daily average of 42 feeding events ±1·1 ($F_{4,3} = 1{\cdot}3$, $P = 0{\cdot}43$). The generalist, however, decreased the number of meals by *c*. 7% between the control diet and the juniper diets ($F_{4,3} = 8{\cdot}6$, $P = 0{\cdot}05$), dropping from an average of 46 ± 2·0 feeding events on the control diet to 43 ± 1·5 on the 90% juniper diet. All juniper diets differed from the control condition with respect to meal number, but meal number did not vary across concentrations (Bonferroni corrected post hoc tests, *P*'s $< 0{\cdot}05$).

WATER CONSUMPTION

Water intake did not differ between individuals of each species that persisted throughout the feeding study ($F_{1,12} = 2{\cdot}44$, $P = 0{\cdot}14$). There was a strong juniper concentration effect ($F_{4,48} = 12{\cdot}3$, $P < 0{\cdot}01$) and a significant interaction effect ($F_{4,48} = 4{\cdot}7$, $P < 0{\cdot}03$) on water intake. Although there were no significant differences in water intake in the specialist across juniper concentrations, the generalist increased water intake with increasing juniper intake (Fig. 5). In addition, the generalists that were removed from the trial because of excessive mass loss drank significantly less than those who maintained body mass through the final day of the trial ($F_{1,9} = 8{\cdot}86$, $P = 0{\cdot}02$, Fig. 5b).

ALPHA-PINENE INTAKE

The specialist consumed more alpha-pinene per meal than the generalist ($F_{1,12} = 14{\cdot}3$, $P = 0{\cdot}03$, Fig. 6). Alpha-pinene intake was also affected by the concentration of juniper in the diet. Alpha-pinene intake per meal significantly increased with increasing concentrations of juniper for both the specialist and the generalist ($F_{3,36} = 27{\cdot}3$, $P < 0{\cdot}01$). There is also a significant interaction between species and concentration ($F_{3,36} = 9{\cdot}7$, $P < 0{\cdot}01$). The specialist increased alpha-pinene intake at every concentration until 90% juniper, whereas the generalist increased intake of alpha-pinene per meal between the control and 50% juniper diet but

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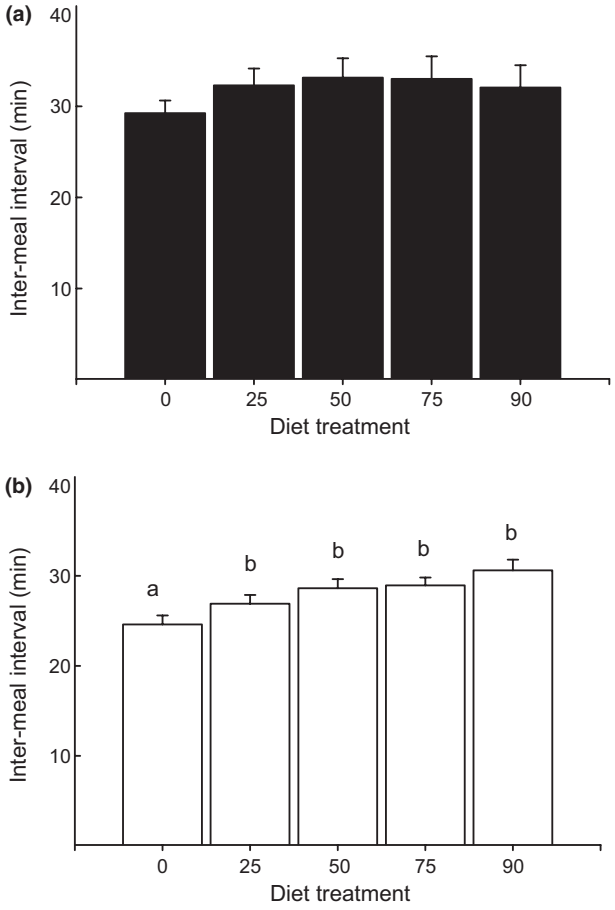

Fig. 4. Inter-meal interval (mean ± SE) with increasing concentrations of juniper for the specialist, *Neotoma stephensi* (a, black bars) and the generalist *Neotoma albigula* (b, white bars). The diet treatment represents the per cent of the diet that was juniper (by dry weight). Different letters represent significant differences (Bonferroni corrected $P < 0{\cdot}05$).

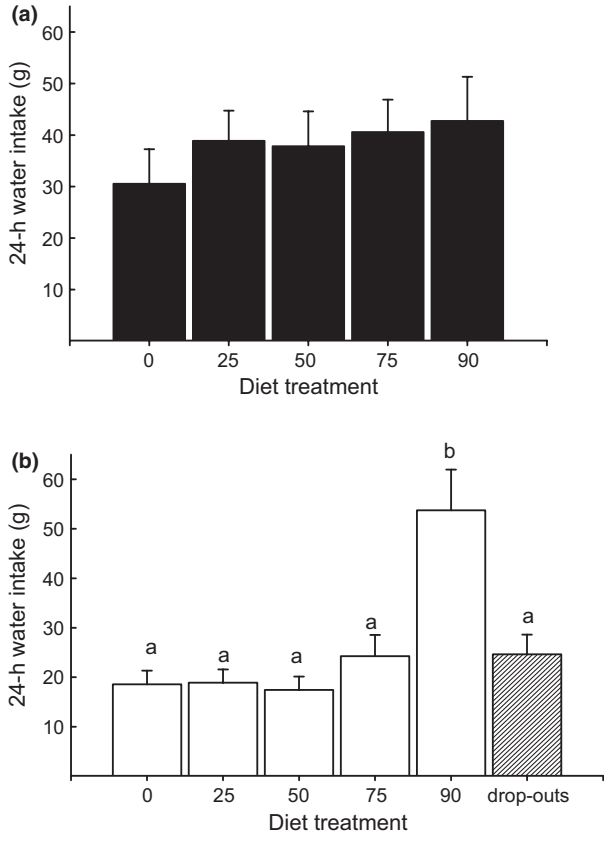

Fig. 5. Total water intake (mean ± SE) with increasing concentrations of juniper for the specialist, *Neotoma stephensi* (a, black bars), and the generalist *Neotoma albigula* (b, white bars). The diet treatment represents the per cent of the diet that was juniper (by dry weight). Different letters represent significant differences (Bonferroni corrected $P < 0{\cdot}05$).

maintained alpha-pinene intake on the 50%, 75% and 90% juniper treatments.

PREDICTORS OF RETENTION

In *N. albigula,* water intake at the time of removal from the experiment was the only significant predictor of an animal's ability to remain in the feeding trial (Table 2). Individuals who remained in the trial almost doubled their water intake from the control diet to the 90% juniper. Other factors, such as total intake during the previous day, meal size during the previous day, starting body mass and average water intake during the trial, were not significant predictors in the Cox proportional hazards model. This analysis was not conducted on *N. stephensi* because no individuals were removed from the trial.

Discussion

To avoid toxicosis, mammalian herbivores must either avoid large doses of potentially toxic PSCs or have the capacity to

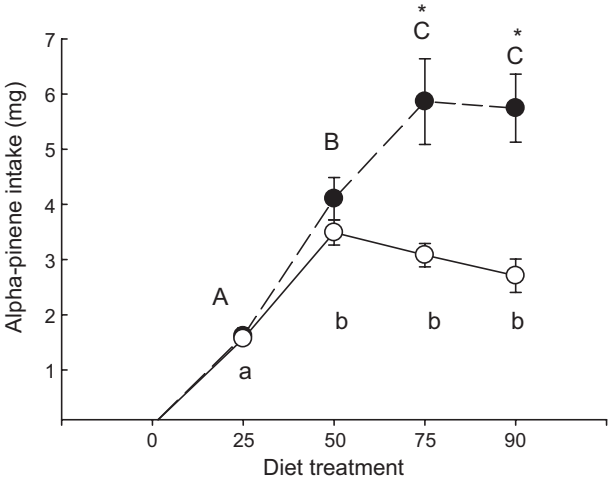

Fig. 6. Alpha-pinene intake for the specialist, *Neotoma stephensi* (black circles), and the generalist, *Neotoma albigula* (white circles). The diet treatment represents the per cent of the diet that was juniper (by dry weight). Letters (capital for *N. stephensi* and lower case for *N. albigula*) indicate significant differences from previous intakes, across treatments. Asterisks indicate significant differences between species at a particular treatment (Bonferroni corrected $P < 0{\cdot}05$).

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Table 2. Water intake significantly predicted feeding trial completion

Predictor	Regression coefficient	SE (coef)	z	P
Water intake at time of failure	−1·97	0·84	−2·35	0·02
Average water intake	−0·12	0·08	−1·58	0·11
Body mass at start of trial	0·02	0·02	1·34	0·18
Total intake at time of failure	−0·037	0·11	−0·33	0·74
Meal size at time of failure	−2·04	5·55	−0·37	0·71

Cox proportional hazards models were used to test behavioural parameters for predictive value for completion of the feeding trial, i.e. animals maintained >90% of the starting body mass. Only the generalist was included in this analysis since all specialists completed the trial.

biotransform ingested PSCs. We predicted that the generalist herbivore, *N. albigula*, would be more at risk of toxicosis and would modulate feeding and drinking behaviours on a PSC containing diet. In comparison, we predicted that the specialist herbivore, *N. stephensi*, would not be at risk of toxicosis when given its preferred diet and thus would not modify its behaviour. Two possible feeding modifications were predicted for the generalist in response to increasing PSC concentrations: a decrease meal size or an increase IMI. These modifications are not mutually exclusive and could be used in concert. We found that while the specialist showed no behavioural modification, the generalist used both modifications as dietary PSC concentration increased.

Neotoma stephensi showed no significant behavioural modifications (e.g. total intake, meal size, IMI, water intake) when fed increasing concentrations of juniper. This was not surprising given that under natural conditions *N. stephensi* consumes a diet of >90% juniper foliage (Vaughan 1982). Therefore, the highest dose of juniper consumed by *N. stephensi* was likely below its biotransformation capacity. This specialist is physiologically suited to consume high quantities of juniper without behavioural modification. Evidence to date indicates that the capacity of the gut efflux transporters in the intestine of *N. stephensi* is more than twofold greater than that of *N. albigula* (Green *et al*. 2004). This difference in gut transporter capacity may explain why *N. stephensi* absorbs less toxin across the gut tissue and excretes more unchanged parent compound in the faeces compared the generalist (Sorensen & Dearing 2003; Sorensen, Turnbull & Dearing 2004). In addition, the specialist exhibits higher activity and greater expression of biotransformation enzymes for detoxification of PSCs compared to the generalist (Haley *et al*. 2007; Skopec, Haley & Dearing 2007).

Specialist herbivores such as ringtail possums (*Pseudocheirus peregrinus*), koalas (*Phascolarctos cinereus*) and some populations of desert woodrats (*Neotoma lepida*) regulate PSC intake when fed foliage from their preferred plant species with differing PSC concentrations (Lawler *et al*. 1998; Sorensen, Heward & Dearing 2005b; Wiggins *et al*. 2006; Marsh, Wallis & Foley 2007). In these studies, total intake varied up to 30% as PSCs increased. In our study, we did not see significant modification by the specialist; however, the specialist did show a non-significant decrease in meal size between 75% and 90% juniper, which led to the maintenance of alpha-pinene dose between 75% and 90% juniper diets. Although our study was not designed to examine how specialists respond to variation in PSCs among individuals of their preferred plant species, the decrease in meal size observed for *N. stephensi* is consistent with the type of selectivity documented for possums and koalas. It is possible that some juniper trees have PSC levels that exceed *N. stephensi*'s biotransformation ability. If so, we expect that at such concentrations, *N. stephensi* would exhibit behavioural modifications like those observed in the marsupial specialists. Perhaps *N. stephensi's* preference for particular juniper trees is driven by differences in PSC concentration (Vaughan 1982). In contrast, the generalist has a lower tolerance for juniper (Dearing, Mangione & Karasov 2000; Sorensen, McLister & Dearing 2005a) and altered its feeding behaviour in a manner consistent with the regulation model (Foley, Iason & McArthur 1999; Torregrossa & Dearing 2009a). It decreased total intake and meal size, increased IMI and increased water intake when juniper was added to the diet.

We propose that the behavioural modifications displayed by the generalist were because of the increasing levels of PSCs in the juniper treatments rather than differences in nutrient content. The juniper diets have higher gross energy contents than the control diet (Table 1); however, this is caused by the essential oils (terpenes) in juniper, which are energy-rich but unavailable energy substrates for woodrats. Sorensen, Heward & Dearing (2005b) demonstrated that the efficiency of energy metabolism was one-third lower in woodrats consuming a juniper diet compared with a control diet. Thus, the juniper diets in our study were effectively lower in available energy than the control diet. Nitrogen content also decreased with increasing amounts of juniper in the diet. If woodrats were foraging to maintain the energy and nitrogen intakes observed for the control diet, then they should have increased intake with increasing levels of dietary juniper (Booth 1974; Hirsch & Collier 1974; Kanarek 1976; Aparecida de Franca *et al*. 2009). The observed decrease in intake and meal size for the generalist is unexplainable from a strictly nitrogen (Dearing, Mangione & Karasov 2000; Dearing, McLister & Sorensen 2005) or energy perspective but is consistent with increasing concentrations of PSCs.

The generalist's decrease in meal size as the per cent juniper increased in the diet resulted in the generalist consuming less alpha-pinene than was consumed by the specialist. Furthermore, the generalist maintained a constant dose of alpha-pinene across the three highest juniper concentrations (50%, 75, and 90% juniper). The ability to maintain a constant PSC intake suggests that the generalist was capable of detecting the concentration of alpha-pinene in the diet and regulating intake in accordance with a physiologically acceptable level.

The generalist also regulated intake through modifications of IMI in response to the addition of PSCs to the diet. While it has been proposed that generalists will increase IMI in response to increasing PSCs in the diet, the two other studies

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testing the regulation model on a generalist herbivore did not report results for IMI (Wiggins *et al.* 2003; Boyle *et al.* 2005). These results suggest that *N. albigula* needed more time between meals to metabolize the doses of PSCs ingested.

Interestingly, the juniper concentration that elicited a change in behaviour of the generalist differed for meal size and IMI. The IMI may be the first response to changes in PSCs as it changed at a lower concentration of juniper than did meal size (25% vs. 50%). The generalist ingested 2-mg alpha-pinene/meal on the 25% juniper diet compared with no alpha-pinene in the control diet; the IMI was 2 min longer on average on the juniper treatment compared to the control diet. However, there was no change in IMI between 25% and 50% juniper diets, which contributed an additional 1·5 mg of alpha-pinene/meal. This was surprising, as we expected an additional increase in IMI with the increased dose of alpha-pinene. However, if we consider alpha-pinene as a proxy for the numerous PSCs in juniper, we may not have increased the PSCs enough between 25% and 50% to warrant an increase in IMI. The clearance time of alpha-pinene in the generalist is very close to the IMI recorded on control diet (~25 min). Sorensen & Dearing (2003) reported that *N. albigula* cleared more than 75% of circulating alpha-pinene in 20 min when given an oral dose > twice that of the intakes recorded here. Therefore, a baseline IMI of 25 min may be sufficient to allow clearance of alpha-pinene. There was no change in IMI at higher concentrations either, however, the alpha-pinene intake per meal remained constant between the 50% and 90% juniper treatments because of the decrease in meal size; therefore, a change in IMI would not be necessary.

In addition, increased water intake appeared to be a component of the ability of *N. albigula* to tolerate increasing quantities of juniper. The individuals able to maintain body mass throughout the trial, all increased water intake with increasing concentrations of juniper in the diet, whereas animals that were unable to maintain body mass did not. Water intake was the only significant predictor of an animal's ability to persist in the trial. Although an increase in water intake by *N. albigula* has been previously documented (Dearing, Mangione & Karasov 2002), our study demonstrated that woodrats with greater water intake were better able to maintain body mass on diets with increased PSCs. It is unlikely that mass maintenance was achieved through increased water retention. Previous work revealed the diuretic effects of juniper; urine output of woodrats increased linearly with water intake on diets with increasing juniper concentrations (Dearing, Mangione & Karasov 2002). Rather, the ability of individuals to maintain hydration through increased water consumption may facilitate biotransformation and clearance of PSCs in juniper. For example, activity of glutathione-s-transferase, a biotransformation enzyme up regulated by *N. albigula* on juniper diets (Haley *et al.* 2007; Skopec, Haley & Dearing 2007), is depressed when laboratory rats are water restricted (Kim *et al.* 2001). In addition, it is less likely that biotransformed metabolites will be reabsorbed into circulation from the kidneys if the animal is in water balance (Shitara, Horie & Sugiyama 2006).

These data suggest that water consumption and the ability of generalists to consume PSCs may be more tightly linked than previously considered. This could have major consequences on foraging behaviour and fitness for herbivores in arid climates. Interestingly, the generalists who were able to maintain body mass drank the same volume of water as the specialists on the 90% juniper diet, whereas the generalists that were unable to maintain body mass did not increase water intake. The variability of the generalist's response may be due to variability in kidney function or the sensitivity of the angiotensin–renin system.

Our data support the regulation model and provide the first evidence that generalist herbivores regulate intake of PSCs by decreasing meal size, altering IMI and increasing water consumption. Furthermore, these data imply that there is a relationship between biotransformation capacity and regulation, as species with a greater capacity for biotransformation did not behaviourally regulate PSC intake while the species with the lower biotransformation capacity did (Haley *et al.* 2007). A similar relationship has been reported in the literature on alcohol consumption. Voluntary intake of alcohol, in animals, is highly correlated with the activity of liver alcohol dehydrogenase (Kulkosky 1985). Although this evidence is correlational, the tie between biotransformation capacity and behavioural regulation seems promising. Future experimental studies that can block biotransformation function are needed to understand the mechanisms of detection and regulation.

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