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CACHING AS A BEHAVIORAL MECHANISM TO REDUCE TOXIN INTAKE

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We hypothesized that caching could be a mechanism to remove volatile secondary compounds from a plant-based diet. This mechanism has been demonstrated in 1 herbivore and has been hypothesized as a widespread mechanism for reduction of intake of toxins. We examined this hypothesis in both the field and the laboratory by documenting the ability of herbivorous rodents to manipulate alpha-pinene, the major terpene in juniper (*Juniperus osteosperma*). First, we dismantled middens of *Neotoma albigula* and found that cached plant material was devoid of alpha-pinene, whereas surrounding trees contained alpha-pinene. In laboratory trials, we examined the ability of woodrat species (*N. albigula*, *N. lepida*, and *N. bryanti*) to reduce toxin intake by storing food before consumption. Each species responded differently when offered terpene-treated pellets. *N. albigula* controlled terpene intake by increasing reliance on the terpene-free cache. *N. lepida* controlled terpene intake by decreasing total intake but did not change cache consumption. *N. bryanti* did not regulate terpene intake. All 3 species abandoned more food in the foraging cage when the food contained terpene. In an additional laboratory trial with *N. albigula*, we determined that increased abandonment was not related to cache size.

Key words: caching, herbivore, hoarding, plant secondary compound

Caching or large-scale food storage by animals has traditionally been viewed as a behavioral mechanism to provide food during shortages (Dearing 1997a; Post 1993; Post and Reichman 1991; Vander Wall 1990). However, caching might serve an alternative function, that is, allowing time for degradation of plant secondary compounds that are potentially toxic (Dearing 1997b; Gliwicz et al. 2006; Roy and Bergeron 1990). For example, pikas (*Ochotona princeps* and *Ochotona hyperborea*) preferentially cache plants high in tannins, presumably for later consumption when tannin levels decrease (Dearing 1996, 1997b, 1997c; Gliwicz et al. 2006). These studies on pikas documented 2 phenomena. First, phenolics degrade during storage, and 2nd, pikas preferentially eat low-phenolic food and cache high-phenolic food. Examination of these data suggests that pikas manipulate toxin intake via storage. However, it was not determined whether pikas consumed the cached food after the toxins had decayed.

To explore the generality of detoxification via storage and expand on previous studies, we investigated caching as a behavioral mechanism for the regulation of toxin intake in 3 rodent herbivores in the genus *Neotoma*. Commonly known as woodrats or packrats, members of the genus *Neotoma* collect

plant material that they store for future consumption in stick-piles called middens. We examined the potential manipulation of juniper *Juniperus osteosperma*, an evergreen containing high levels of monoterpenes (Schwartz et al. 1980). The volatile nature of terpenes enhances loss during storage. We hypothesized that members of the genus *Neotoma* would cache terpene-containing food and delay consumption until after the terpenes volatilized. We further hypothesized that when fed a terpene-containing diet, members of the genus *Neotoma* should preferentially eat food stored in their caches because the stored food will have lower concentrations of terpenes. Finally, we hypothesized that animals will be selective when feeding from caches and choose the oldest food to maximize terpene loss.

To investigate these hypotheses we used 3 species of *Neotoma*: the white-throated woodrat (*N. albigula brevicauda*—Macêdo and Mares 1988), the desert woodrat (*N. lepida lepida*—Patton et al. 2008), and Bryant's woodrat (*N. bryanti* formerly known as *N. lepida intermedia*—Patton et al. 2008). *N. albigula* and the population of *N. lepida* used in this study regularly encounter juniper in their natural habitat and include juniper in their diet (Cameron 1971; Dial 1988; Stones and Hayward 1968). Although the population of *N. bryanti* that we used does not have juniper in its natural environment, it consumes salvia (*Salvia apiana*), which is also high in terpenes (Atsatt and Ingram 1983).

Terpenes are a particularly good candidate for manipulation during storage because they are both toxic and volatile. The

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terpene that is found in juniper, alpha-pinene, can cause central nervous system depression, contact dermatitis, lung function impairment, liver and kidney cysts, and death (Falk et al. 1990; Savolainen and Pfaffli 1978; Spearling et al. 1967). However, once foliage is removed from the plant, the terpene concentration of the removed needles decreases with time (Isidorov et al. 2003).

We conducted 3 experiments to examine the role of caching in terpene manipulation. We 1st conducted a field survey to compare terpene levels in juniper stored by *N. albigula* versus juniper freshly collected from the environment. We then executed 2 controlled laboratory experiments to examine the relationship between terpene content and caching behavior. The 1st was a laboratory feeding trial that included all 3 woodrat species and was designed to document cache use and to determine whether or not woodrats would increase consumption from the cache when faced with a diet containing alpha-pinene. The 2nd study included only *N. albigula* and explored the interaction between caching activity, cache size, and terpene content.

MATERIALS AND METHODS

Experiment 1: alpha-pinene levels of natural caches.—In February 2006, we partially and temporarily dismantled 20 woodrat middens in Castle Valley, Utah (38°30'N, 109°18'W). Middens were selected to be similar in size (approximately 1–1.5 m³) and all of the middens selected were associated with a juniper tree. All middens had fresh woodrat feces and fresh cactus pads visible from the exterior, which are signs that a woodrat currently resides within the midden. The middens were assumed to belong to individuals from the species *N. albigula* because that is the only species that we have trapped on several trapping surveys of this site. The exterior layers were removed from 1 side of the midden until all interior chambers were visible and samples of the caches could be removed from the midden. All visible juniper was removed from caching chambers and placed into sealed plastic bags. Fresh samples for comparison were collected from 3 trees within 30 m of the midden, which is within the foraging radius of woodrats (Finley 1990). We also collected a sample from the tree(s) that provided structural support for the midden. All samples were immediately stored on dry ice for transportation to the University of Utah, where they were kept in a –20°C freezer until analysis. In the laboratory, samples were ground by mortar and pestle while submerged in liquid nitrogen. Alpha-pinene was extracted from samples in hexanes and quantified by gas chromatography, using a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Palo Alto, California) based on the methods as described in Sorenson et al. (2004).

Experiment 2: species comparison of the effects of alpha-pinene on caching behavior.—This study was conducted on all 3 *Neotoma* species. *N. albigula* was collected from Castle Valley, Utah (38°30'N, 109°18'W); *N. lepida* was collected from White Rocks, Utah (40°28'N, 109°55'W), and *N. bryanti*

was collected from Casper's Wilderness Area, California (33°31'N, 117°33'W). All animals were considered adults as indicated by body mass (>100 g at the time of trapping) and were maintained in quarantine until they tested negative for Sin Nombre virus using an enzyme-linked immunosorbent assay as described in Dearing et al. (1998). Animals were maintained before testing on high-fiber rabbit chow pellets (Teklad 2031, Teklad, Indianapolis, Indiana) and water ad libitum. Animals were acclimated to a 12L:12D cycle. At the conclusion of these studies all animals were maintained in captivity. All experimental protocols followed guidelines approved by the American Society of Mammalogists (Gannon et al. 2007) and were approved by the University of Utah Institutional Animal Care and Health Committee (protocol 07-02015).

Diet preparation.—High-fiber rabbit chow pellets (1.27 cm diameter; Harland Teklad TD.05191, Teklad) were uniquely dyed to permit dating of cached food. Food coloring (Sur la Table, Seattle, Washington) was added to pellets by mixing the color with 10 ml of water and rolling 10 pellets in a tray containing the colored liquid. Untreated pellets were approximately 3 g each and the average absorption of water using this method was 0.32 g ± 0.08 SD of water per gram of pellet.

We added alpha-pinene to the subset of pellets termed "terpene pellets" hereafter. To add the alpha-pinene, 0.5 ml of alpha-pinene and 10 ml of water were sonicated to form an emulsion and applied to the pellets in the same manner described to dye the nontoxic pellets. All pellets were stored in plastic bags and frozen before presentation. A subset of pellets from each day was ground and tested for alpha-pinene content, using the method described by Sorenson et al. (2004). At the time of presentation, the average alpha-pinene content was 3 mg ± 0.3 SD per gram of pellet. This dose is ingestible and safe for generalist species of *Neotoma* (Sorenson 2003). Pellets prepared with this method lost terpenes quickly and alpha-pinene was undetectable after 24 h using the gas chromatography analysis described earlier. Thus, all pellets remaining in the cage after 24 h, that is, cached food, were considered terpene-free.

Diet presentation.—During testing, animals were housed individually in cages specially designed to promote hoarding (Wood and Bartness 1996). A standard wire-bottom rat cage (24 × 20 × 29 cm) was connected by 8 cm of polyvinyl chloride tubing to a standard mouse cage (20 × 31 × 13 cm). The rat cage acted as a "foraging cage" and contained water ad libitum. The mouse cage acted as a "caching cage" and contained bedding and a paper towel for nest building. At the start of the dark period, approximately 35 g of food was presented in a food cup in the foraging cage for 12 h; we will refer to this as the "presented" diet. To encourage caching behavior presentation time was restricted (Bartness and Clein 1994). At the start of the light period, pellets remaining in the food bowl and in or under the foraging cage were collected, weighed, and classified as "abandoned." Twenty-four hours after food presentation, food in the caching cage was removed, weighed, and classified as "cached." All cached food was returned to the animal's cache. The grams of abandoned and cached pellets were measured directly. The amount of food

eaten from the cache was calculated each day by comparing the present contents of the cache to the cache contents on the previous day. Total intake was estimated by adding the number of pellets eaten from the presented diets (calculated by subtracting abandoned pellets and cached pellets from the total presented) and adding the amount of food eaten from the cache. Because terpenes were only measurable in the presented diets, terpene-pellet intake is equivalent to the intake of presented diet. To explore the animals' ability to limit terpene intake, we compared each animal's consumption of terpene pellets to an intake predicted from its behavior on a nontoxic diet. Predicted intake is equivalent to each animal's consumption of presented diet while on the terpene-free pellets, therefore representing the number of terpene pellets we could expect the animals to consume if they do not alter foraging and caching behavior. Dry weights were calculated for a subset of each day's pellets at the time of presentation and 24 h later. All measures were corrected for water loss.

Caching trial.—During a pretrial period, animals were given all possible colors, presented at random, over 3 days to verify that animals would not preferentially abandon or cache pellets with respect to color. None of the 3 species of woodrats in this study had color preferences (*N. albigula*: number of pellets cached by color, $F = 1.4$, $df. = 8, 4$, $P = 0.2$, number of pellets abandoned by color, $F = 0.7$, $df. = 8, 4$, $P = 0.69$; *N. lepida*: number of pellets cached by color, $F = 0.67$, $df. = 8, 4$, $P = 0.72$, number of pellets abandoned by color, $F = 1.03$, $df. = 8, 4$, $P = 0.42$; *N. bryanti*: number of pellets cached by color, $F = 0.96$, $df. = 8, 4$, $P = 0.5$, number of pellets abandoned by color, $F = 1.4$, $df. = 8, 4$, $P = 0.25$).

All animals were given terpene-free pellets for 3 days followed by terpene-treated pellets for 5 days ($n = 12$ individuals/species). Differences between types of pellets cached in a 12-h presentation period were compared using an analysis of covariance with total cache size as a covariant. Because we were interested in when animals chose to eat from the cache, only animals that cached an average of 10% or more of the presented diet were included in the analysis (*N. albigula*, $n = 11$ [7 females and 4 males]; *N. lepida*, $n = 9$ [5 females and 4 males]; *N. bryanti*, $n = 5$ [1 female and 4 males]). Animals caching <10% per day were considered noncachers and as such were severely limited in their ability to use their cache.

To determine whether woodrats preferentially used the oldest food in their cache, we analyzed the final night of the experiment using Ivlev's electivity index as presented in Kam et al. (1997). Each pellet was placed in an age class based on day of presentation (1–8 nights old). We calculated the proportion of the total cache that was composed of each age class (cache proportion). We also calculated the proportion of food consumed from the cache that represented each age class (intake proportion). We estimated the index as $(\text{intake proportion} - \text{cache proportion}) / (\text{intake proportion} + \text{cache proportion})$. This index allows us to identify preference while compensating for differences in availability. Ivlev's index produces a positive number if the food item is preferred and a

negative number if the food item is avoided. We calculated this index for each animal and compared each age class's electivity indexes by analysis of variance (ANOVA).

Experiment 3: the interaction of cache size and terpene treatment in *N. albigula*.—This experiment was conducted on the same *N. albigula* used in experiment 2; animals were maintained and housed in the same manner as previously described. On the 1st day of testing, 25 g of terpene-free pellets, dyed black, were placed in the caching cage of each animal. To test whether a large cache influenced the amount of food abandoned per day, animals were divided into 2 treatment groups ($n = 6$ per group). The 1st group was presented with 35 g of terpene pellets for the first 2 nights. The following 2 nights, animals were presented with terpene-free pellets. The 2nd group received the same treatments in the reverse order; that is, terpene-free pellets followed by terpene pellets. This order allowed us to examine caching behavior independent of cache size. In this trial, diets were removed during the dark cycle after 8 h of presentation. Data were analyzed by repeated-measure ANOVA with order of diet presentation as an independent factor. All animals were included in the analysis, because all animals were given a substantial cache at the start of the experiment and were therefore capable of using it at any time during the experiment ($n = 12$; 7 females and 5 males).

RESULTS

Experiment 1: alpha-pinene levels of natural caches.—Midden interiors were highly structured. Of the 20 middens dismantled, 12 had obvious and segregated nest chambers, and 18 had segregated food storage chambers. Seven also had 2 or more food chambers that were distinct from other areas of the midden, and 7 contained a chamber that contained cactus needles and feces almost exclusively. No juniper from the midden interiors had detectable alpha-pinene (i.e., >0.25 mg/g of sample), whereas juniper from the surrounding trees contained a modest amount of alpha-pinene, averaging $1.6 \text{ mg} \pm 0.5 \text{ SD}$ per gram (dry weight) of sample.

Experiment 2: species comparison of the effects of toxic diets on caching behavior.—Cache sizes did not differ among the 3 woodrat species on the final day of the experiment ($F = 3.04$, $df. = 2, 17$, $P = 0.07$), but the individual variation in cache size was large and there was a trend for *N. lepida* to have smaller caches than the other species (mean \pm SD, *N. albigula*: 48.4 ± 39.8 g, *N. lepida*: 25.5 ± 16.5 g, *N. bryanti*: 73.3 ± 32.4 g).

Neotoma albigula showed no change in total intake when given terpene or terpene-free pellets ($F = 2.13$, $df. = 1, 88$, $P = 0.15$; Fig. 1A). However, these animals did increase the amount of food consumed from the cache when given terpene pellets ($F = 4.9$, $df. = 1, 77$, $P = 0.03$; Fig. 1B). The increase was independent of cache size (covariate $F = 0.02$, $df. = 1, 77$, $P = 0.89$). The increased use of the cache resulted in consumption of fewer terpene pellets (presented pellets) than had been observed on a terpene-free diet; therefore, animals consumed less terpene than predicted ($F = 15.5$, $df. = 1, 88$,

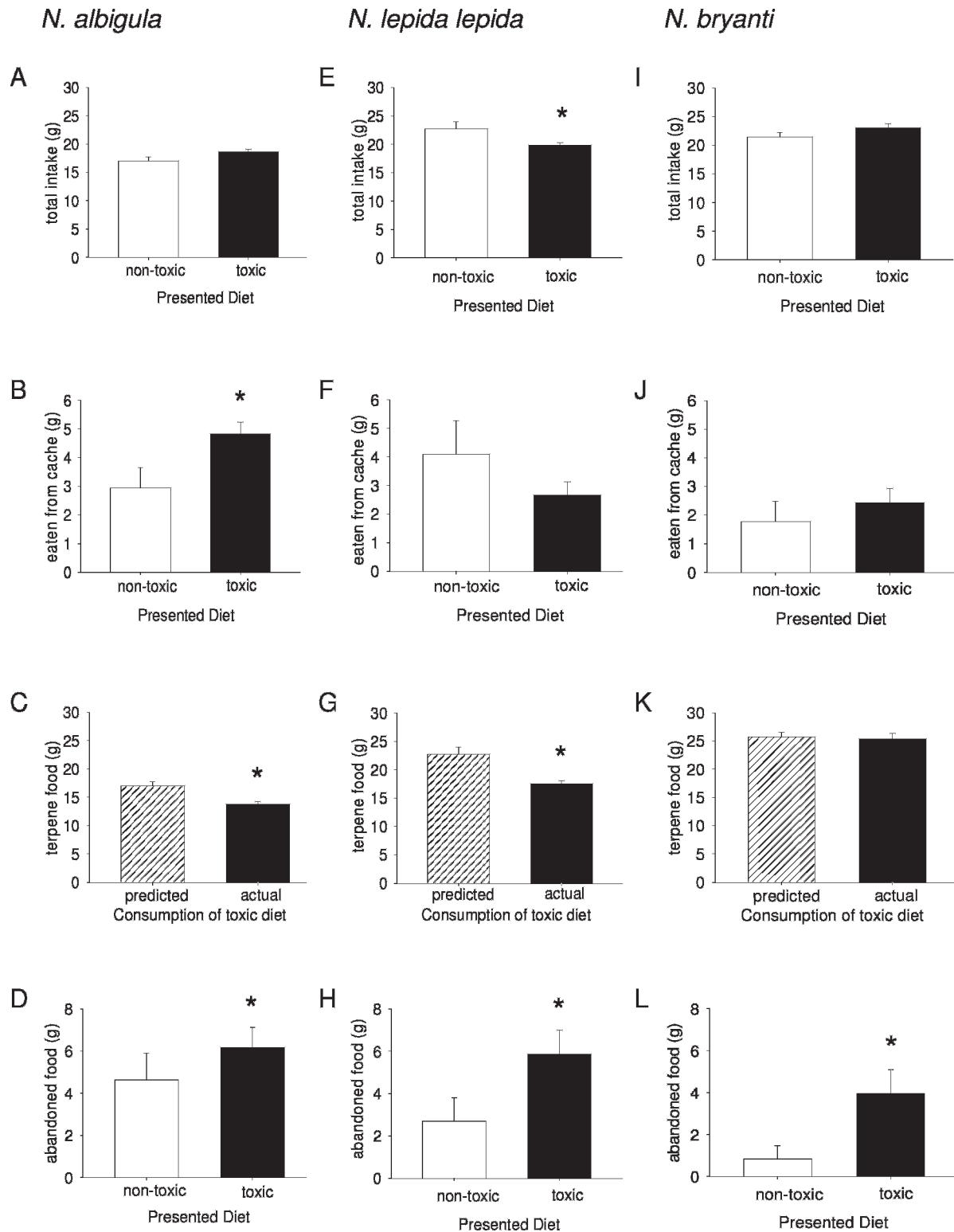


FIG. 1.—Columns represent species, *Neotoma albigula* (panels A–D), *N. lepida lepida* (panels E–H), and *N. bryanti* (panels I–L), respectively. Rows represent 24-h intake of the groups on each diet (panels A, E, and I), the amount in grams eaten from the cache on each diet (panels B, F, and J), the comparison between predicted and actual terpene intake (panels C, G, and K), and the amount of food abandoned on each diet (panels D, H, and L), as read from top to bottom. Terpene-free diet (open bars) is compared to terpene-containing diet (black bars). Predicted intake of terpenes, which is equivalent to presented intake on terpene-free diets (striped bars), is compared to the actual intake of terpene pellets (black bars). * $P < 0.05$.

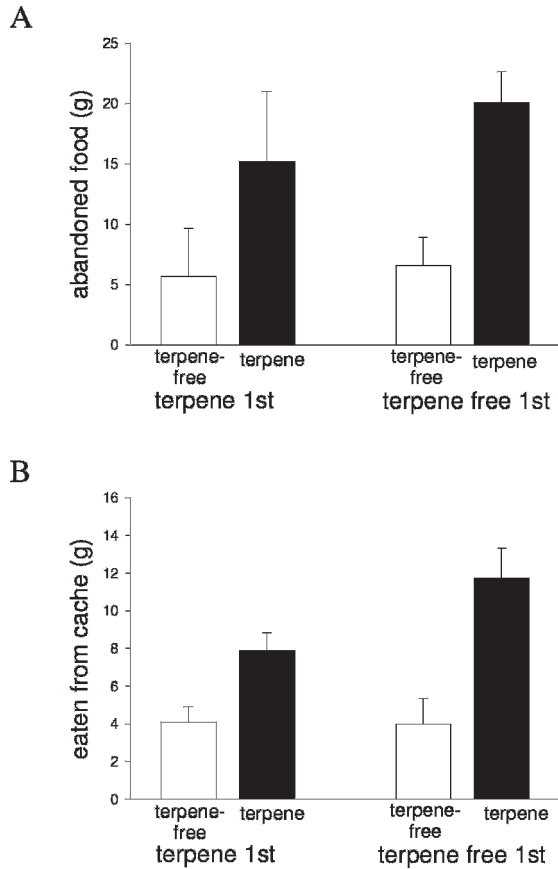


FIG. 2.—A) The amount (in g) of food abandoned when presented with terpene-free (open bars) or terpene-treated (black bars) pellets. The “terpene 1st” bars represent *Neotoma albigula* that were given terpene-treated pellets for the first 2 days of the study and terpene-free pellets for the subsequent 2 days. “Terpene-free 1st” represents the group receiving these same treatments in the opposite order. B) The amount of food (in g) eaten from the cache during the presentation of terpene-free or terpene-treated food. Labeling is identical to panel A.

$P < 0.01$; Fig. 1C). However, there was no significant selectivity based on the age of food in the cache ($F = 0.74$, $d.f. = 7$, 80 , $P = 0.63$). Animals foraged on foods in the cache based on availability. Furthermore, woodrats abandoned more food when offered terpene pellets ($F = 8.4$, $d.f. = 1$, 87 , $P = 0.005$; Fig. 1D). Cache size was a significant covariate; as cache size increased, food was more likely to be abandoned (covariate $F = 30.9$, $d.f. = 1$, 87 , $P < 0.01$).

Neotoma lepida decreased total intake when given terpene pellets ($F = 15.2$, $d.f. = 1$, 43 , $P < 0.01$; Fig. 1E), but did not adjust cache use ($F = 2.4$, $d.f. = 1$, 37 , $P = 0.13$; Fig. 1F); cache size was not a significant covariate ($F = 0.53$, $d.f. = 1$, 37 , $P = 0.47$). *N. lepida* appeared to prefer cached items >5 days old, although this trend was not significant ($F = 2.03$, $d.f. = 7$, 64 , $P = 0.064$). Animals decreased intake by consuming fewer presented pellets when the pellets contained terpene compared to when the presented diet was terpene free, which led to a lower than predicted total intake of terpenes ($F = 20.48$, $d.f. = 1$, 52 , $P < 0.01$; Fig. 1G). Food was more likely

to be abandoned if it contained terpenes and if the cache was large ($F = 12.3$, $d.f. = 1$, 42 , $P = 0.001$, covariate $F = 11.1$, $d.f. = 1$, 42 , $P < 0.01$; Fig. 1H).

Neotoma bryanti did not adjust total intake based on type of pellets presented ($F = 0.1$, $d.f. = 1$, 38 , $P = 0.76$; Fig. 1I) nor did these animals change how many pellets they ate from the cache ($F = 2.0$, $d.f. = 1$, 32 , $P = 0.17$, covariate $F = 2.5$, $d.f. = 1$, 32 , $P = 0.13$; Fig. 1J). Furthermore, *N. bryanti* did not show selectivity based on age of food in the cache ($F = 0.66$, $d.f. = 7$, 32 , $P = 0.70$) nor did these animals decrease intake of terpene-free pellets compared to the predicted intake ($F = 0.074$, $d.f. = 2$, 17 , $P = 0.79$; Fig. 1K). However, they did abandon more food (i.e., cache less) when given terpene pellets than when presented with terpene-free pellets ($F = 13.3$, $d.f. = 1$, 37 , $P = 0.01$, cache covariate $F = 9.7$, $d.f. = 1$, 37 , $P = 0.01$; Fig. 1L).

Experiment 3: the interaction of cache size and toxin treatment in N. albigula.—The type of pellet (terpene versus terpene-free) presented significantly affected all 3 measures: caching, consumption from cache, and abandonment. However, order of presentation did not significantly affect any measure. When given terpene pellets, animals cached less (diet $F = 4.74$, $d.f. = 1$, 20 , $P = 0.04$, order $F = 0.045$, $d.f. = 1$, 20 , $P = 0.83$, interaction $F = 0.81$, $d.f. = 1$, 20 , $P = 0.378$), abandoned more ($F = 7.42$, $d.f. = 1$, 20 , $P = 0.013$, order $F = 0.02$, $d.f. = 1$, 20 , $P = 0.9$, interaction $F = 0.67$, $d.f. = 1$, 20 , $P = 0.42$; Fig. 2A), and ate more from their cache ($F = 21.90$, $d.f. = 1$, 20 , $P < 0.001$, order $F = 2.26$, $d.f. = 1$, 20 , $P = 0.15$, interaction $F = 2.33$, $d.f. = 1$, 20 , $P = 0.13$; Fig. 2B).

DISCUSSION

Storage of food for future use is a complex behavior used by a wide variety of animals. We investigated 1 of the aspects of this behavior, the manipulation of toxins through storage. Because the 3 species used here have the same general ecology, in that they are herbivorous animals that store plant material for future use, we predicted that they would behave similarly with respect to the caching of toxic foods and use of the cache after toxins volatilized. Specifically, we predicted that all 3 species would reduce the level of toxins in food by storing the food before consumption to allow for toxin volatilization. Surprisingly, we observed 3 entirely different strategies. Only the caching behavior of *N. albigula* fit our prediction. Examination of these data suggests that *N. albigula* detected terpenes in the diet and used storage as a method of decreasing terpene intake. However, neither *N. lepida* nor *N. bryanti* used caching as a detoxification mechanism. *N. lepida* readily cached 10% of the food presented but was conservative in cache use in that these animals did not increase cache use to compensate for the decrease in intake of consumed food when offered terpene pellets. In contrast, *N. bryanti* was not a particularly avid cacher and those that did cache rarely used stored food. It is possible the observed differences in caching behavior are driven by disparate environmental factors in their habitats.

Three lines of evidence support the hypothesis that *N. albigula* manipulates the level of toxin in its diet through the storage of food. The 1st line of evidence is anecdotal; the highly organized nature of middens of *N. albigula* suggests that these woodrats are capable of complex behaviors, which may include planning for future events. For example, in many of the middens, a large amount of chamber space was dedicated to feces and cactus needles. Because much of the interiors were defended by cactus needles embedded into the walls, we speculate that that needles and fecal material could be stored for future fortification. Second, juniper collected from midden interiors had no detectable alpha-pinene. This finding is consistent with the prediction that animals store food until it is no longer toxic. Third, we found that *N. albigula* increased consumption from the cache when faced with a toxic diet. In short, *N. albigula* regularly added food to and subtracted food from its cache. This finding is consistent with the hypothesis that *N. albigula* could use its cache as a nontoxic resource. In addition, this pattern of cache use seems robust because alterations of cache size and foraging time in our 3rd experiment did not change the patterns we saw in caching behavior or cache use (Fig. 2).

Our 2nd prediction was that the value of a cached food will change with time (Gendron and Reichman 1995) and that animals would choose the oldest food in the cache to maximize terpene loss. We found no evidence that *N. albigula* was selective when feeding from the cache. There are 2 likely reasons for the lack of preference when feeding from the cache. The 1st is that caches were removed but replaced entirely daily for measurements. This disruption may have altered some cue to the age of the food item. Furthermore, terpenes were not detectable in the cache and therefore food items in the cache may not be changing in value. Therefore, it is unsurprising that animals removed food from the cache at random.

Neotoma lepida detected terpenes in the diet but in contrast to *N. albigula*, it did not regulate terpene intake through storage and instead responded by decreasing total intake. This difference is surprising because *N. lepida* has experience with juniper (Cameron 1971; Stones and Hayward 1968) and laboratory studies demonstrate that *N. lepida* is physiologically capable of feeding on juniper (Mangione et al. 2000). Furthermore, both species exhibit similar caching behaviors; however, caches of *N. lepida* tended to be smaller than those of *N. albigula* at the conclusion of this experiment. We believe this difference in part an artifact of *N. lepida* having a slightly larger total intake per day than *N. albigula*. The major difference between the 2 species is that *N. lepida* was less willing to consume food from its cache.

There have been several demonstrations of animals adjusting caching behavior in response to varying environmental factors, including community structure (Muñoz and Bonal 2007), foraging distance (Post and Reichman 1991), and perishability (Jansen et al. 2006). This plasticity in caching behavior may allow animals, both within and between species, to deal with varying conditions. For example, eastern gray

squirrels (*Sciurus carolinensis*) eat rather than cache species of acorns that germinate immediately regardless of their levels of tannins (Smallwood et al. 2001). Germination during storage is akin to spoiling. To squirrels, food scarcity may be a larger constraint than the antinutritional effects of tannins and therefore they adjust their behavior to maximize food intake over minimizing tannin intake. *N. lepida* may be making a similar decision, to ingest a toxic food rather than risk food scarcity by depleting the cache.

Winter is a difficult time for many small mammals, and a large cache maybe a priority for *N. lepida*. Post et al. (2006) reported that 2 species of *Neotoma*, *N. floridana* and *N. micropus*, rely heavily on caches during winter. Although these species decrease in body mass between autumn and winter as food abundance wanes, they increase in body mass between winter and spring, suggesting a reduction in energy expenditure (foraging) and an increase in consumption of food from the cache. Examination of data from the Western Regional Climate Center (www.wrcc.dri.edu) indicates that *N. lepida* encounters temperatures below freezing for longer periods of time than *N. albigula*; the annual minimum temperature is 10°F lower for *N. lepida*. Long, cold winters may require a reliable and adequate long-term food store, and this could be reflected in the reluctance of *N. lepida* to remove food from its cache during the summerlike temperatures it was exposed to during the experiments. Furthermore, it is notable that although *N. lepida* did not increase cache use when presented a terpene-treated diet, there was a trend for *N. lepida* to prefer cached items >5 days old. This trend is difficult to interpret because of investigator effects and the fact that it is unlikely that the caches were changing in nutritional value. However, this trend may imply a cache management strategy aimed at minimizing spoilage (Gendron and Reichman 1995). If this is the case, it would be consistent with our postulate that *N. lepida* is attempting to maximize the future value of their cache for winter use.

The strategy of *N. lepida* of regulating toxin intake by decreasing total intake may be typical for a generalist herbivore. In nature, when an herbivore encounters a toxic food that necessitates such a reduction in consumption, it may incorporate other plants into its diet (Freeland and Jansen 1974). Freeland and Jansen (1974) argued that diet mixing is a strategy for generalist herbivores to avoid toxicosis from any 1 plant. In short, animals are predicted to alternate the ingestion of secondary compounds from various plants to avoid overloading any 1 set of biotransformation pathways in the liver. They postulated that there are many biotransformation pathways and each has a threshold for particular compounds; if this limit is surpassed the herbivore could experience negative consequences. However, by switching the plants it ingests, and therefore compounds, a different biotransformation pathway will be used, and the herbivore will avoid toxicosis (Freeland and Jansen 1974). There are experimental examples of this strategy, such as brushtailed possums (*Trichosurus vulpecula*), which eat more in a day when offered foods processed by different biotransformation

pathways (Dearing and Cork 1999; Marsh et al. 2006). We suggest that because the *N. lepida* in this trial was unable to choose a new food type, its only appropriate response was to limit food intake.

Neotoma bryanti exhibited a 3rd behavioral strategy. Although these animals were similar to *N. lepida* in limited cache use, they did not decrease intake of the terpene pellets and were poor cachers in the laboratory compared to the other woodrats in this study. The individual *N. bryanti* that chose to cache stored significant quantities of presented food (32%). However, in the initial screen less than one-half of the individuals cached at least 10% of the food offered (5 of 12). Those that did cache rarely consumed food stored in caches (Fig. 1J) and showed no preference for older foods.

The population of *N. bryanti* used in this study was not trapped in a desert habitat like *N. lepida* and *N. albigula*, but from a more mesic area with higher humidity (Macêdo and Mares 1988; Patton et al. 2008). Under these conditions, long-term storage could result in fungal or bacterial spoiling, hence caching may not be a suitable strategy for these woodrats. There is evidence that cachers attempt to maximize storage time. *N. floridana* selects dry foods over fresh foods in trials that vary in competition and foraging distance (Post and Reichman 1991). Mountain beavers (*Aplodontia rufa*) air-dry vegetation to reduce spoilage (Karban et al. 2007) and fox squirrels (*Sciurus niger*) will spend more energy foraging for nuts with greater storage value (Kotler et al. 1999). It is not surprising that animals show a caching preference for less-perishable foods because spoilage represents lost energy. Perhaps *N. bryanti* has restricted caching to avoid spoilage. However, because there was considerable variation in the caching behavior of this species and because so few were willing to cache in the laboratory, we are limited in our ability to interpret the behavior of this species.

Last, *N. bryanti* did not regulate terpene intake via a reduction in intake. The lack of regulation may be because in nature these animals feed on *Salvia*, which contains alpha-pinene as well as other terpenes (Emboden and Lewis 1967). Thus, this woodrat may have the ability to detoxify higher concentrations of terpenes than those used in the experiments. It is possible that either or both *N. lepida* and *N. bryanti* would exhibit behaviors similar to *N. albigula* if given a diet higher in terpenes.

In summary, caching behavior is immensely complex. The rationale that animals use to decide what to cache and what to consume is not well understood. Only recently have investigators begun to address the physiological controls of food storage (Day and Bartness 2004; Keen-Rhinehart and Bartness 2005) and the roles of environmental constraints are still being explored (Post and Reichman 1991). In the data presented here, all 3 closely related species experienced the same controlled laboratory environment yet we have described 3 very different caching strategies. We suggest that animals modify their caching behavior in response to natural or perceived environmental constraints. The environmental history may dictate whether animals should follow long- or

short-term storage strategies. Furthermore, we have shown that toxin manipulation through caching is not restricted to the pika (Dearing 1997b, 1997c); however, our results suggest that manipulation of toxins may be limited to particular ecological circumstances. First, because not all plant toxins are reduced by storage (Wood 2005), this strategy will be restricted to plants that show toxin degradation over time. Second, we propose that toxin manipulation through food storage may be limited to scenarios where toxins are more critical to the animals' fitness than food availability. The diversity of species of woodrats as well as habitats in which they occur will be useful in future investigations to test these hypotheses through inter- and intraspecific comparisons.

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