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INTERPOPULATION DIFFERENCES IN TOLERANCE TO CREOSOTE BUSH RESIN IN DESERT WOODRATS (*NEOTOMA LEPIDA*)

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Abstract. Plant secondary metabolites (PSMs) can affect survival, reproduction, and distribution of herbivores. Individuals with a high capacity to tolerate PSMs will experience fewer and smaller adverse effects than less tolerant individuals. Theoretically, the capacity to tolerate PSMs can be acquired during development, modulated during adulthood, or genetically fixed. We studied tolerance to phenolic resin from creosote bush (*Larrea tridentata*) in two populations of desert woodrats as a first step in understanding phenotypic habituation and genetic adaptation of this species to creosote resin. One population was from Mojave desert habitat where woodrats eat creosote bush, and the other from the Great Basin desert, where creosote bush is not present and woodrats consume mainly juniper (*Juniperus osteosperma*). For >1 mo in the laboratory, woodrats from both populations were fed rabbit chow with increasing amounts of phenolic resin extracted from creosote bush until they lost body mass or showed any sign of sickness. Woodrats from the Mojave population maintained body mass at higher concentrations of resin and remained in the experiment longer. There were no differences between populations in food intake across all resin levels; however, maximum resin intake was 25% higher for the Mojave population. Food intake decreased with increasing resin intake. Glucuronic acid excretion in urine, one indicator of detoxification capacity, did not differ between populations. Water consumption increased with increased levels of creosote resin in the diet in woodrats from both populations. The results are consistent with the idea of differential tolerance to creosote bush phenolic resin in desert woodrat populations. Woodrats appear to be a promising natural system to study the developmental or genetic factors underlying vertebrate adaptation to plant secondary metabolites.

Key words: *creosote bush; desert woodrats; detoxification; glucuronic acid; Great Basin; Mojave Desert;* Larrea tridentata*;* Neotoma lepida*; phenolic resin; plant secondary metabolites; tolerance.*

INTRODUCTION

The ability to cope with or tolerate PSMs (plant secondary metabolites) has profound implications for animal population ecology by influencing diet selection (Freeland and Janzen 1974, Belovsky and Schmitz 1991), mortality, and hence population dynamics (Haukioja 1980, Bryant et al. 1991). Tolerance to a PSM can be measured as the highest intake of the compound (as mass per unit time or as percentage of diet) that permits survival, ability to maintain body mass, or normal growth. In insects tolerance to PSMs differs between populations within species (Fox and Morrow 1981, Futuyma and Peterson 1985, Ayres and Scriber 1994). Intraspecific tolerance to secondary compounds has not been investigated in mammals, and the desert woodrat (*Neotoma lepida*) is a good species to study potential intraspecific differences in tolerance to PSMs. Woodrats of this species are widely distributed, in all North American deserts except the Chihuahuan. *Neotoma lepida* occurs in many habitat types, i.e., creosote bush, pinyon–juniper woodland, chaparral, oak woodland, and coastal scrub (Cameron and Rainey 1972). Populations in these habitats have diets dominated by different plant species with different secondary metabolite composition. *Opuntia* spp. are rich in oxalates (Shirley and Schmidt-Nielsen 1967, Justice 1985), *Juniper* spp. are rich in terpenes (Schwartz et al. 1980, Adams et al. 1981), and *Larrea tridentata* (creosote bush) is rich in phenolics. Leaves of creosote bush contain 10–25% dry mass of a phenolic resin. The resin is composed of 40% dry mass of NDGA (nordihydroguaiaretic acid) and the remainder of the resin is a complex mixture of partially *o*-methylated flavones and flavonols (Mabry et al. 1977, Rhoades and Cates 1976).

There are few species of animals that feed on creosote bush (Meyer and Karasov 1991). Creosote bush phenolic resin is known to deter feeding by arthropods and to complex with protein in vitro (Rhoades and Cates 1976). Laboratory rats fed NDGA at 0.5, 1, or 3% of food dry mass, produced cysts in the kidney and vacuolation of kidney tubular epithelium (Grice et al. 1968, Goodman et al. 1970). Animals that naturally feed on creosote bush are very selective regarding the age and part of the plant upon which they feed. For example, jackrabbits (*Lepus californicus*) and *N. lepida*

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prefer the mature over the young leaves presumably because phenolic resin levels are lower in the mature ones (Hayden 1966, Meyer and Karasov 1989, Ernest 1994).

We focused our study of differential tolerance to PSMs on two particular populations of desert woodrats: (1) individuals from three sites in the Mojave Desert (hereafter called Mojave woodrats) and (2) individuals from two sites in the Great Basin desert (hereafter called Great Basin woodrats). The composition of plant species in both habitats and consequently the diets of these two populations of desert woodrats differed greatly between the two habitats. Mojave woodrats eat creosote bush, whereas Great Basin woodrats rely mostly on juniper (*Juniperus osteosperma*) (A. M. Mangione, *personal observation*). We measured tolerance of woodrats as defined by maintenance of mass balance when ingesting creosote bush resin and/or the lack of any signs of adverse effects.

Because differences in tolerance might relate to differences in detoxification, we also measured the excretion of uronic acids (principally glucuronic acid), the most important conjugation metabolites in mammals (Sipes and Gandolfi 1991). We predicted that Mojave woodrats would excrete more glucuronic acid per unit dry mass of resin consumed.

Our hypothesis is that Mojave woodrats will show higher tolerance to creosote resin than Great Basin woodrats. We predict that Mojave woodrats will maintain body mass at higher levels of resin and ingest more resin than Great Basin woodrats.

Methods and Materials

Field site and sample collection

Woodrats from the Mojave Desert were trapped at three different sites near Beaver Dam in Grand County, Utah (37°06′ N, 113°58′ W). Woodrats from the Great Basin were trapped in two locations 70 km apart at Jericho, Juab County, Utah (39°57′ N, 112° 22′ W) and Dugway, Tooele County, Utah (40°19′ N, 112°57′ W).

The vegetation at Beaver Dam was primarily composed of creosote bush, black brush (*Coleogyne ramosissima*), Joshua tree (*Yucca brevifolia*), and desert almond (*Prunus fasciculata*) and the more scarce cholla (*Opuntia* sp.). At both Jericho and Dugway, Utah, sites juniper (*Juniperus osteosperma*) was the dominant tree and big sagebrush (*Artemisia tridentata*) was the dominant bush; there was no creosote bush present in these habitats.

Woodrats were captured between 10 April and 4 June 1996 using Tomahawk and Sherman live traps baited with peanut butter and oats. Cotton for bedding was placed in traps. Two to three traps were placed at woodrat middens that showed some degree of recent woodrat activity, i.e., fresh plant clippings or feces. To avoid trapping diurnal mammals, traps were opened at 1600 in the afternoon and checked immediately after sunrise. All woodrats were transported to the animal facility at the University of Utah and were kept in quarantine closets for 2–5 mo until they were all tested for Sin Nombre hantavirus (Dearing et al. 1998), and then were transported to the Department of Wildlife Ecology, University of Wisconsin, Madison, Wisconsin. To confirm that woodrats ate creosote bush in the wild, fecal pellets of woodrats from both populations were analyzed. Fecal samples were collected from traps and from the plastic bag in which the animals were weighed, and were processed following Williams (1969). The presence of creosote bush fragments in feces of 10 individuals from each population was checked, and fragments were found in all of those from the Mojave Desert but none from the Great Basin. Diet was quantified in five individuals from each population; thirty microscope fields were observed per animal and relative frequency was calculated as the number of fields in which a fragment of a particular plant was present divided by the total number of fields observed times one hundred. Creosote bush fragments were present in fecal pellets of 21.2 ± 3.9% of wild-caught Mojave woodrats and 0% of Great Basin woodrats.

Creosote bush for resin extraction was collected on 10 April 1996 between 1600 and 1800 at the Mojave site from a stand of creosote bush outside the trapping areas. A mixture of young and mature foliage was clipped with scissors from twigs ≤0.3 cm in diameter. The foliage was transported on dry ice to the University of Utah.

Animal housing and diet preparation

Woodrats were housed in metal cages (47 × 30 × 21 cm) with screened bottoms. The animals were provided with cotton bedding and a ceramic bowl in which to nest. The room was kept at 21°C and 65% relative humidity. When not involved in experiments, the woodrats were fed rabbit chow ad libitum (Harlan Teklad 8630, Madison, Wisconsin, USA): 17.5% crude protein, 22.02% acid detergent fiber (ADF), 2.5% fat, and 7.5% water. A slice of apple was given to each animal once a week.

Phenolic resin was extracted from creosote bush foliage by soaking the tissue in diethyl ether for 45 min (1:5, wet leaf mass:volume). The extract was filtered (Whatman no. 4 paper; Whatman Incorporated, Fairfield, New Jersey) and evaporated at 40°C for 2–4 h. Extraction of 10.1 kg wet mass of creosote bush yielded 685 g of dense, viscous resin, which was stored at −25°C for ≤7 mo. The resin-treated diets were prepared as follows: a known amount of resin (depending on the desired concentration of resin in the diet) was dissolved in a volume of 95% aqueous ethanol equal to 25% of the mass of the ground rabbit chow used in the treatment. Control diet was prepared in the same manner without the addition of resin. The ethanol was needed, otherwise the resin cannot be mixed with the chow. The resin/ethanol solution and the chow were

thoroughly mixed and then dried at room temperature in a fume hood overnight. Once the mix was dry (as confirmed gravimetrically), the powder was pelleted. Because both heat and water can alter the properties of phenolics in diets (Price et al. 1980) and alter diet palatability (Lindroth et al. 1984, Dietz et al. 1994) we blanketed the pelletizer with a plastic bag filled with crushed ice to minimize heating during pelleting. The amount of water used to make the pellets (both control and resin-treated diets) was minimized by adding 7–10% (volume/mass) of a 60% ethanol solution. The final pellets were dried at room temperature and stored in the freezer. Because the pellets contained little water, and we expelled most air from the bag prior to sealing, there was negligible condensation on the frozen pellets.

Feeding trials and experimental design

The experimental protocols were approved by the Research Animal Resources Center (RARC), University of Wisconsin, Madison, Wisconsin.

Nine woodrats from the Mojave Desert, six males and three females, and eight woodrats from the Great Basin, three males and five females (five from Dugway, Tooele County, Utah, and three from Jericho, Juab County, Utah) were exposed to increasing levels of resin in the chow (expressed as percentage of pellet dry mass). The animals were first offered rabbit chow with 0% resin and then switched sequentially to 1, 2, 3, 5, 7, and 9% of resin in the chow. During all treatments, fresh food and water were offered ad libitum daily.

The concentrations and sequence were selected to allow the animals to habituate to the resin and to evaluate differences between populations at both low and high concentrations of resin. The animals were exposed to each concentration for 6 d. Body mass was measured daily and animals were removed from the experiment if they lost 15% of their initial body mass or showed signs of sickness such as swaying or not responding to sound and tactile stimuli. Water was supplied in bottles and water intake was measured by weighing the bottles daily and correcting for spillage and evaporation. Food intake was calculated daily by subtracting the dried orts (uneaten food) from the amount of food offered every day. Daily values of resin intake were calculated as the product of the amount of dry matter ingested per day times the proportion of resin in the diet at that particular level. Maximum resin intake for each individual was calculated as the mean of the individual's two highest daily resin intakes, which always occurred on consecutive days.

Urine and feces were collected during days 2, 4, and 6 of each 6-d treatment. On these days woodrats were restricted to a portion of their cage (16 × 19 × 20 cm) with a funnel and feces deflector that allowed for separate collection of urine and feces. Pilot experiments showed that there were no differences in food intake when woodrats were in either section of the cage. Urine unpolluted by food or feces drained into an iced plastic vial to minimize bacterial growth and evaporation.

To account for one of the most important modes of detoxification in mammals, we measured uronic acid conjugates (principally glucuronic acid) in urine of woodrats from both populations. Urine samples from the last (sixth) day of treatments 0% and 2% resin in the diet were analyzed following Jakubas et al. (1993) and Blumenkrantz and Asboe-Hensen (1973).

Statistical analyses

Body mass, intake rates of dry matter and water on the last 2 d of each 6-d treatment, and resin intake were analyzed with two factors, population (Great Basin vs. Mojave) and site nested within populations (two sites from the Great Basin and three sites from Mojave desert), with repeated measures (Wilkinson 1997). We used the last two days of food and water intake because when there was variation within the treatment the woodrats were habituated by the last two days. Orthogonal contrasts (Wilkinson 1997) were used to isolate differences between the two populations at specific treatment levels. Production of glucuronic acid in the urine was compared between populations using nested analysis of covariance (ANCOVA) with resin intake as a covariate.

The period of time that woodrats from the two populations were able to remain in the experiment, which we considered a good indicator of the animals' capacity to cope with creosote bush resin, was compared using Kaplan-Meier survival analysis (Wilkinson 1997 [SYSTAT]). This test estimates survival probability, or in other words the chances that an animal will remain in the experiment longer than time t (t is the time at which the first animals are removed from the experiment). Thus, survival probability was calculated each time an animal was removed from the experiment. The survival probability at day 0 is 1 for all the animals and it is 0 the last day, when no animals remained in the experiment.

In all cases values are expressed as mean ± one standard error (n = number of woodrats). A value of $P < 0.05$ was considered significant, and $0.05 \leq P \leq 0.1$ was taken to indicate a trend.

Results

Body masses of woodrats at the beginning of the resin trial (initial body mass on day one of treatment 0% resin) were not significantly different between populations (120.8 ± 8.0 and 130.9 ± 9.0 for Mojave (n = 9 woodrats) and Great Basin (n = 8 woodrats), respectively; nested ANOVA $F_{1,12} = 2.26$, $P = 0.16$) or among sites ($F_{1,12} = 2.9$, $P = 0.14$). Body masses at the beginning of the experiment were not significantly different from those at time of capture ($F_{1,12} = 0.24$, $P = 0.6$).

The mean length of time the animals were able to remain in the experiment (Fig. 1) was significantly lon-

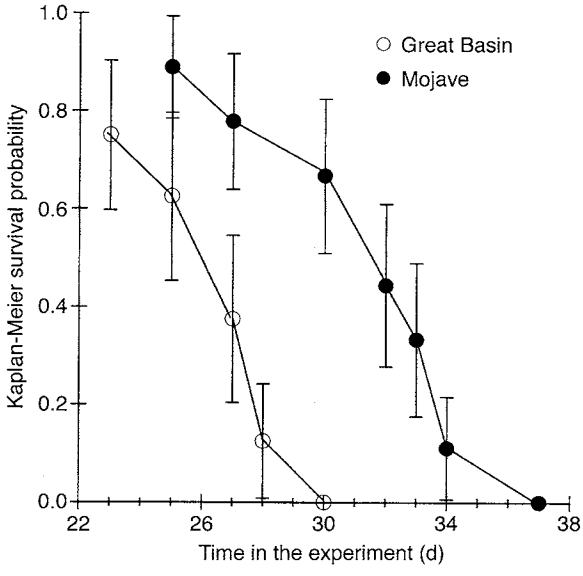

FIG. 1. Survivorship (i.e., retention) of woodrats (*Neotoma lepida*) in the resin-tolerance experiment. Woodrats were removed from the experiment when they lost 15% of their initial body mass or showed any sign of sickness. Values are mean probability ± 1 SE of survivorship (i.e., retention) in the experiment. Probability until day 22 was 100%; therefore, means from day 23 until day 37 were plotted.

ger for Mojave woodrats than for Great Basin woodrats ($\chi^2_1 = 7.88$, $P = 0.005$). By the end of 5% resin treatment, or day 30, Mojave wooodrats still had a 66.9% probability of remaining in the experiment, whereas the Great Basin woodrats had zero probability of remaining. Only one animal from Mojave population remained in the experiment until the first day of the 9% resin treatment.

Mojave woodrats maintained body mass at higher concentrations of resin in the diet. Over the treatments 0% to 3% resin the body mass of Great Basin woodrats, but not Mojave woodrats, decreased with increasing percentage of resin in the diet, as indicated by the significant interaction between population and time (Table 1, Fig. 2). Site within population was not significant (Table 1). Mojave woodrats started losing mass at 5% resin whereas Great Basin animals started losing body mass at 2% resin in the diet (Fig. 2). Great Basin woodrats lost body mass during treatment 2% (days 13 to 18; $F_{5,30} = 3.16$, $P = 0.0021$) and during treatment 3% (days 19 to 23; $F_{4,24} = 3.42$, $P = 0.0024$). This pattern was not observed in Mojave woodrats, which maintained constant body mass during the same periods (days 13 to 18; $F_{5,30} = 0.53$, $P = 0.99$, and days 19 to 23; $F_{4,24} = 0.48$, $P = 0.74$)

Over the treatments 0% to 3% resin in diet, woodrats reduced food intake with the increase in resin level (Fig. 3a), there were no significant differences between sites within populations, but there was a trend for a different response between the two populations as in-

TABLE 1. Nested analysis of variance in tolerance of desert woodrats (*Neotoma lepida*) from two populations to increasing levels of creosote bush (*Larrea tridentata*) resin in their diets.

Variable	*F*	df	*P*
Change in body mass (%)			
Population	6.94	1, 12	0.022*
Site(Population)	1.04	3, 12	0.41
Time	4.72	21, 252	0.001*
Time × Population	4.42	21, 252	0.001*
Time × Site(Population)	0.62	63, 252	0.99
Dry matter intake			
Population	0.88	1, 12	0.36
Site(Population)	1.31	3, 12	0.31
Treatment	10.68	3, 36	0.001*
Treatment × Population	2.365	3, 36	0.087
Treatment × Site(Population)	1.15	9, 36	0.352
Resin intake			
Population	2.54	1, 12	0.137
Site(Population)	0.74	3, 12	0.544
Treatment	204.63	2, 24	0.001*
Treatment × Population	3.64	2, 24	0.041*
Treatment × Site(Population)	0.58	6, 24	0.743
Maximum resin intake			
Population	9.54	1, 12	0.009*
Site(Population)	1.54	3, 12	0.25
Water intake			
Population	8.69	1, 11	0.013*
Site(Population)	0.52	3, 11	0.67
Treatment	8.27	3, 33	0.001*
Treatment × Population	1.91	3, 33	0.14
Treatment × Site(Population)	0.59	9, 33	0.79

Notes: The two factors in the ANOVA are "Population" and "Site" nested within Population. The factors "Time" and "Treatment" were analyzed as repeated measures. Interactions are represented with a × symbol.

* *P* values are significant at the 0.05 level.

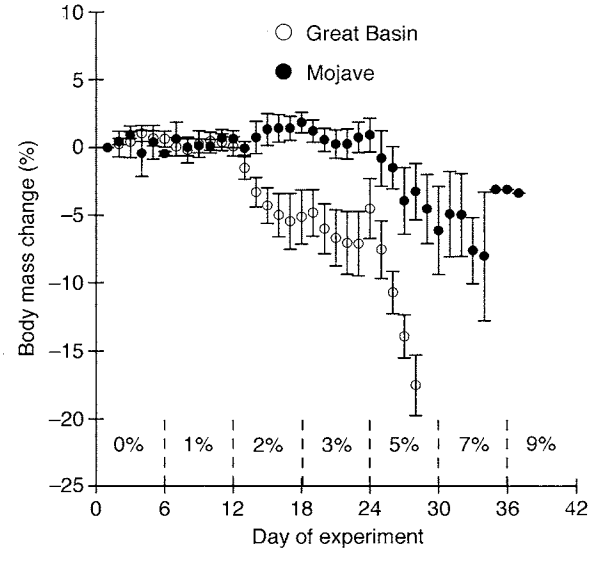

FIG. 2. Percentage body mass change (mean ± 1 SE) from initial body mass over time. The percentage of resin in the diet is shown between the dashed vertical lines on the *x*-axis of the graph.

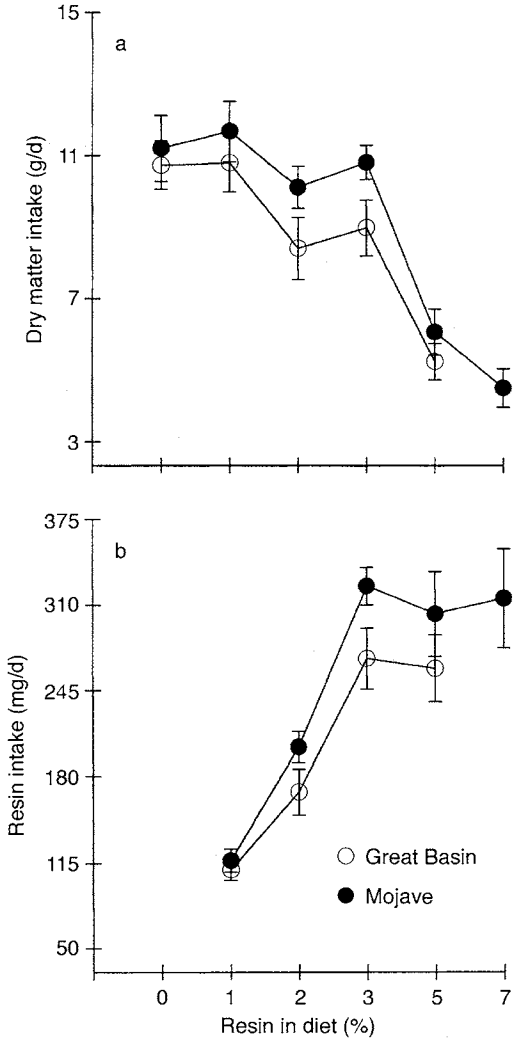

FIG. 3. Variation in (a) food intake and (b) resin intake with percentage of resin in the diet. Values are means ± 1 SE.

dicated by a nearly significant interaction (Table 1). When the populations were analyzed separately Great Basin woodrats showed a significant decline in food intake with the increase in resin level ($F_{3,18} = 15.4$, $P < 0.001$) whereas there was no significant decline for Mojave woodrats ($F_{3,18} = 1.68$, $P = 0.2$).

Resin intake increased between treatments 1% and 3% in both populations with no significant differences in resin intake between populations (Fig. 3b) or between sites within populations (Table 1), but with possibly a slight difference in pattern as indicated by a significant interaction between treatments and population (Table 1). Between treatments 3% and 5%, resin intake did not change (treatment $F_{1,10} = 1.11$, $P = 0.31$; population $F_{1,10} = 1.59$, $P = 0.23$; site {population} $F_{3,10} = 1.13$, $P = 0.38$), and dry matter intake significantly declined (treatment $F_{1,13} = 86.1$, $P < 0.001$; population $F_{1,13} = 2.48$, $P = 0.139$) (Fig. 3a, b).

The maximum resin intake of Mojave woodrats was 25.5% higher than that of Great Basin woodrats (Tables 1 and 2), with no differences in maximum resin intake between sites within populations. The difference occurred because all but one of the Great Basin woodrats reached their maximum resin intake at 3% resin treatment whereas 45% of Mojave woodrats reached their maximum resin intake at levels >3% (Table 2). The difference did not occur because of differences in food intake rate; the corresponding food intake rates did not differ significantly between populations ($F_{1,12} = 0.55$, $P = 0.47$) or between sites within populations ($F_{3,12} = 0.5$, $P = 0.7$, Table 2).

The amount of glucuronic acid in urine increased 13–16× from 0% to 2% resin (Mojave, 5.7 ± 1.1 to 93.8 ± 10.8 mg/d and Great Basin, 5.1 ± 1.0 to 67.1 ± 10.1 mg/d). Among animals eating 2% resin diet, resin intake rate had an effect on glucuronic acid excretion rate ($F_{1,10} = 7.4$, $P = 0.022$; Fig. 4). At this treatment level, there were no differences between populations ($F_{1,10} = 0.14$, $P = 0.710$) and there were no site effects ($F_{3,10} = 0.45$, $P = 0.72$) or interaction effect between population and resin intake ($F_{1,10} = 0.11$, $P = 0.74$) . The treatment 2% was selected because there were no differences in resin intake or food intake between populations at this resin level but changes in body mass differed between populations.

There was a significant difference in water intake due to increasing resin levels in the diet (Table 1, Fig. 5). Great Basin woodrats ingested more water per day than Mojave woodrats and water intake increased with the percentage of resin. The difference between populations was apparent throughout the experiment. Interpopulation differences in water intake were significant at 2% and 3% resin ($P < 0.05$; Fig. 5). Also, water intake of Great Basin woodrats increased between 0% and 3% resin ($F_{3,15} = 7.47$, $P = 0.003$; no site effect $F_{1,5} = 0.33$, $P = 0.59$) but this was not the case for Mojave woodrats, for which water intake remained constant during the same period ($F_{3,18} = 1.39$, $P = 0.27$; no site effect $F_{2,6} = 0.56$, $P = 0.59$). Mojave woodrats did not significantly increase water intake until the 5% treatment ($F_{4,16} = 6.28$, $P = 0.003$; no site effect $F_{2,4} = 1.16$, $P = 0.4$).

DISCUSSION

Tolerance to creosote bush resin

To our knowledge, this is the first experimental evidence for interpopulation differences in tolerance to a plant secondary metabolite in a mammalian species. Our results support the hypothesis that Mojave woodrats are more tolerant to creosote resin than Great Basin woodrats. Mojave woodrats maintained body mass until the last day of 3% resin in the diet (day 24) and started losing mass at 5% resin level (day 25). In contrast, on every diet treatment higher than 1% resin, Great Basin woodrats lost body mass (Fig. 2). Hence,

TABLE 2. Maximum resin intake rate of woodrats and the corresponding feeding rate of woodrats fed diets with resin content of 3% or more of food dry mass.

Woodrat number	Maximum resin intake (mg/d)	Food intake (g/d)	Diet resin concentration (%)†
Mojave population			
42	301.8	10.1	3
49	348.0	11.6	3
53	328.5	10.9	3
61	395.6	13.2	3
51	288.9	9.6	3
48	423.8	8.5	5
60	394.1	7.9	5
45	329.6	6.6	5
59	470.2	6.7	7
Mojave mean ± SE	364.5 ± 20.1	9.5 ± 0.7	
Great Basin population			
34	246.3	8.21	3
85	382.7	12.8	3
87	209.1	7.0	3
89	204.8	6.3	3
94	243.4	8.1	3
118	262.4	8.7	3
88	316.8	10.55	3
33	308.3	6.16	5
Great Basin mean ± SE	271.7 ± 21.3	8.5 ± 0.8	

† Diet resin level (percentage of food dry mass) at which maximum resin intake was reached.

Mojave woodrats were able to ingest three times more resin than Great Basin woodrats without losing body mass (324 ± 16 mg resin/d at 3% resin intake for Mojave woodrats vs. 109 ± 7 mg resin/d at 1% for Great Basin woodrats).

One of the seeming paradoxes of our study was that Great Basin woodrats were losing body mass during treatments 2% and 3% (Fig. 2) even though their feeding rates were not significantly lower (Fig. 3a and Table 1). Three possibilities are that their feeding rates were different but we lacked the power to detect this, that Great Basin woodrats digested less dietary energy at the 2% and 3% resin treatments, or that Great Basin woodrats had an increase in daily energy expenditure produced by an increment in cost of detoxification. We favor the first explanation because Great Basin woodrats, but not Mojave woodrats, decreased their feeding

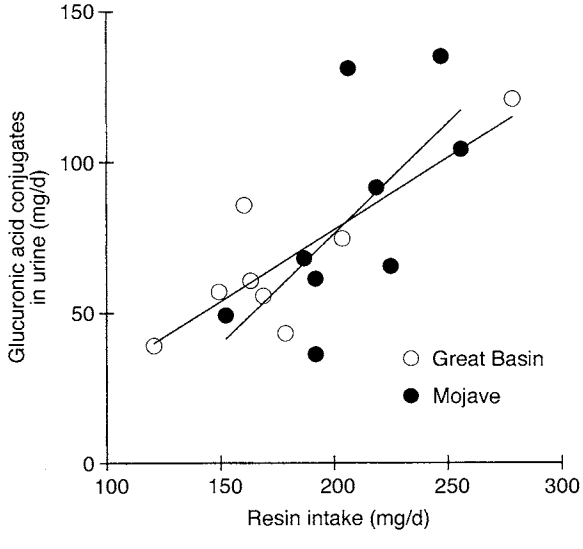

FIG. 4. Relationship between total glucuronic acid conjugates found in urine and resin intake for woodrats from both populations on the sixth day following a switch to a diet containing 2% resin.

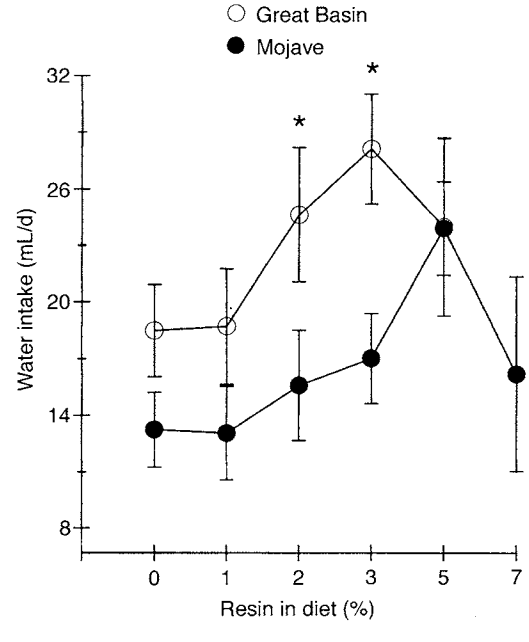

FIG. 5. Daily water intake (mean ± 1 SE) in response to percentage of resin in the diet. Asterisks indicate significant differences ($P < 0.05$) between populations (using contrasts between populations at each treatment).

rates during treatments 2% and 3% (Fig. 3a). We doubt the second explanation because Meyer and Karasov (1989) found no evidence that resin ingestion at any level inhibited digestive efficiency in Mojave woodrats, though they did not test other populations. The last explanation seems less likely because we observed no significant difference in glucuronic acid excretion between the populations (Fig. 4). While it is possible that higher detoxication costs associated with excretion of other unmeasured conjugates such as hippuric acid and sulfates (Caldwell 1980, Sipes and Gandolfi 1991) by Great Basin woodrats caused greater mass loss, the few studies so far in mammals indicate energy costs of detoxication are relatively low (Guglielmo et al. 1996).

What sets an individual's maximum tolerable intake of a PSM? One hypothesis is that the maximum tolerable intake of a PSM is related to taste aversion (Epple et al. 1996, Wang and Provenza 1997). Alternative explanations are that some animals can ingest more PSM because they absorb less, or that the maximum tolerable intake relates to the animal's capacity to biotransform and eliminate the PSM once it is absorbed (Freeland and Jansen 1974). All the explanations are plausible in the case of woodrat population differences in tolerance of resin. The resin is certainly aversive in choice tests with woodrats (Meyer and Karasov 1989), though in the absence of any alternative food the initial aversion can be overcome to creosote bush resin in particular (Meyer and Karasov 1991) and PSMs in general (e.g., Lawler et al. 1999). To our knowledge it is not known for any PSM whether or at what point a limit is reached in ability to habituate to the aversive nature of the PSM. We did not measure directly the absorption of PSMs from creosote bush resin in the two woodrat populations, but from the similar slopes and intercepts of the regressions of glucuronic acid production vs. resin intake (Fig. 4) we infer that the absorption is probably similar in the two populations. Finally, between-population differences in capacity to detoxify resin could exist by glucuronidation at higher resin intakes than we measured (Fig. 4) or by different detoxication pathways. We are currently assessing excretion of glucuronides and other detoxification products (i.e., hippuric acid and sulfate conjugates) at all resin levels.

Physiological responses to creosote resin

Our sampling of urine on day 6 of each resin treatment level (Fig. 4) seems adequate to measure detoxication metabolites. This is because the time for the enzymes involved in the detoxification processes to reach maximal induction in a number of species is 3–5 d (Sipes and Gandolfi 1991, Siess et al. 1996) and this was also the time for detoxification metabolites to appear at a maximum and steady state in the urine of common ringtail possum (*Pseudocheirus peregrinus*) fed *Eucalyptus radiata* (McLean et al. 1993). Woodrats on day 6 of treatment 2% had already spent 11 d in contact with resin. The variation we observed in glucuronic acid production among individuals was positively correlated with resin intake rate (Fig. 4), which probably varied among individuals partly because of differences in food intake rate related to body size differences.

Traditionally PSMs have been studied with regards to their effects on digestibility, absorption, and energy balance of herbivores (Thomas et al. 1988, Holechek et al. 1990, Robbins et al. 1991, Karasov et al. 1992). Only a few studies have considered possible impacts on water requirement or water balance (Mole et al. 1990, Jakubas et al. 1993). In our study, both populations of desert woodrats increased water intake with increasing levels of phenolic resin, though at different levels (Fig. 5, Table 1). The increase in water intake was not in compensation for a urine higher osmotic load generated by greater food ingestion, because food intake decreased across the experiment (Fig. 3a). But urine osmotic load might be increased if woodrats losing body mass catabolized body protein, which can result in higher levels of urea that have to be excreted in the urine (Giesecke et al. 1989). Alternatively, perhaps higher water intake was in compensation for diuresis caused by resin phenolics, because other phenols like flavonoids have diuretic effects in rats (Galati et al. 1996). An alternative explanation is that the increase in water intake relates to sensory properties of the resin. As in the case of other PSMs, creosote resin may cause irritation in the mouth, which is sensed by the trigeminal nerve, and water may alleviate this irritation (Silver 1987). Whatever the causal mechanism for higher water intake, for desert dwelling animals such as desert woodrats, water is a scarce and critical resource and creosote bush resin may compromise their water budget. Both direct and indirect effects of PSMs on water balance have important ecological and physiological implications that require further analysis.

Ecological and evolutionary implications

Great Basin woodrats had a lower tolerance to creosote bush resin than Mojave woodrats and tolerance did not improve over many weeks. Kaplan-Meier survival analysis indicated that Great Basin woodrats' chances of survival during this experiment were reduced early in time compared to Mojave woodrats' chances of survival. We wonder what would be the fate in the wild of Great Basin woodrat individuals in contact with a novel PSM such as creosote bush phenolic resin. Individuals that do not respond quickly enough to an encounter with a novel PSM, as a result of either competition or dispersal events, may have reduced chances of dispersal, migration, and survival. Desert woodrats occur in pinyon–juniper woodlands (Durrant 1952, Stones and Hayward 1968) that are distributed in the Great Basin and as islands surrounded by desert scrub vegetation and chaparral across the American southwest (Vaughan 1982, Lamolino et al. 1989). This

habitat constitutes a strong barrier for terrestrial mammals usually restricted to forests (Brown 1978, Lamolino et al. 1989). Perhaps differences in tolerance to PSMs should be considered another variable that might explain isolation and distribution of mammals and perhaps creosote bush resin has an effect on the distribution and dispersal of *Neotoma lepida* in the Mojave and Great Basin deserts.

Either developmental or evolutionary time of exposure to a PSM can play a key role in setting a trait, such as tolerance, in a population. In an example of developmental (or phenotypic) habituation, Distel and Provenza (1991) showed that goats 6 wk old that fed on a tannin-bearing plant, blackbrush (*C. ramosissima*), ingested 27% more blackbrush than did inexperienced goats when tested 9 mo later. Rabbit pups raised in contact with juniper odor, from fecal pellets or through lactation from mothers fed on juniper, showed a greater preference for their mothers' diets at weaning than pups never exposed to juniper odor (Bilkó et al. 1994). In an example of apparent evolutionary adaptation, rat kangaroos (*Bettongia penicilliata*) still in contact with vegetation (such as *Gastrolobium* spp.) containing fluoroacetate (a potent PSM) exhibited a smaller physiological response to fluoroacetate than congenerics separated from contact with fluoroacetate-bearing vegetation 7000 yr ago (Mead et al. 1985).

It would seem that there has been sufficient time for similar local evolutionary adaptation among woodrat populations. Carbon dating of vegetative fragments found in woodrat middens indicates that woodrats in the Mojave desert have been exposed to creosote bush during the last 10 000 yr (Van Devender 1977, Van Devender and Spauldin 1979), while woodrats in the Great Basin have been exposed to pinyon–juniper vegetation for the last 35 000 yr, during which time there is no evidence of the presence of creosote bush in that region (Thompson 1990).

Habitats are currently being transformed by several factors such as livestock grazing, timber cutting, water diversion, fire suppression, and human recreational activities (McDonald and Brown 1992), which can alter food quality and availability for herbivores. On a broader scale, habitat changes caused by global warming and increased atmospheric levels of CO_2 may modify both the availability and chemical composition of plants in ways that might not be tolerated by some herbivores (Lindroth et al. 1993, Kinney et al. 1997, Lawler et al. 1997, Penuelas et al. 1997, Dury et al. 1998, Penuelas and Estiarte 1998). There is a lack of information regarding the physiological bases for differences in tolerance to plant secondary metabolites by wild mammals. To fill this gap we need to define wildlife's limits in tolerance to PSMs and describe the extent to which these limits are genetically fixed or are phenotypically plastic. This information will help us predict the responses of wild mammals to both natural and anthropogenically induced changes in plant secondary metabolites.

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