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Source: *Physiological and Biochemical Zoology*, Vol. 81, No. 5 (September/October 2008), pp. 584-593
Published by: The University of Chicago Press
Stable URL: http://www.jstor.org/stable/10.1086/589951
Accessed: 30/05/2013 12:30

"Pharm-Ecology" of Diet Shifting: Biotransformation of Plant Secondary Compounds in Creosote (*Larrea tridentata*) by a Woodrat Herbivore, *Neotoma lepida*

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Accepted 5/8/2008; Electronically Published 8/27/2008

ABSTRACT

Diet switching in mammalian herbivores may necessitate a change in the biotransformation enzymes used to process plant secondary compounds (PSCs). We investigated differences in the biotransformation system in the mammalian herbivore, *Neotoma lepida*, after a radical shift in diet and secondary compound composition. Populations of *N. lepida* in the Mojave Desert have evolved over the past 10,000 years to feed on creosote (*Larrea tridentata*) from an ancestral state of consuming juniper (*Juniperus osteosperma*). This dietary shift represents a marked change in the dietary composition of PSCs in that creosote leaves are coated with phenolic resin, whereas juniper is high in terpenes but lacks phenolic resin. We quantified the enzyme activity of five major groups of biotransformation enzymes (cytochrome P450s, NAD(P)H:quinone oxidoreductase, glutathione conjugation, sulfation, and glucuronidation) recognized for their importance to mammalian biotransformation for the elimination of foreign compounds. Enzyme activities were compared between populations of Mojave and Great Basin woodrats fed control and creosote diets. In response to creosote, the Mojave population had greater levels of cytochrome P450s (CYP2B, CYP1A) and glutathione conjugation liver enzymes compared with the Great Basin population. Our results suggest that elevated levels of cytochrome P450s and glutathione conjugation enzymes in the Mojave population may be the underlying biotransformation mechanisms that facilitate feeding on creosote.

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Physiological and Biochemical Zoology 81(5):584–593. 2008. 1522-2152/2008/8105-8015$15.00
DOI: 10.1086/589951

Introduction

For herbivores, food ingestion can have deleterious consequences (Lindroth 1988; Meyer et al. 1989; McArthur et al. 1991; Foley and McArthur 1994; Iason and Mason 1996; Dearing et al. 2001, 2002; Boyle and Dearing 2003; McLister et al. 2004; Sorensen et al. 2005*b*) because, at every meal, herbivores confront food items containing plant secondary compounds (PSCs) that are potentially toxic. The physiological consequences of PSC ingestion are numerous and include disturbance of acid-base homeostasis, emetic stimulation, mineral wasting, central nervous system depression, and kidney and liver lesions (Koppel et al. 1981; Hedenstierna et al. 1983; Lindroth and Batzli 1984; Lindroth et al. 1986; Foley and Hume 1987; Thomas et al. 1988; Meyer and Karasov 1989; Iason and Palo 1991; McArthur and Sanson 1991, 1993; Levin et al. 1992; Foley and McArthur 1994; Iason and Murray 1996). The ability of herbivores to ingest large quantities of PSCs is primarily governed by biotransformation ("detoxification") enzymes (Berenbaum 2002; Despres et al. 2007; Li et al. 2007). Much more is known about the biotransformation mechanisms employed by insect herbivores compared with their mammalian counterparts (Dearing et al. 2005; Despres et al. 2007; Li et al. 2007).

One of the greatest challenges for an herbivore when shifting from one plant diet to another is the metabolism of novel PSCs (Foley and McArthur 1994; Foley et al. 1999; Sorensen et al. 2005*a*). The desert woodrat, *Neotoma lepida*, presents a unique opportunity to evaluate changes in biotransformation mechanisms used by mammalian herbivores when an evolutionary diet shift occurs. Populations of *N. lepida* in the Mojave Desert have undergone significant dietary changes. Paleontological evidence of vegetative fragments from fossil woodrat middens indicates that the ancestral populations of woodrats in Mojave Desert probably fed on juniper (*Juniperus osteosperma*). Between 8,000 and 10,000 years ago, creosote (*Larrea tridentata*) became established and expanded in the Mojave Desert, replacing juniper (Spaulding 1991). Populations currently residing in the Mojave Desert can forage extensively on creosote, which can constitute up to 75% of their diet. The leaves of creosote produce a phenolic resin (Cameron and Rainey 1972;

Meyer and Karasov 1989) composed of numerous flavonols and partially *O*-methylated flavones, with the primary phenolic, nordihydroguaiaretic acid (NDGA), comprising 40% of the dry mass of total resin (Cameron and Rainey 1972; Meyer and Karasov 1989). Creosote resin and NDGA are well recognized as feeding deterrents in mammalian and insect herbivores and cause marked physiological harm and even death (Goodman et al. 1970; Lira-Saldivar 2003; Arteaga et al. 2005). Because the PSCs in creosote are highly toxic and chemically different from the ancestral diet of juniper, woodrats consuming creosote presumably evolved novel biotransformation mechanisms during the expansion of creosote.

Woodrats from the Mojave differ in their ability to ingest and process creosote compared with populations that continue to feed on juniper and are evolutionarily naive to creosote. Juniper is characterized by high levels of terpenes and lacks a phenolic resin such as that in creosote. In laboratory feeding trials, woodrats from the Mojave population voluntarily ingested three times more creosote resin compared with a population from the Great Basin Desert that feeds on juniper. These two populations of *N. lepida* also excreted different levels of urinary metabolites when fed a creosote diet, indicating that they detoxify creosote differently (Mangione et al. 2000). Moreover, the set of mRNAs encoding for biotransformation enzymes in the liver produced in Mojave woodrats consuming a diet of creosote in the wild was different from the set found in Great Basin woodrats consuming juniper (Lamb et al. 2001). Taken together, this evidence strongly suggests that the difference in tolerance to creosote resin between these two closely related populations is probably the result of recently evolved biotransformation mechanisms in the Mojave population for the biotransformation and elimination of the high concentration of NDGA and other phenolics found in creosote.

We took a comparative approach to determine the biotransformation mechanisms used by *N. lepida* consuming creosote. We compared the liver enzyme activities of individuals from two populations of *N. lepida* fed control and creosote diets. We focused on the liver because it is the primary site of biotransformation of xenobiotics and because of the results of previous work implicated hepatic enzymes in particular (Lamb et al. 2001; Mangione et al. 2000). The population from the Great Basin Desert was naive to creosote, whereas the population from the Mojave Desert had previous ecological and evolutionary exposure to creosote. We quantified the enzyme activities of five major classes of biotransformation enzymes plus three specific cytochrome P450 isozymes that are known to biotransform either PSCs or pharmaceuticals. We compared enzyme activities of woodrats consuming creosote with those of animals fed a toxin-free diet to evaluate induced versus constitutive enzyme levels.

We generated hypotheses with respect to putative changes in the biotransformation systems of Mojave and Great Basin woodrats based on available information on the metabolism of creosote's primary component, NDGA, in rodent model species. In laboratory rats, kidney disease and cysts occur when animals are exposed to 0.5%–3% (dry weight) of NDGA (Goodman et al. 1970). In contrast, Mojave woodrats consume anywhere between 3.3% and 7.8% (dry weight) of NDGA daily (Mangione 1999). NDGA can inhibit cytochrome P450 1A (CYP1A) activity in vitro, and there is some evidence that UDP-glucuronosyltransferase (UGT) is used in the metabolism of NDGA in mammalian models (Capdevila et al. 1988; Agarwal et al. 1991; Lambert et al. 2002). Furthermore, glutathione-S-transferase (GST) is important in the metabolism of NDGA because glutathione depletion contributes to the apoptosis associated with NDGA exposure (Tang and Honn 1997). On the basis of the documented biotransformation of creosote resin, particularly the main component NDGA, we hypothesized that creosote resin would inhibit CYP1A activity in both Mojave and Great Basin woodrats and that the Mojave woodrats would have higher GST and UGT activity levels to biotransform creosote resin compared with Great Basin woodrats.

Material and Methods

Study system. Neotoma lepida were trapped in the Mojave and Great Basin Deserts. Individuals from the Mojave Desert were trapped near Beaver Dam Wash, Utah (37°06′N, 113°58′W). Individuals from the Great Basin Desert were trapped near Jericho, Utah (39°57′N, 112°22′W). Animals were transported to the University of Utah Animal Facility, housed in individual cages (48 cm × 27 cm × 20 cm) with bedding and cotton batting, and put on a 12L : 12D photoperiod for at least 3 mo before the experiments to allow for hantavirus testing. All animals were maintained on high-fiber rabbit chow (Harland Teklad formula 2031) and water ad lib. before experimentation. All procedures were approved by the University of Utah IACUC (04–02012). From this point, *N. lepida* from the Mojave Desert will be referred to as "Mojave woodrats," and those from the Great Basin Desert will be referred to as "Great Basin woodrats."

Dietary treatment and resin extraction. Creosote (*Larrea tridentata*) leaves, young and old, were collected from several bushes (>10) at trapping sites in the Mojave Desert. Foliage was frozen at −20°C until resin extraction. Resin was extracted by submersing foliage in ethyl ether (1 : 6, wet leaf mass : volume solvent) for 45 min. The foliage was removed and remaining solvent was run through Whatman filter paper (grade 1) to remove large particles. Ethyl ether was evaporated using a rotovap until the resin was highly viscous. Any remaining ethyl ether was evaporated from resin using a vacuum pump for over 48 h. The dry extracted resin was 4.7% the dry weight of leaves. Creosote resin was stored at −20°C for less than 3 mo before use.

The creosote treatments were prepared by dissolving the desired concentration of creosote resin into a volume of ethyl acetate equal to 25% of the dry weight of the rabbit chow to which it was added. To control for any residual effects of the solvent, we added ethyl acetate without resin to the control diet. Ethyl acetate was evaporated from all diets in a fume hood,

and total evaporation was confirmed gravimetrically. Diet treatments were stored at −20°C and used within 2 wk.

Individuals from each population were fed either a control diet or the treatment diet. Total food intake was measured daily. The control treatment, lacking any creosote resin, was fed to a group in each population (Mojave, $N = 9$; Great Basin $N = 5$) for 5 d to measure levels of constitutive biotransformation enzymes. Another set of woodrats were given the diet containing 5% creosote resin (Mojave, $N = 11$; Great Basin, $N = 7$). This diet containing 5% approximated the concentration of resin in creosote leaves (4.7% dry weight). Animals were first acclimated to creosote resin for 3 d on a low-resin treatment of 2.5% resin followed by 5% creosote resin diet for 7 d before they were killed. Three days of exposure to a compound is generally recognized in pharmacological studies as more than sufficient time to fully induce enzyme activity (Parkinson 1996; Bomhard et al. 1998; Meredith et al. 2003).

Microsomal preparation and enzyme assays. We used conventional enzyme activity assays to measure the amount of functioning hepatic enzymes among treatment groups. Enzyme activities were determined in subcellular fractions isolated from whole-liver tissue. After the animals completed dietary treatments, they were killed with CO_2. The liver was immediately perfused in situ by injecting cold isotonic saline into the hepatic portal vein. After the gall bladder was removed, the liver was excised and weighed. Microsomal and cytosolic fractions were prepared by differential ultracentrifugation as described by Franklin and Estabrook (1971) and stored at −80°C until assayed for activity. Protein concentrations were determined colorimetrically by the method of Lowry (1951) to standardize enzyme activity to protein concentration. We measured total cytochrome P450 (CYP) because this pathway is the most abundant and used biotransformation pathway (Danielson 2002). In addition, we measured three specific isozymes of the P450 pathway (CYP1A, CYP2B, and CYP3A) that were included either for their known role in biotransformation of PSC or because they are common enzymes in pharmacological studies (Gregus et al. 1983; Rosenthal and Berenbaum 1992; Hiroi et al. 1995; Parkinson 1996; Pass et al. 1999, 2001; Danielson 2002; Liukkonen-Anttila et al. 2003; Sivapathasundaram et al. 2003). Three conjugation enzymes were assayed: UGT, GST, and sulfotransferase (SULT). UGT and GST represent the major conjugation enzymes in mammals (Dutton 1980; Daniel 1993). SULT is considered an important alternative to glucuronidation for phenolics and was included for this reason (Parkinson 1996).

Cumulative CYP concentration was determined from microsomes using the CO difference spectrum on a spectrophotometer by the method of Omura and Sato (1964), which measures the reduced P450:CO complex characteristic of all CYP isozymes cumulatively. CYP1A activities were determined by the method of Klotz et al. (1984), which measures the rate of resorufin production during the metabolism (*O*-deethylation) of 7-ethoxyresorufin (Sigma Chemical, St. Louis, MO). CYP2B activities were determined by the method of Lubet et al. (1985) that measures the rate of resorufin production during the metabolism (dealkylation) of pentoxyresorufin (Sigma Chemical). CYP3A activities were determined by testosterone 6β–hydroxylation (Sigma Chemical), where the testosterone metabolite (6β–hydroxytestosterone) was separated by HPLC and quantified from its absorbance at 236 nm (Guengerich et al. 1986). Cytosolic NAD(P)H:quinone oxidoreductase (QOR) activities were determined by the dicoumarol-inhibited rate of reduction of 2,6-dichlorophenolindophenol (Aldrich Chemical, Milwaukee, WI) by NADH at pH 7.4 detected with spectrophotometry (Benson et al. 1980).

Hepatic microsomal UGT activities were determined with spectrophotometry (Bock et al. 1983) in reactions containing detergent (0.05% Triton X-100; Sigma Chemical) and measured the UDP glucronic acid–dependent disappearance rate of *p*-nitrophenol (Sigma Chemical). Cytosolic GST activities were determined with spectrophotometry (Habig and Jakoby 1981), detecting the change in absorbance at 340 nm upon conjugation of glutathione to 1-chloro-2,4-dinitrobenzene (CDNB, a general reference substrate; Aldrich Chemical). SULT cytosolic activities were determined by detecting the 3′-phosphoadenosine 5′-phosphosulfate (PAPS)–dependent disappearance rate of *p*-nitrophenol (Sigma Chemical) with spectrophotometry (Sekura et al. 1981).

The probe substrates used in the assays are considered iso-

Table 1: Means ± 1 SE of body mass, food intake, and liver mass from woodrats fed control diet or 5% creosote resin diet

	Mojave Woodrats		Great Basin Woodrats	
Variable	Control Diet	Creosote Diet	Control Diet	Creosote Diet
Body mass (initial)	154.1 ± 11.6	129.7 ± 9.9	142.5 ± 14.6	145.6 ± 12.4
Body mass (final)	158.1 ± 11.2^{A}	126.4 ± 9.6^{B}	148.6 ± 14.3^{A}	128.1 ± 12.1^{B}
% change in body mass	2.42 ± 1.8^{A}	1.82 ± 1.6^{A}	3.5 ± 2.3^{A}	−9.57 ± 1.96^{B}
Dry matter intake[a]	10.93 ± .8^{A}	6.53 ± .7^{B}	10.3 ± 1.1^{A}	5.02 ± .9^{B}
Liver mass	4.21 ± .5	3.83 ± .4	4.2 ± .5	3.75 ± .5

Note. Different letters (A, B) denote means significantly different ($P < 0.05$) as determined by Fisher's least significant difference within the same row.

[a] Calculated from an average of 7 d on 5% creosote diet.

Table 2: Summary of ANOVAs and ANCOVAs for body mass, food intake, and liver mass

Source of Variation	*F*	df	*P*
Body mass (initial):			
Population	.031	1, 27	.861
Diet	.76	1, 27	.391
Population × diet	1.255	1, 27	.272
Body mass (final):			
Population	.107	1, 27	.746
Diet	4.788	1, 27	.037
Population × diet	.219	1, 27	.644
Percentage change in body mass:			
Population	2.951	1, 27	.097
Diet	20.06	1, 27	.000
Population × diet	5.223	1, 27	.030
Dry matter intake:			
Population	1.369	1, 26	.253
Diet	21.07	1, 26	.000
Population × diet	.425	1, 26	.520
Covariate (body mass)	2.983	1, 26	.096
Liver mass:			
Population	.091	1, 26	.765
Diet	3.481	1, 26	.073
Population × diet	.204	1, 26	.655
Covariate (body mass)	.266	1, 26	.610

zyme specific. For example, isozyme CYP1A catalyzes the metabolism of 7-ethoxyresorufin; SULT enzymes catalyze the metabolism of *p*-nitrophenol. Although these protocols were designed and validated in laboratory rats, they are often used in disparate species. Given that laboratory rats are in the same family (Muridae) as woodrats (*Neotoma*), the use of these assays likely provide a valid estimate of enzyme-specific activities. The probe substrates, CDNB and *p*-nitrophenol, are relatively nonspecific substrates for GST and UGT isozymes respectively. However, CDNB generally targets the alpha class of GST in liver assays and *p*-nitrophenol targets UGT1As. The SULT substrate targets SULT1, used for phenol metabolism.

Statistical analysis. Body mass (g) and the activities of each biotransformation enzyme analyzed were compared using a two-way ANOVA, with population and diet as the independent variables. When significant ($P < 0.05$), a post hoc Fisher's least significant difference (LSD) for pairwise comparisons was conducted. Because there were differences in body mass over time between the groups and because body mass affects food intake and liver mass, ANCOVA was used to compare food intake as well as liver mass using body mass as a covariate and diet and population as the independent factors. For one assay (QOR), a Kruskal-Wallis test was used to address the issue of heteroscedasticity with each species, with diet treatment as the grouping variables and activity as the dependent variable. Systat 10 (SPSS, Chicago, IL) was used for analysis.

Results

Body mass, food intake, and liver weight. There was no statistically significant difference in initial body mass. The final body mass in both populations was significantly lower for animals fed creosote than for those fed the control diet (Table 1). The percentage change from initial to final body mass was significantly different between creosote and control-fed woodrats. The significant interaction is explained by the Great Basin woodrats fed creosote losing significantly more body mass than any other group (Fisher's LSD $P < 0.005$). Woodrats of both populations on the creosote treatment ingested nearly half as much diet as woodrats fed the control treatment (Table 1).

Diet and not differences in body mass resulted in differences in intake (Table 2). There was not a significant effect of population or diet on liver mass; however, diet was marginally significant (Table 2; $P = 0.07$). The liver mass of woodrats on the creosote diet was ~10% less than that of woodrats on the rabbit chow diet (Table 1).

Enzyme activity assays. NAD(P)H:QOR activity was heteroscedastic as determined by the Kruskal-Wallis test ($P = 0.019$) with QOR activity as the dependent variable. The Kruskal-Wallis test statistic was 9.946 with 3 df. Data plotted (Fig. 1) represent the medians and the median absolute deviations to represent central tendency and variation. The difference in medians is the result of the Great Basin control treatment having a lower value than all other groups.

Cytochrome P450s. Mojave woodrats had higher total cytochrome P450 concentrations than Great Basin woodrats (Fig. 1). Cytochrome P450 concentration was constitutively 30% higher in Mojave woodrats compared with Great Basin woodrats (Table 3). Dietary treatment did not alter the level of total cytochrome P450 concentration in either population.

Cytochrome P450 isozymes (CYP1A, CYP2B, and CYP3A). Population and diet treatment influenced levels of CYP1A activity (Table 3). Populations did not differ in constitutive expression. However, Mojave woodrats induced CYP1A levels nearly twofold when fed the creosote treatment. In contrast, Great Basin woodrats did not induce CYP1A when fed creosote (Fig. 2). Population but not diet significantly influenced CYP2B activity (Table 3). CYP2B activity was constitutively different; Mojave woodrats had 1.5-fold-higher levels than Great Basin woodrats, and there was not induction with diet treatment (Fig. 2). CYP3A activity levels did not differ between or within the two populations fed control or creosote diet (Fig. 2).

UDP-glucuronosyltransferases. Diet treatment significantly affected UGT activity, but population did not (Table 3). There were no constitutive differences, and both populations significantly induced UGT activity to a similar degree, ~40% from constitutive levels, when fed a creosote diet (Fig. 3).

Sulfotransferase. SULT activity was not different between or within Mojave or Great Basin woodrats. Furthermore, levels of SULT activity were not influenced by diet as there was no significant difference in either diet treatment (Fig. 3). There was a significant interaction effect (Table 3), which was followed

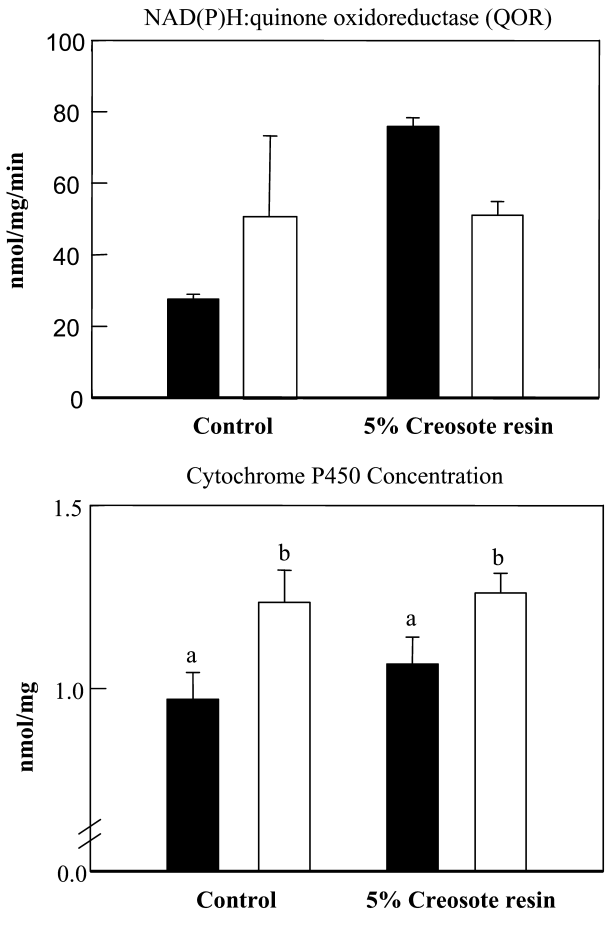

Figure 1. Hepatic functionalization enzymes in *Neotoma lepida* from the Great Basin Desert (*solid*) and the Mojave Desert (*open*) fed control or 5% creosote resin diets. Data for NAD(P)H:quinone oxidoreductase are represented as the median + median absolute deviation. Data for cytochrome P450 concentration are represented as the mean + SE. Bars with different letters (*a*, *b*) denote means significantly different ($P < 0.05$) as determined by Fisher's least significant difference.

with a post hoc Fisher's LSD and explained by a significant reduction in activity of Mojave woodrats from control treatment to creosote treatment ($P = 0.009$).

Glutathione-S-transferase. Population but not diet significantly influenced levels of GST activity (Table 3). Constitutive GST activities were nearly twofold higher in Mojave woodrats than in Great Basin woodrats and did not change on the creosote diet (Fig. 3).

Discussion

Little information exists on the biotransformation mechanisms implemented by mammalian herbivores to detoxify and eliminate PSC. This research investigated the response of biotransformation enzymes to a creosote diet to elucidate mechanisms used by Mojave woodrats to metabolize the PSC in creosote that may have allowed a dietary shift from juniper to creosote. From knowledge of NDGA metabolism in laboratory rodents, we predicted that both Mojave and Great Basin woodrats would have low levels of CYP1A activity and that the Mojave woodrats would have higher GST and UGT activity levels than Great Basin woodrats. In contrast to our predictions, Mojave woodrats consuming creosote increased the activity of CYP1A, and both populations induced UGT to a similar extent on creosote treatment. Woodrats from the Great Basin, whose natural diet does not contain creosote, did not elicit a biotransformation enzyme response, with the exception of increased UGT, to a diet containing creosote resin. The limited ability to induce biotransformation enzymes could explain why they are unable to maintain food intake and body mass in long-term feeding trials as compared with Mojave woodrats (Mangione et al. 2000). The trend toward body mass loss in Great Basin woodrats ($P = 0.097$) over 7 d fed 5% creosote in this study may have also become statistically significant as seen in previous studies if dietary treatments were carried out long term. In addition, there were major differences in constitutive enzyme activities between Great Basin and Mojave woodrats, with Mo-

Table 3: Summary of ANOVAs and ANCOVAs for biotransformation enzyme assays

Source of Variation	*F*	df	*P*
Cytochrome P450 concentration:			
Population	17.142	1, 28	.000
Diet	.533	1, 28	.471
Population × diet	.274	1, 28	.605
CYP1A (EROD):			
Population	11.449	1, 28	.002
Diet	3.839	1, 28	.060
Population × diet	6.362	1, 28	.018
CYP2B (PROD):			
Population	13.648	1, 28	.001
Diet	.011	1, 28	.919
Population × diet	.475	1, 28	.496
CYP3A:			
Population	.635	1, 28	.432
Diet	2.974	1, 28	.096
Population × diet	3.009	1, 28	.094
UGT:			
Population	2.566	1, 28	.120
Diet	19.23	1, 28	.000
Population × diet	.401	1, 28	.532
SULT:			
Population	1.59	1, 28	.218
Diet	.710	1, 28	.407
Population × diet	6.574	1, 28	.016
GST:			
Population	20.12	1, 28	.000
Diet	.081	1, 28	.779
Population × diet	.819	1, 28	.373

Note. EROD = 7-ethoxyresorufin; PROD = pentoxyresorufin; UGT = UDP-glucuronosyltransferase; SULT = sulfotransferase; GST = glutathione-S-transferase.

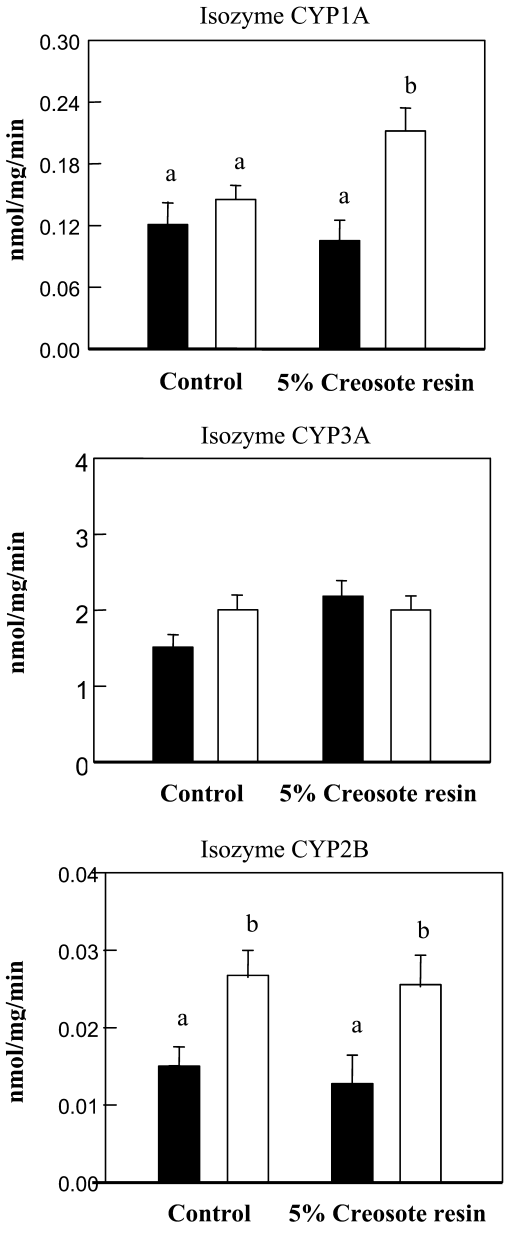

Figure 2. Hepatic cytochrome P450 isozymes in *Neotoma lepida* from the Great Basin Desert (*solid*) and the Mojave Desert (*open*) fed control or 5% creosote resin diets. Data are represented as the mean + SE. Bars with different letters (*a*, *b*) denote means significantly different ($P < 0.05$) as determined by Fisher's least significance difference.

jave woodrats having higher overall cytochrome P450 concentration and higher activity levels of isozyme CYP2B and GST. The following paragraphs will present an evaluation of potentially significant biotransformation enzymes used by the Mojave woodrats along with ecological implications of these enzymatic changes.

Cytochrome P450s play an integral part in mammalian and insect xenobiotic metabolism because of the vast number of isozymes that metabolize a diversity of compounds. Mojave woodrats had higher concentrations of overall cytochrome P450s (a measure that includes all CYP isozymes) than Great Basin woodrats, indicating that cytochrome P450s, as a whole, may be important in the metabolism of NDGA or other PSC in creosote (Fig. 1). The specific isozyme CYP1A was induced by a creosote diet in Mojave woodrats. Previous in vitro research indicates that NDGA negatively affects CYP1A activity, but in vivo, its induction indicates that it may play an important role in NDGA metabolism (Capdevila et al. 1988; Agarwal et al. 1991). The specific isozyme CYP2B was constitutively higher

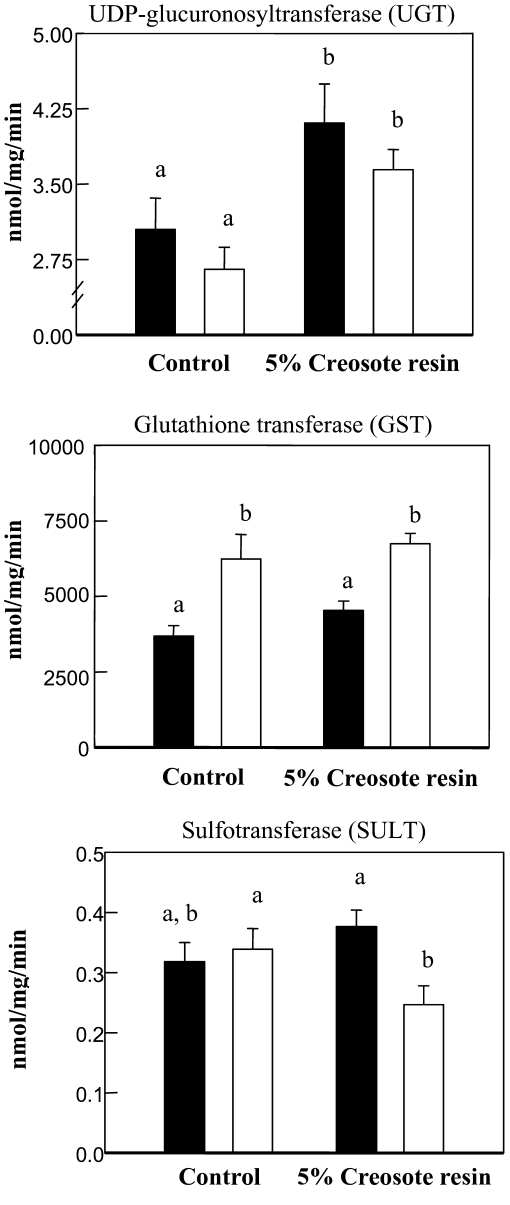

Figure 3. Hepatic conjugation enzymes in *Neotoma lepida* from the Great Basin Desert (*solid*) and the Mojave Desert (*open*) fed control or 5% creosote resin diets. Data are represented as the mean + SE. Bars with different letters (*a*, *b*) denote means significantly different ($P < 0.05$) as determined by Fisher's least significant difference.

in Mojave woodrats compared with Great Basin woodrats. There is some evidence that CYP2B is elevated in Great Basin woodrats feeding on juniper (Lamb et al. 2001). The lack of induction of CYP2B activity by a creosote diet in either population does not preclude the induction of CYP2B activity when consuming different plant compounds. CYP1A and CYP2B are important in the metabolism of a number of PSCs, including terpenoid and phenolic compounds (Austin et al. 1988; Debersac et al. 2001; Rahden-Staron et al. 2001; Szaefer et al. 2003). Therefore, these cytochrome P450 isozymes may be important to Mojave woodrats for the metabolism of creosote PSCs, and the lower activity in Great Basin populations may explain their lower tolerance for creosote as evidenced by decreased food intake and increased mass loss in long-term feeding trials (Mangione et al. 2000).

Higher GST activity may also contribute to the elevated tolerance for creosote in Mojave woodrats. It is possible that elevated GST activity is used to complete the metabolism and elimination of NDGA metabolites. Glutathione (the conjugate used by GST) depletion contributes to apoptosis associated with NDGA exposure (Tang and Honn 1997). GST is recognized for conjugating a number of reactive metabolites and is likely to further metabolize the orthoquinone derivative of NDGA (Griffith 1997). Metabolism of NDGA produces an orthoquinone metabolite similar to that produced by acetaminophen metabolism (Grice et al. 1968; De Smet 1993). At low doses, the orthoquinone metabolite of acetaminophen (N-acetyl-*p*-benzoquinone imine [NAPQI]) is efficiently detoxified, typically by glutathione conjugation catalyzed by GST. At high doses of acetaminophen, glutathione becomes depleted, leading to oxidative stress caused by NAPQI accumulation (Slattery et al. 1987; Harman et al. 1991). Similar oxidative stress is found with NDGA when glutathione is depleted in in vitro studies (Committee on the Framework for Evaluating the Safety of the Dietary Supplements 2004). Thus, a diet containing high levels of NDGA from creosote has the potential to result in the accumulation of a toxic orthoquinone metabolite unless glutathione reserves and GST activity in the liver are great enough to fully metabolize and eliminate the orthoquinones produced.

Synthesis of glutathione in the liver is rate limited by the γ-GCS enzyme. Other studies support the hypothesis that glutathione production may be maintained at a higher level in Mojave woodrats than in Great Basin woodrats. In free-ranging woodrats feeding on natural diets, Mojave woodrats consuming creosote expressed two times more γ-GCS than Great Basin woodrats and five times more than laboratory rats feeding on chow (Lamb et al. 2001). The Mojave woodrat population may maintain high glutathione such that the twofold-higher GST activity does not result in depletion of glutathione, thereby protecting Mojave woodrats from toxicity when consuming a diet principally composed of creosote.

Both populations decreased intake when fed 5% creosote, and both decreased body mass as a result. A diet containing 5% creosote resin in this study was approximately equivalent to a 100% creosote foliage diet, and this concentration is slightly higher than typically consumed by Mojave woodrats. In our 1-wk study, there was not a significant difference in body mass change of the two populations on the creosote diet. There was, however, a significant interaction effect explained by the Great Basin woodrats' greater loss of mass on a creosote diet than any other group (Fisher's LSD $P < 0.005$). Dry matter intake did not explain this difference. An increase in the energetic costs of biotransforming creosote may explain the decrease in mass of the Great Basin woodrats. Other long-term feeding trials demonstrated that Great Basin woodrats cannot consume high levels of creosote without excessive and life-threatening body mass loss (Mangione et al. 2000). Enzyme activity assays indicate that the lack of tolerance in the Great Basin woodrats may be the result of a lack of enzymatic response to creosote. The Great Basin woodrats fed creosote increase only UGT and no other biotransformation enzymes. The constitutive and induced differences between these two populations offer insight into why these two closely related populations have different tolerances for the same diet of creosote.

Theory predicts that herbivore feeding behavior is driven by constraints on evolving a biotransformation system capable of metabolizing the large quantities of a single type of toxin present in a diet of a single species of plant (Freeland and Janzen 1974). This research suggests that a population of an herbivorous woodrat, *Neotoma lepida*, appears to have developed novel biotransformation enzyme regulation in response to PSC found in creosote over an evolutionary short period of time. Research presented here investigated several biotransformation enzymes, recognized for their importance to mammalian biotransformation and is one of the most extensive in evaluating the biotransformation enzymes influenced by dietary PSC. The major differences discovered in Mojave woodrats are enzyme activities that are constitutively higher, particularly CYP2B and GST, in addition to induced responses of CYP1A. Determining the relative importance of each of these enzymes requires functional studies such as cellular expression and inhibitor studies that block the individual actions of these isozymes. Other physiological mechanism such as intestinal efflux transports (e.g., *p*-glycoprotein) should also be investigated for their potential role in allowing creosote resin ingestion. The phylogeny of *N. lepida* suggests that creosote feeding may have evolved multiple times (Patton and Alvarez-Castaneda 2004). Thus, studies on multiple populations in the Mojave are needed to determine whether *N. lepida* has evolved multiple responses to a creosote diet.

Acknowledgments

We thank committee members Dr. G. Yost, Dr. F. Goller, and Dr. D. Feener for their invaluable input. We thank Dr. J. Sorensen, Dr. M. Skopec, and Ann-Marie Torregrossa for critical reading of the manuscript. We also thank Dr. J. Sorensen and undergraduates in Dr. Dearing's lab, especially Elizabeth Birdsall and Katie Young, for assistance with feeding, tissue collec-

tion as well as collection and maintenance of woodrats. Research was supported by the National Science Foundation (grant IBN-023402).

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