J Chem Ecol (2007) 33:2111–2122
DOI 10.1007/s10886-007-9371-5

# Xenobiotic Metabolism of Plant Secondary Compounds in Oak (*Quercus Agrifolia*) by Specialist and Generalist Woodrat Herbivores, Genus *Neotoma*

**Shannon L. Haley · John G. Lamb · Michael R. Franklin · Jonathan E. Constance · M. Denise Dearing**

Received: 6 March 2007 / Revised: 5 July 2007 / Accepted: 11 September 2007 /
Published online: 11 October 2007


**Abstract** The challenge of consuming plant compounds that are recognized to have toxic physiological effects is an unavoidable consequence of an herbivorous diet and requires mechanisms to metabolize and eliminate them after consumption. We took a pharmacological approach to understanding how an oak (*Quercus agrifolia*) specialist (*Neotoma macrotis*) and generalist (*N. lepida*) herbivores process the same dietary toxins. Oak contains polyphenolic compounds considered toxic to most other mammals. *N. macrotis* includes up to 85% of oak in their diet. *N. lepida* includes oak as a portion of the diet but is considered a generalist in areas where sympatric with *N. macrotis*. Xenobiotic metabolizing enzyme activities of *N. macrotis* and *N. lepida* were investigated after animals were fed a 70% oak diet and a toxin-free control diet. Biotransformation activities of five major enzymes [cytochrome P450s (CYP), NAD(P)H/quinone oxidoreductase (QOR), UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT), and glutathione S-transferase (GST)] and three specific CYP isozymes (CYP1A, CYP2B, and CYP3A) were investigated. The results indicate that, with the exception of CYP2B induction, *N. macrotis* and *N. lepida* enzyme activities are not changed by an oak diet. The major differences in enzyme activities were constitutive. The specialist, *N. macrotis*, had higher constitutive activity of QOR, UGT, and GST. The generalist, *N. lepida*, had higher constitutive activity levels of CYP1A and SULT.

**Keywords** Cytochrome P450 monooxygenases · Conjugation · *Neotoma* · Specialist · Herbivore

S. L. Haley (✉) · M. D. Dearing
Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA
e-mail: haley@biology.utah.edu

J. G. Lamb · M. R. Franklin · J. E. Constance
Department of Pharmacology and Toxicology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA

## Introduction

Many herbivores are exposed to a diverse array of plant secondary compounds (PSC) naturally present in their diet. Many of these are recognized for their toxicity and generate physiological damage when consumed. The detoxification limitations hypothesis (Freeland and Janzen 1974) proposed that xenobiotic metabolism is the principal physiological mechanism responsible for neutralizing the harmful effects of PSC. This hypothesis suggested also that the paucity of mammalian dietary specialists is the result of constraints on the evolution of a xenobiotic metabolizing system capable of eliminating the large concentrations of similar toxins present in a specialist diet (Freeland and Janzen 1974; Marsh et al. 2006).

Previous studies on biotransformation mechanisms suggest that specialists differ intrinsically from generalists in enzymatic metabolism of PSC. Specialist herbivores appear to rely more on enzymes that add a functional group (Phase 1) and less on those that add an endogenous conjugate (Phase II) compared to generalist herbivores (McLean et al. 1993, 2001; McLean and Foley 1997; Boyle et al. 2001). In these studies, several species of marsupials that specialize on *Eucalyptus* had higher Phase I activity and lower Phase II, when exposed to *Eucalyptus* PSC compared to generalists (McLean and Foley 1997; Boyle et al. 1999, 2001). Additionally, *Eucalyptus* specialists expressed different levels of specific classes of Phase I enzymes (i.e., cytochrome P450s) than generalists exposed to the same compounds (Bolton and Ahokas 1997; Stupans et al. 1999).

We evaluated the prediction that a eutharian specialist woodrat (*Neotoma macrotis*) had higher levels of Phase I and lower levels of Phase II enzyme activities than a sympatric generalist woodrat (*N. lepida*). Both woodrats include oak (*Quercus* spp.) leaves in their diet. *Neotoma macrotis* is an oak specialist with up to 85% of its diet being *Quercus agrifolia* (Atsatt and Ingram 1983; Matocq 2002). In lab studies, populations of *N. macrotis* selected *Q. agrifolia* leaves over foliage from other dominant species (Atsatt and Ingram 1983). *Neotoma lepida* includes oak as a portion of their diet but is considered a generalist. In areas where *N. lepida* is sympatric with *N. macrotis*, the diet is primarily *Opuntia occidentalis*, cactus, and *Salvia apiana* (Cameron 1971; Atsatt and Ingram 1983). Oak is high in phenolics that are considered toxic to most other mammals (Atsatt and Ingram 1983; Lindroth and Batzli 1984; Thomas et al. 1988; Iason and Murray 1996). The differential consumption of oak by the specialist and generalist is predicted to be a result of xenobiotic metabolizing capabilities (Atsatt and Ingram 1983).

We took a comparative pharmacological approach to determine the xenobiotic metabolizing mechanisms used by the specialist and generalist when consuming oak foliage. We investigated the biotransformation activities of five major enzymes and three specific isozymes that include members from the two classes of xenobiotic metabolizing enzymes: Phase I and Phase II. The Phase I enzymes assayed were oxidation (cytochrome P450s; CYP) and reduction (NAD(P)H: quinone oxidoreductase; QOR). In addition to overall CYP concentration, catalyzed oxidations characteristic of three isozymes (CYP1A, CYP2B, and CYP3A) were included either for their role in biotransformation of PSC in a variety of animal systems and/or because they are common enzymes in pharmacological studies (Gregus et al. 1983; Hiroi et al. 1995; Parkinson 1996; Pass et al. 1999, 2001; Danielson 2002; Liukkonen-Anttila et al. 2003; Sivapathasundaram et al. 2003a). The Phase II enzymes assayed were UDP-glucuronosyltransferase (UGT), glutathione S-transferase (GST), and sulfotransferase (SULT), each chosen for their common role in the biotransformation of a variety of compounds (Gregus et al. 1983; Sivapathasundaram et al. 2003b). UGT and GST represent the major Phase II enzymes in mammals (Dutton 1980; Daniel 1993). SULT is an important alternative to glucuronidation for phenolics (Parkinson 1996).

## Materials and Methods

*Study System* *Neotoma macrotis* and *N. lepida* were trapped at Caspers Wilderness Park in Orange County, CA, USA. The animals were transported to the University of Utah Animal Facility, housed in individual cages (48×27×20 cm) with bedding and cotton batting and put on a 12–12 hr L/D cycle for at least 3 months before experiments to allow for Sin Nombre hantavirus screening. All the animals were maintained on standard rabbit chow (Harland Teklad formula 2031) and water ad libitum before experimentation. All procedures were approved by the University of Utah IACUC.

*Dietary Treatments* The animals were fed one of two diets (control or 70% oak). Control diet consisted of the same rabbit chow used for the maintenance diet with the exception that it was in powdered form. *Quercus agrifolia* used in diet treatments was stripped from several trees (>10) at sites where animals were trapped in California and stored at −20°C until use. Foliage was homogenized by grinding in a Waring blender (model CB-5) with dry ice until it passed through a 1-mm screen. Homogenization of the oak was necessary to control for oak diet intake but may have caused some chemical oxidation of the PSC present in the foliage. Oak diets were prepared by adding ground oak to ground rabbit chow on a percent dry weight basis of each component. The diets that contained oak were prepared daily to minimize the potential for chemical degradation. Total food intake (g dry matter) was measured each day. For the control treatment, the specialist, *N. macrotis* ($N$=7), and generalist, *N. lepida* ($N$=7), were fed the control diet of rabbit chow for 5 days to determine constitutive levels of xenobiotic metabolizing enzymes. Animals in another group of specialists ($N$=9) and generalists ($N$=7) that were given the 70% oak treatment diet were first acclimated to a 25% oak diet for 3 days, followed by 50% oak diet for 1 days, and then fed 70% oak diet for 3 days. Before treatment, all the animals were given a control diet that consisted of standard rabbit chow (Harland Teklad) for several weeks.

*Microsomal Preparation and Enzyme Assays* Enzyme activity assays were used to measure activity of hepatic enzymes among species and treatment groups. Enzyme activities were determined in subcellular fractions isolated from whole liver tissue. On the last day of each dietary treatment, the animals were killed with $CO_2$. The liver was immediately perfused in situ by injecting cold isotonic saline into the hepatic portal vein, and the gall bladder was removed. The liver was then removed 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.

Cytosolic NAD(P)H/quinone oxidoreductase (QOR) activities were determined by the dicoumarol-inhibited rate of reduction of 2, 6-dichlorophenolindophenol (Aldrich Chemical Co., Milwaukee, WI, USA) by NADH at pH 7.4 detected with spectrophotometry (Benson et al. 1980). Overall CYP concentration was determined from microsomes by using the carbon monoxide difference spectrum on a spectrophotometer by the method of Omura and Sato (1964) that measures the reduced P450/CO complex characteristic of all CYP isozymes cumulatively. CYP1A activities were determined by the method of Klotz et al. (1984) that measures the rate of resorufin production during the metabolism (*O*-deethylation) of 7-ethoxyresorufin (EROD; Sigma Chemical Co., St. Louis, MO, USA). CYP2B activities were determined according to Lubet et al. (1985) by measuring the rate of

resorufin production during the metabolism (de-alkylation) of pentoxyresorufin (PROD; Sigma). CYP3A activities were determined by testosterone 6$\tilde{\beta}$ hydroxylation (Sigma) where the testosterone metabolite (6$\tilde{\beta}$ hydroxytestosterone) was separated by high-performance liquid chromatography and quantified from its absorbance at 236 nm (Guengerich et al. 1986).

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

The probe substrates used in the assays are considered isozyme specific. For example, isozyme CYP 1A 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 suborder (Myomorpha) as woodrats, the use of these assays is likely to 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 UGT1A's. The SULT substrate targets SULT1 that is used for phenol metabolism.

*Statistical Analysis* Body mass and the activities of each detoxification enzyme analyzed were compared by using a two-way analysis of variance (ANOVA) with species and diet as the independent variables. When significant ($P<0.05$), a post hoc Fisher's LSD for pair-wise comparisons was conducted. Because there were differences in body mass, an ANCOVA was used to compare dry matter intake and liver mass by using body mass as a covariate, and diet and species as the independent factors. Systat 10 (SPSS, Chicago, IL, USA) was used for analysis.

## Results

*Body Mass, Dry Matter Intake, and Liver Mass* The body mass of the specialist was approximately 20% greater than the generalist (Table 1). As a result of the difference, food intake was analyzed with body mass as a covariate. Dry matter intake did not differ between

**Table 1** Mean body mass, dry matter intake (DMI) and liver mass from the specialist, *N. macrotis* and the generalist, *N. lepida* fed control diets or diets containing 70% oak (*Quercus agrifolia*; data represented as means±1 SE)

| Variable | *N. macrotis* (specialist) | | *N. lepida* (generalist) | |
|---|---|---|---|---|
| | Control diet | Oak diet | Control diet | Oak diet |
| Body mass (g) | 208±15.4$^{a}$ | 204±13.6$^{a}$ | 161±15.4$^{b}$ | 162±15.4$^{b}$ |
| Dry matter intake (g) | 10.5±1.5$^{a}$ | 24.7±1.3$^{b}$ | 11.5±1.5$^{a}$ | 22.5±1.5$^{b}$ |
| Liver mass (g) | 5.05±0.36$^{a}$ | 4.15±0.32$^{b}$ | 5.59±0.36$^{a}$ | 4.94±0.36$^{b}$ |

$^{a,b}$ Denote means that are significantly different ($P<0.05$) as determined by Fisher's LSD

the specialist and generalist (Table 2). However, both species had twofold higher intake when consuming oak diet compared to control diet (Table 1). Liver mass was not different between the specialist and generalist, but liver mass was significantly smaller in animals on the oak treatment compared to control groups in both species irrespective of body size (Table 2).

*Cytochrome P450 Concentration* Overall, there were no significant cytochrome P450 concentration differences among the treatment groups (Fig. 1). However, there was a trend for the generalist, *N. lepida*, to have higher activity than the oak specialist (Table 3).

*Quinone Oxidoreductase* The specialist had higher QOR activity than the generalist, *N. lepida* (Fig. 1). Specifically, the levels of constitutive QOR activity were more than two times higher in the specialist compared to the generalist and did not change on oak diet (Table 3).

*Cytochrome P450 Isozymes* Activity levels for the specific isozymes CYP1A, CYP2B, and CYP3A varied among the groups (Fig. 2). CYP1A activities in the generalist were constitutively 30% higher than that of the specialist (Fig. 2). Neither the specialist nor generalist significantly increased CYP1A activity between control and oak diet (Table 3). CYP2B activities in the control treatment groups were similar (Fig. 2). However, there was a significant effect of diet on CYP2B activities (Table 3). Both the specialist and generalist increased activity by more than 20% on the oak treatment. Finally, CYP3A activity levels were not different between the specialist and generalist fed control or oak diets (Table 3).

*UDP-Glucuronosyltransferases* Levels of UGT activity were constitutively different between the specialist and generalist (Fig. 3). UGT activity in the specialist was nearly two times higher compared to the generalist. Levels of UGT did not change with diet treatment groups and remained higher in the oak specialist compared to the generalist (Table 3).

*Sulfotransferase* Levels of SULT activity were constitutively different between the specialist and generalist (Fig. 3). SULT activity in the generalist was more than two times higher than the specialist in both treatments (Table 3). There was no change in SULT activity by either the specialist or the generalist from control to oak diets.

**Table 2** Summary of *F*-values, degrees of freedom (*df*) and *P* values for ANOVA analysis of body mass and ANCOVA analyses of food intake and liver mass among *N. macrotis* and *N. lepida* in two treatment groups: control diet and 70% oak diets

| ANOVA/ANCOVA | | | |
|---|---|---|---|
| Source of variation | *F* | *df* | *P* |
| Body mass | | | |
| Species | 8.555 | 1,26 | 0.007 |
| Diet | 0.006 | 1,26 | 0.939 |
| Species X diet | 0.043 | 1,26 | 0.836 |
| Dry matter intake | | | |
| Species | 0.137 | 1,25 | 0.714 |
| Diet | 78.10 | 1,25 | <0.001 |
| Species X diet | 1.281 | 1,25 | 0.268 |
| Covariate (body mass) | 0.070 | 1,25 | 0.793 |
| Liver mass | | | |
| Species | 2.918 | 1,25 | 0.100 |
| Diet | 5.237 | 1,25 | 0.031 |
| Species X diet | 0.136 | 1,25 | 0.716 |
| Covariate (body mass) | 31.89 | 1,25 | <0.001 |

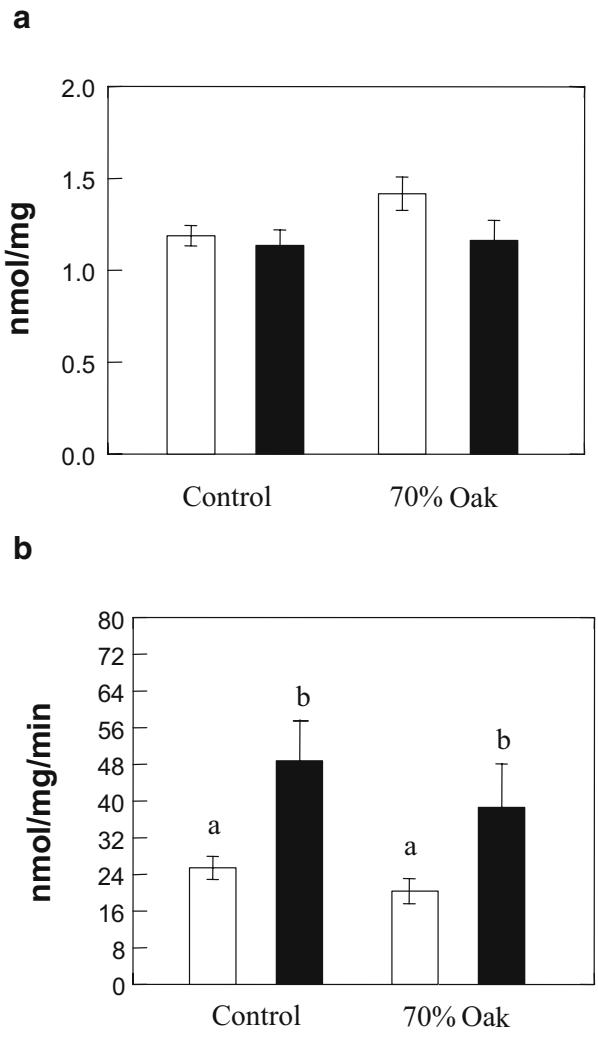


**Fig. 1** Hepatic Phase I enzymes (mean±SE) in the specialist, *N. macrotis* (*filled bar*) and the generalist, *N. lepida* (*open bar*) fed control diets or diets containing 70% oak (*Quercus agrifolia*): **a** Cytochrome P450 Concentration; **b** NAD(P)H/quinone oxidoreductase (QOR) activity. Data are represented as bars and different letters (*A* and *B*) denote means that are significantly different ($P<0.05$) as determined by Fisher's LSD

*Glutathione Transferase* Levels of constitutive GST activity were significantly higher in the specialist compared to the generalist (Table 3). There was a change in GST activity in the generalist when consuming the oak diet treatment (Fig. 3). The generalist significantly decreased GST activity by approximately 25% from the control diet compared to the oak diet treatment group (Table 3).

## Discussion

Previous research suggests that mammalian xenobiotic metabolism is the primary physiological mechanism that permits specialist feeders to consume high levels of a limited class of plant secondary compounds (PSC). We predicted that the mammalian oak specialist would have higher activities of Phase I xenobiotic metabolizing enzymes and

**Table 3** Summary of *F* values, degrees of freedom (*df*) and *P* values for ANOVA analysis of xenobiotic metabolizing enzyme assays among *N. macrotis* and *N. lepida* in two treatment groups: control diet and 70% oak diets

| ANOVA | | | |
|---|---|---|---|
| Source of variation | *F* | *df* | *P* |
| QOR | | | |
| Species | 18.90 | 1,26 | <0.001 |
| Diet | 0.777 | 1,26 | 0.386 |
| Species X diet | 0.059 | 1,26 | 0.810 |
| Cytochrome P450 concentration | | | |
| Species | 3.46 | 1,26 | 0.074 |
| Diet | 2.39 | 1,26 | 0.134 |
| Species X diet | 1.515 | 1,26 | 0.229 |
| CYP1A (EROD) | | | |
| Species | 10.006 | 1,26 | 0.004 |
| Diet | 1.184 | 1,26 | 0.287 |
| Species X diet | 0.824 | 1,26 | 0.372 |
| CYP2B (PROD) | | | |
| Species | 1.462 | 1,26 | 0.237 |
| Diet | 15.02 | 1,26 | 0.001 |
| Species X diet | 0.023 | 1,26 | 0.881 |
| CYP3A | | | |
| Species | 0.431 | 1,26 | 0.517 |
| Diet | 0.469 | 1,26 | 0.500 |
| Species X diet | 1.456 | 1,26 | 0.238 |
| UGT | | | |
| Species | 74.95 | 1,26 | <0.001 |
| Diet | 0.365 | 1,26 | 0.551 |
| Species X diet | 0.681 | 1,26 | 0.417 |
| SULT | | | |
| Species | 19.78 | 1,26 | <0.001 |
| Diet | 1.594 | 1,26 | 0.218 |
| Species X diet | 1.783 | 1,26 | 0.193 |
| GST | | | |
| Species | 72.23 | 1,26 | <0.001 |
| Diet | 6.247 | 1,26 | 0.019 |
| Species X diet | 1.057 | 1,26 | 0.313 |

lower activities of Phase II enzymes than the generalist. However, both the specialist and generalist had high levels of different Phase I enzyme activities. Furthermore, the specialist had higher overall levels of Phase II enzyme activities than the generalist in two of the three Phase II enzymes assayed. There were few differences in enzyme activity levels from control diet to an oak diet. There was only one enzyme induced by oak diet, CYP2B, and it by little more than 20% in both specialist and generalist. In addition, the generalist appeared to suppress GST activities by approximately 30% on an oak diet, although there was not a significant interaction effect. There were major differences in constitutive enzyme activity between the species. The specialist, *N. macrotis*, had higher activity of QOR, UGT, and GST. QOR and UGT activities were both twofold higher, and GST activities were 37% higher than the generalist when both were consuming control diet. When both species were consuming an oak diet, GST activities were twofold higher in the specialist than the generalist. The generalist, *N. lepida*, had higher constitutive activity levels of CYP1A and SULT. CYP1A activities were approximately 30% higher, and SULT activities nearly threefold higher than the specialist.

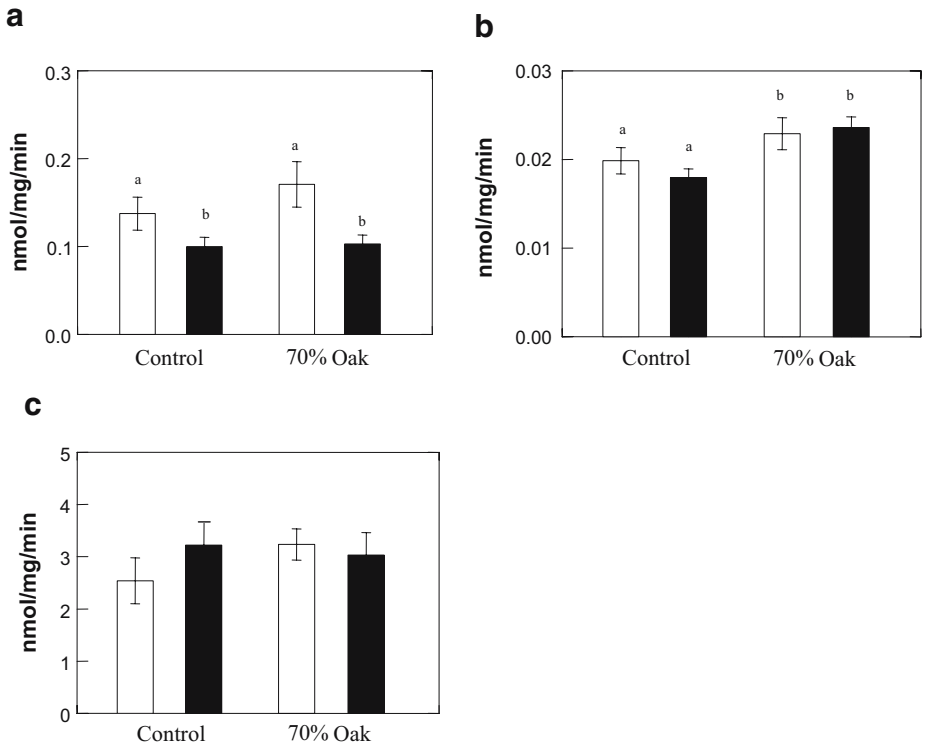


**Fig. 2** Hepatic cytochrome P450 isozyme activities (mean±SE) in the specialist, *N. macrotis* (*filled bar*), and the generalist, *N. lepida* (*open bar*), fed control diets and diets containing 70% oak (*Quercus agrifolia)*: **a** Isozyme CYP1A; **b** Isozyme CYP2B; **c** Isozyme CYP3A. Data are represented as the mean+SE. Bars with different letters (*A* and *B*) denote means that are significantly different ($P<0.05$) as determined by Fisher's LSD

Oak expresses high levels of phenolic PSC (Atsatt and Ingram 1983). Phenolics, including tannins, are known to be deterrent or toxic to herbivores. Their toxic effects have been demonstrated in a number of plant–herbivore interactions, and protective mechanisms are required to allow for their consumption (Feeny 1970; Haukioja et al. 1985; Rhoades 1985; Rossiter et al. 1988). Tannins may act as digestability reducers and bind dietary protein during digestion, resulting in elevated levels of fecal nitrogen. Since both species have similar nitrogen retention efficiencies, the digestability reducing effect of tannins and the level of protection provided by proline-rich salivary proteins is also likely similar (Atsatt and Ingram 1983). Tannins are only a portion of the total polyphenols that have toxic potential in oak. Furthermore, their effect is not limited to digestability reduction because they can be degraded and absorbed into circulation resulting in toxicity. In either case, xenobiotic metabolizing enzymes may either enhance or reduce the harmful effects of oak phenolics (Foley and McArthur 1994).

The fate of most absorbed phenolics involves biotransformation by Phase I oxidation (most notably carried out by cytochrome P450s). CYP1A was 30% higher in the generalist compared to the oak specialist, but activity was not induced with oak diet. A number of plant compounds including caffeine, indole-3-carbinol, and warfarin are metabolized by CYP1A (Ayalogu et al. 1995; Vang et al. 1999; Kuo et al. 2006). CYP2B was induced by around 20% in both the specialist and the generalist. Elevation of this enzyme on an oak

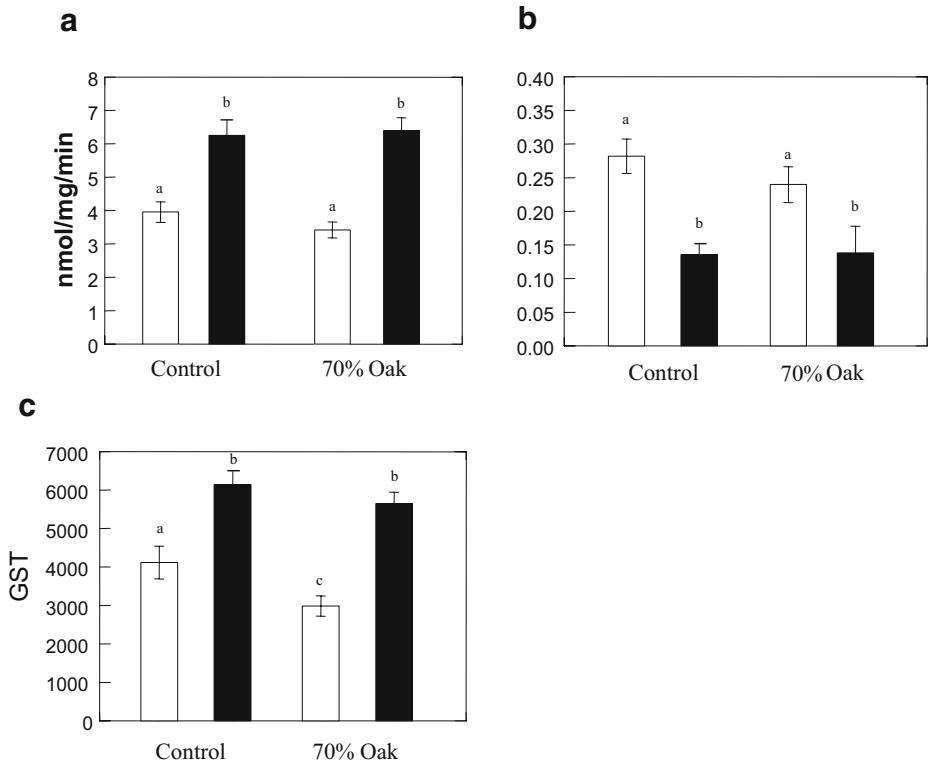


**Fig. 3** Hepatic conjugation enzyme activities (mean±SE) in the specialist, *N. macrotis* (*filled bar*), and the generalist, *N. lepida* (*open bar*), fed control diets and diets containing 70% oak (*Quercus agrifolia*): **a** UDP-glucuronosyltransferase (UGT); **b** Sulfotransferase (SULT); **c** Glutathione transferase (GST). Data are represented as the mean+SE. Bars with different letters (*A*, *B* and *C*) denote means that are significantly different ($P<0.05$) as determined by Fisher's LSD

diet rich in polyphenolics is somewhat surprising because a number of plant phenolics (e.g., tannic, ellagic, and protocatechuic acids) are known to inhibit the activity of CYP2B (Szaefer et al. 2003).

The induction of CYP2B by both the specialist and generalist indicates that both have similar capacities to induce this enzyme when consuming an oak diet. In a field study, the specialist had a higher rate of CYP2B activity, as measured by in vivo hexobarbital clearance rates, than the generalist (Dearing et al. 2006). The enhanced ability of the specialist to metabolize hexobarbital under field conditions is not surprising due to the greater consumption of oak in the wild. Therefore, while the generalist consumes mostly *Opuntia occidentalis*, cactus, and *Salvia apiana* in its natural habitat, it would have lower levels of CYP2B activity. However, the activities observed here indicate that the generalist has the capacity, when consuming oak, to increase CYP2B activity to an extent similar to the oak specialist.

The differences in the pattern of conjugation enzymes used by the two species could explain their natural diet preferences. The oak specialist had higher constitutive activity levels of UGT, whereas the generalist had higher levels of SULT. Species variation in the rates of glucuronidation (UGT) and sulfation (SULT) can lead to differences in tolerance for phenolic compounds because there are differences in capacity for conjugation between these two Phase II enzymes (Parkinson 1996). SULT requires PAPS (3′-phosphoadenosine-

5′-phosphosulfate) as a sulfate donor to conjugate xenobiotics, and PAPS is in limited supply. Thus, SULT activity in vivo has a relatively low capacity. UGT's cofactor, UDP-glucuronic acid, is not as limited as PAPS, and UGT has a higher capacity as a result (Klaassen and Boles 1997). This difference may be more profound when oak is a constant diet component. The cost or toxic effect of chronic intake of a particular compound can be different from that produced by short-term exposure, particularly with regard to cofactor abundance. In a diet containing high levels of phenolics that are consumed chronically, the difference may alter a species' ability to specialize.

While the constitutive activity differences may explain oak tolerance, any inference regarding these differences should be viewed cautiously, as they were not altered by a change from control to oak diet. Many of the differences measured between the specialist and generalist were constitutive. These may be due to species phylogenetic distance and not necessarily short-term dietary behavior. The fact that few of the enzymes analyzed were affected by the change from control diet to oak diet constricts inference concerning which enzymes metabolize the PSC in oak. Many xenobiotic metabolizing enzymes are also important in the metabolism of endogenous compounds (e.g., hormones, bilirubin, and bile acids; Parkinson 1996). Therefore, while differences in xenobiotic enzymes likely influence oak consumption, assays to look specifically at oak phenolic metabolism as influenced by these specific enzymes will be necessary. The reduced size of the liver in both the specialist and generalist may indicate that mechanisms other than hepatic xenobiotic metabolizing enzymes are involved. For example, some intestinal proteins (multi-drug resistant proteins) prevent the absorption of compounds into circulation (Bodo et al. 2003).

Although a theory has emerged may postulate that specialists rely more heavily on Phase I than Phase II xenobiotic metabolizing enzymes in comparison to generalists, this research indicates that it is not a widespread pattern broadly applied to all specialist/generalist comparisons. Although this study included many of the major xenobiotic metabolizing enzymes used by mammals, and although we found differences between the specialist and generalist species, future work with inhibitors and cloning of specific genes may be necessary to tease apart the role these enzymes play.

**Acknowledgement** We thank committee members Dr. G. Yost, Dr. F. Goller, Dr. D. Feener for their invaluable input. We also thank Dr. J. Sorensen, Dr. M. Skopec and Ann-Marie Torregrossa for critical reading of the manuscript and the undergraduates in Dr. Dearing's lab, especially Katie Young and John Matthews, for assistance with feeding, tissue collection as well as collection and maintenance of woodrats. Research was supported by National Science Foundation (NSF IBN 023402).

## References

ATSATT, S. R., and INGRAM, T. 1983. Adaptation to oak and other fibrous, phenolic-rich foliage by a small mammal, *Neotoma fuscipes*. *Oecologia* 60:135–142.

AYALOGU, E. O., SNELLING, J., LEWIS, D. F., TALWAR, S., CLIFFORD, M. N., and IOANNIDES, C. 1995. Induction of hepatic CYP1A2 by the oral administration of caffeine to rats: lack of association with the Ah locus. *Biochim. Biophys. Acta* 1272:89–94.

BENSON, A. M., HUNKELER, M. J., and TALALAY, P. 1980. Increase of NAD(P)H:quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. *Proc. Natl. Acad. Sci. USA* 77:5216–20.

BOCK, K. W., BURCHELL, B., DUTTON, G. J., HANNINEN, O., MULDER, G. J., OWENS, I. S., SIEST, G., and TEPHLY, T. R. 1983. UDP-glucuronosyltransferase activities. Guidelines for consistent interim terminology and assay conditions. *Biochem. Pharmacol*. 32:953–5.

BODO, A., BAKOS, E., SZERI, F., VARADI, A., and SARKADI, B. 2003. The role of multidrug transporters in drug availability, metabolism and toxicity. *Toxicol. Lett.* 140–141:133–143.

BOLTON, R. M. and AHOKAS, J. T. 1997. Ontogenetic expression of detoxification enzymes in an Australian marsupial, the brushtail possum (*Trichosurus vulpecula*). *Comp. Biochem. Physiol.* 118B:239–247.

BOYLE, R., MCLEAN, S., FOLEY, J. E., and DAVIES, N. W. 1999. Comparative metabolism of dietary terpene, *p*-cymene, in generalist and specialist folivorous marsupials. *J. Chem. Ecol.* 25:2109–2127.

BOYLE, R., MCLEAN, S., FOLEY, W., DAVIES, N.W., PEACOCK, E.J., and MOORE, B. 2001. Metabolites of dietary 1,8-cineole in the male koala (*Phascolarctos cinereus*). *Comp. Biochem. Physiol. C Toxicol. Pharmacol*. 129:385–95.

CAMERON, G. N. 1971. Niche overlap and competition in woodrats. *J. Mammal.* 52:111.

DANIEL, V. 1993. Glutathione S-transferases: gene structure and regulation of expression. *Crit. Rev. Biochem. Mol. Biol.* 28:173–207.

DANIELSON, P. B. 2002. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. *Curr. Drug Metab.* 3:561–97.

DEARING, M. D., SKOPEC, M. M., and BASTIANI, M. J. 2006. Detoxification rates of wild herbivorous woodrats (*Neotoma*). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 145: 419–422.

DUTTON, G. J. 1980. Glucuronidation of Drugs and Other Compounds. Boca Raton: CRC Press.

FEENY, P. P. 1970. Seasonal changes in Oak leaf tannins and nutrients as a cause of spring feeding in winter-moth caterpillars. *Ecology* 51:656–681.

FOLEY, W. J., and MCARTHUR, C. 1994. The effects and costs of allelochemicals for mammalian herbivores: an ecological perspective, pp. 370–391, in D. J. Chivers, P. Langer (ed.). The Digestive System in Mammals: Food, Form and Function. Cambridge: Cambridge University Press.

FRANKLIN, M. R., and ESTABROOK, R. W. 1971. On the inhibitory action of mersalyl on microsomal drug oxidation: a rigid organization of the electron transport chain. *Arch. Biochem. Biophys.* 143: 318–29.

FREELAND, W. J., and JANZEN, D. H. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. *Am. Naturalist* 108:269–289.

GREGUS, Z., WATKINS, J. B., THOMPSON, T. N., HARVEY, M.J., ROZMAN, K., and KLAASSEN, C. D. 1983. Hepatic phase I and phase II biotransformations in quail and trout: comparisons to other species commonly used in toxicity testing. *Toxicol. Appl. Pharmacol.* 67:430–441.

GUENGERICH, F. P., MULLER-ENOCH, D., and BLAIR, I. A. 1986. Oxidation of quinidine by human liver cytochrome P-450. *Mol. Pharmacol.* 30:287–95.

HABIG, W. H. and JAKOBY, W. B. 1981. Assays for differentiation of glutathione S-transferases. *Methods Enzymol.* 77:398–405.

HAUKIOJA, E., NIEMELA, P., and SIREN, S. 1985. Foliage phenols and nitrogen in relation to growth, insect damage, and ability to recover after defoliation, in the mountain birch *Betula pubescens* ssp. *tortuosa*. *Oecologia* 65:214–222.

HIROI, T., MIYAZAKI, Y., KOBAYASHI, Y., IMAOKA, S., and FUNAE, Y. 1995. Induction of hepatic P450s in rat by essential wood and leaf oils. *Xenobiotica* 25:457–67.

IASON, G. R. and MURRAY, A. H. 1996. The energy costs of ingestion of naturally occurring nontannin plant phenolics by sheep. *Physiol. Zool.* 69:532–546.

KLAASSEN, C. D. and BOLES, J. W. 1997. Sulfation and sulfotransferases 5: the importance of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) in the regulation of sulfation. *FASEB J* 11:404–18.

KLOTZ, A. V., STEGEMAN, J. J., and WALSH, C. 1984. An alternative 7-ethoxyresorufin O-deethylase activity assay: a continuous visible spectrophotometric method for measurement of cytochrome P-450 monooxygenase activity. *Anal. Biochem.* 140:138–45.

KUO, Y. H., LIN, Y. L., DON, M. J., CHEN, R. M., and UENG, Y. F. 2006. Induction of cytochrome P450-dependent monooxygenase by extracts of the medicinal herb *Salvia miltiorrhiza*. *J. Pharm. Pharmacol.* 58:521–7.

LINDROTH, R. L., and BATZLI, G. O. 1984. Plant phenolics as chemical defenses: effects of natural phenolics on survival and growth of prairie voles (*Microtus ochrogaster*). *J. Chem. Ecol.* 10:229–244.

LIUKKONEN-ANTTILA, T., HONKANEN, H., PELTOKANGAS, P., PELKONEN, O., and HOHTOLA, E. 2003. Cytochrome P450 enzyme activity in five herbivorous, non-passerine bird species. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 134:69–77.

LOWRY, O. H. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265–275.

LUBET, R. A., MAYER, R. T., CAMERON, J. W., NIMS, R. W., BURKE, M. D., WOLFF T., and GUENGERICH, F. P. 1985. Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome (s) P-450 by phenobarbital and other xenobiotics in the rat. *Arch. Biochem. Biophys.* 238:43–8.

MARSH, K. J., WALLIS, I. R., ANDREW, R. L., and FOLEY, W. J. 2006. The Detoxification Limitation Hypothesis: Where Did it Come From and Where is it Going? *J. Chem. Ecol*. 32:1247–1266.

MATOCQ, M. D. 2002. Morphological and molecular analysis of a contact zone in the *Neotoma fuscipes* species complex. *J. Mammal.* 83:866–883.

MCLEAN, S., and FOLEY, J. E. 1997. Metabolism of *Eucalyptus* terpenes by herbivorous marsupials. *Drug Metab. Rev.* 29:213–218.

McLean, S., Foley, W. J., Davies, N. W., Brandon, S., Duo, L., and Blackman, A. J. 1993. Metabolic fate of dietary terpenes from *Eucalyptus radiata* in common ringtail possum (*Pseudocheirus peregrinus*). *J. Chem. Ecol.* 19:1625–1643.

McLean, S., Pass, G. J., Foley, W. J., Brandon, S., and Davies, N. W. 2001. Does excretion of secondary metabolites always involve a measurable metabolic cost? Fate of plant antifeedant salicin in common brushtail possum, *Trichosurus vulpecula. J. Chem. Ecol.* 27:1077–89.

Omura, T., and Sato, R. 1964. The carbon monoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* 239:2370–2378.

Parkinson, A. 1996. Biotransformation of xenobiotics. pp. 113–186 *in*: Klaassen CD, editor. Casarett and Doull's Toxicology the Basic Science of Poisons. 5th ed. New York: McGraw-Hills.

Pass, G. J., McLean, S., and Stupans, I. 1999. Induction of xenobiotic metabolising enzymes in the common brushtail possum, *Trichosurus vulpecula*, by *Eucalyptus* terpenes. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* 124:239–246.

Pass, G. J., McLean, S., Stupans, I., and Davies, N. 2001. Microsomal metabolism of the terpene 1,8-cineole in the common brushtail possum (*Trichosurus vulpecula*), koala (*Phascolarctos cinereus*), rat and human. *Xenobiotica* 31:205–221.

Rhoades, D. F. 1985. Offensive–defensive interactions between herbivores and plants: their relevance in herbivore population dynamics and ecological theory. *Am. Naturalist* 125:205–238.

Rossiter, M., Schultz, J. C., and Baldwin, I. T. 1988. Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. *Ecology* 69:267–277.

Sekura, R. D., Duffel, M. W., and Jakoby, W. B. 1981. Aryl sulfotransferases. *Methods Enzymol.* 77:197–206.

Sivapathasundaram, S., Magnisali, P., Coldham, N. G., Howells, L. C., Sauer, M. J., and Ioannides C. 2003a. Cytochrome P450 expression and testosterone metabolism in the liver of deer. *Toxicology* 187:49–65.

Sivapathasundaram, S., Sauer, M. J., and Ioannides, C. 2003b. Xenobiotic conjugation systems in deer compared with cattle and rat. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 134:169–73.

Stupans, I., Kong, S., Kirlich, A., Murray, M., Bailey, E. L., Jones, B. R., and Mckinnon, R. A. 1999. Hepatic microsomal enzyme activity in the koala and tammar wallaby: high 17 beta-hydroxysteroid oxidoreductase activity in koala liver microsomes. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* 123:67–73.

Szaefer, H., Jodynis-Liebert, J., Cichocki, M., Matuszewska, A., and Baer-Dubowska, W. 2003. Effect of naturally occurring plant phenolics on the induction of drug metabolizing enzymes by o-toluidine. *Toxicology* 186:67–77.

Thomas, D. W., Samson, C., and Bergeron, J. 1988. Metabolic costs associated with the ingestion of plant phenolics by *Microtus pennsylvanicus*. *J. Mammal.* 69:512–515.

Vang, O., Frandsen, H., Hansen, K. T., Nielsen, J. B., and Andersen, O. 1999. Modulation of drug-metabolising enzyme expression by condensation products of indole-3-ylcarbinol, an inducer in cruciferous vegetables. *Pharmacol. Toxicol.* 84:59–65.