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Behavioral Responses of a Generalist Mammalian Folivore to the Physiological Constraints of a Chemically Defended Diet

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Abstract Mammalian herbivores, particularly browsers and folivores, encounter and consume a range of plant chemical defenses [plant secondary metabolites (PSMs)] on a regular basis. The physiological regulation of PSM ingestion and the resulting behavioral responses of mammalian herbivores directly affect their feeding decisions and the subsequent foraging strategies that they adopt. Generalist mammalian herbivores are hypothesized to consume a generalized diet because of physiological limitations of their detoxification systems. The consumption of a generalized diet is proposed to enable toxin (PSM) dilution through the use of multiple detoxification pathways. We tested the predictions of the detoxification–limitation hypothesis by offering two chemically different plant species, *Eucalyptus regnans* and *E. globulus*, to a generalist mammalian folivore, the common brushtail possum (*Trichosurus vulpecula*), as single- and mixed-species diets. By feeding more efficiently, brushtail possums benefited more, through increased intake, on the mixed-species diet than on either of the single-species diets. We argue that frequently switching between chemically diverse foliage reduces the physiological constraints imposed by a PSM-rich diet and enables more efficient feeding. The

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behavioral responses of brushtail possums were consistent with the proposed physiological constraints of a chemically defended diet, offering support for predictions of the detoxification–limitation hypothesis. We suggest that feeding behavior of herbivores may be a useful indicator of the physiological constraints imposed by a chemically defended diet.

Keywords Brushtail possum · Detoxification limitations · Eucalypt · Feeding behavior · Herbivore · Plant secondary metabolite

Introduction

By consuming plants, herbivores encounter a range of plant physical and chemical defenses (Feeny, 1970; Bryant and Kuropat, 1980). Plant chemical defenses, or plant secondary metabolites (PSMs), predominantly occur throughout woody plants as highly diverse compounds, both structurally and functionally. Ingestion of PSMs can incur physiological costs that include digestibility reduction (Robbins et al., 1987), toxicity (Pfister et al., 1997), and acidosis (Foley, 1992). To exploit woody plants, mammalian browsers (e.g., roe deer, *Capreolus capreolus*; Tixier and Duncan, 1996) and folivores (e.g., common brushtail possum, *Trichosurus vulpecula*; Kerle, 1984) must be able to process and deal with these chemical defenses. Mammalian browsers and folivores (hereafter referred to as herbivores) are indeed able to subsist on a chemically defended diet through a range of physiological and behavioral adaptations (Cork and Foley, 1991; McArthur et al., 1991).

Following ingestion and absorption, most PSMs must be detoxified to reduce the detrimental effects that they pose. Mammalian herbivores have evolved a series of detoxification pathways that convert absorbed PSMs into more hydrophilic and polar compounds through oxidation, reduction, or hydrolysis (phase I reactions), making them easier to excrete and/or preparing them for conjugation with an endogenous molecule (phase II reactions), again enhancing excretion (Sipes and Gandolfi, 1991). However, there are several limitations of these detoxification processes. Detoxification pathways rely on a series of rate-limited reactions, which, in turn, govern how much a herbivore can ingest per unit of time (Freeland and Janzen, 1974). Mammalian herbivores have been shown to regulate PSM ingestion at or below a proposed threshold level (Pfister et al., 1997; Lawler et al., 1998; Boyle and McLean, 2004). The inability to tightly regulate ingestion below a particular threshold may result in toxicosis, internal malaise, and, if severe enough, may even be fatal to the animal (Freeland and Janzen, 1974). Whereas most PSMs must be detoxified to aid in their excretion, the reactions involved can generate strong organic acids (Sipes and Gandolfi, 1991), which themselves may cause disturbances to a herbivore's acid–base status and, subsequently, disruptions to physiological and biochemical processes (Chilcott and Hume, 1984; Foley, 1992). All acid metabolites must, therefore, be buffered and excreted from the body (Foley et al., 1995). Detoxification of PSMs, through associated metabolic and excretory pathways, is an energetically expensive process (Cork and Foley, 1991; Freeland, 1991).

The capacity to which herbivores efficiently detoxify PSMs governs their subsequent feeding strategies. Only when a herbivore is able to minimize the energetic costs of detoxification (Sorensen et al., 2005), and efficiently metabolize and excrete ingested PSMs (Boyle et al., 1999), is it able to specialize on a relatively

homogenous diet. A generalized feeding strategy is by far the most common feeding approach adopted by mammalian browsers and folivores (Freeland, 1991). The consumption of a generalized diet is hypothesized to ensure adequate nutritional balance (Westoby, 1978). Acting over a much shorter timeframe, the detoxification–limitation hypothesis proposes that a generalized diet of chemically diverse plants enables toxin dilution, through the use of multiple detoxification pathways (Freeland and Janzen, 1974). Consistent with this hypothesis, research has shown that generalist folivores perform better on mixed than single forage diets through increased intake (Dearing and Cork, 1999; Wiggins et al., 2003). Explicit testing of the detoxification–limitation hypothesis is difficult because of limited knowledge of the mechanisms by which herbivores detoxify specific PSMs (Marsh et al., 2005).

Predictions of the detoxification–limitation hypothesis offer insight and understanding into the foraging decisions made by generalist mammalian herbivores. Consumption of a PSM-rich diet imposes direct physiological constraints to the herbivore. These constraints, in turn, should govern how generalists behaviorally respond to their diet, through the selection and mixing of different items. We have recently demonstrated that a folivore's rate of intake, and the frequency of switching between two chemically different plant species, was essential for maximizing intake (Wiggins et al., 2006). These feeding strategies were hypothesized to be directly related to the folivore's blood plasma PSM concentrations, a direct physiological constraint to the herbivore. A herbivore's feeding behavior is likely to be pivotal in how it responds to its dietary physiological constraints and could be used as an indicator of such constraints. Thus, the simultaneous investigation of three factors (intake, physiological responses, and behavioral responses) of herbivores to a mixed diet should enable us to evaluate predictions of the detoxification–limitation hypothesis. We know of no previous studies that have directly integrated these three components concurrently. Such integration is essential for the development of a more thorough understanding of the feeding decisions, and ultimately the foraging strategies, adopted by generalist mammalian herbivores.

We set out to test predictions of the detoxification–limitation hypothesis, in an attempt to explain why generalist mammalian herbivores are able to perform better on a chemically diverse diet than on a single species diet. We offered two chemically different plant species, *Eucalyptus regnans* and *E. globulus* (Wiggins et al., 2006), to a generalist mammalian folivore, the common brushtail possum (*T. vulpecula*), as single- and mixed-species diets. The four specific aims of this study were to (1) demonstrate differences in chemistry between the two eucalypt species; (2) determine whether intake was greater on the mixed- than single-species diets; (3) compare urinary characteristics between diet treatments as a crude indicator of the physiological status of the animals (e.g., pH, titratable ions, ammonia, urea); and (4) quantify differences in feeding behavior relative to maximizing intake.

Methods and Materials

Animals and Diet

Six adult brushtail possums (*T. vulpecula*), three males and three females [3.34 ± 0.59 kg (body weight mean ± SD)], were collected from Hobart (Tasmania,

Australia), housed in a covered outdoor enclosure in individual mesh cages (4.3 × 1.7 × 2.5 m) at the School of Zoology, University of Tasmania, and provided with a nest box and logs for above-ground runways. Animals were maintained on a basal diet that was 18% dry matter (DM), consisting of [as % fresh matter (FM)] 46% apple, 35% silver beet, 10% carrot, 5% lucerne (ground to pass through a 1-mm sieve), and 4% raw sugar. Fruit and vegetable constituents were mixed in a food processor, then combined with dry ingredients. Food was provided at levels sufficient for maintenance (McArthur et al., 2000) and so was freshwater daily.

Feeding Trial

Possums were initially offered 50% of maintenance requirements of a basal diet and *E. regnans* and *E. globulus* sapling foliage *ad libitum* for 5 d, 1 wk before the trial. They received 120% basal diet two nights directly before the trial. Foliage was collected from Geeveston, Tasmania, and stored with stems in freshwater in a 5°C cool room. The feeding trial consisted of three treatment diets fed to possums in a balanced crossover design (Ratkowsky et al., 1993). The treatment diets were (1) *E. regnans* and (2) *E. globulus* single-species diets and (3) *E. regnans* and *E. globulus* mixed diet. Foliage was fed *ad libitum* in 300-g bunches, provided fresh each day. Each treatment was run for 4 consecutive nights, and immediately followed by 4 "rest" nights where possums were offered 120% basal diet to ensure they maintained body weight during the trial. Foliage intake was measured daily and expressed as grams dry matter per kilogram of body mass for each possum [g DM $(\text{kg BM})^{-1}$].

Foliage Properties

Two control bunches of foliage per species per night were pooled and subsampled for physical and chemical analyses to confirm that foliar secondary chemical properties differed. Foliage was assayed for DM, leaf toughness, nitrogen, fiber, oils, and phenolics.

Dry Matter

Subsamples of foliage were oven-dried at 55°C for 48 hr to determine percentage dry matter (% DM).

Leaf Toughness

Five leaves per species per night were sampled at three sections of the leaf (upper, middle, and lower) by using a dual tension gauge portable penetrometer (Chatillon, John Chatillon & Sons, Inc., NY, USA). Units are an index only, expressed as total grams of force required to puncture the thickness of foliage using a 1-g, 0.60-mm pin (Sands and Brancatini, 1991).

Nitrogen

Foliage was oven-dried at 55°C for 48 hr and ground to pass through a 1-mm sieve by using a cyclone grinder, then further oven-dried at 70°C for 24 hr. Following this,

a sulfuric acid and hydrogen peroxide digest was performed following methods of Lowther (1980). Digested samples were colorimetrically analyzed for nitrogen (QuikChem reference 10-107-06-2E, Lachat Instruments, WI, USA) on a continuous flow injector analyzer (QuikChem 800, Lachat Instruments). Results are expressed as % DM.

Fiber

Ground samples were analyzed for plant cell-wall components of neutral detergent fiber, acid detergent fiber, and lignin following methods in the ANKOM$^{200/220}$ Technology Operator's Manual (1997). Results are expressed as % DM.

Total Oils

Fresh-frozen foliage was thawed prior to oil and phenolic assays. Oils were extracted following methods modified from Jones et al. (2002). Briefly, 1 g of foliage was cut into 1-cm^2 pieces and soaked in 10 ml dichloromethane for 1 hr. Extracts were analyzed by combined gas chromatography–mass spectrometry (GC-MS) as detailed in O'Reilly-Wapstra et al. (2004). Total ion currents were determined for the sum of all oil components (total oils) and the heptadecane internal standard. Results for total oils were standardized by dividing by the internal standard. Results are expressed as mg "cineole equivalents" per g DM.

Total Phenolics

One gram of foliage was cut into 1-cm^2 pieces, homogenized with a Polytron Homogenizer (POLYTRON® MR2100, Kinematica AG, Switzerland) in 20 ml acidified (pH 1) methanol (Close et al., 2001), and then boiled for 1.5 min. Samples were extracted overnight at 5°C in the dark, centrifuged at 10,000 rpm for 7 min, and analyzed by high-performance liquid chromatography, detailed in O'Reilly-Wapstra et al. (2004). Differences between major classes of phenolic compounds, the hydrolyzable tannins, and the formylated phloroglucinol compounds (FPCs) only were identified. Representative chromatograms of oils and phenolics were used as a visual reference to demonstrate differences in PSM composition between *E. regnans* and *E. globulus*.

Urine Collection

On night 4 of each treatment, four of the six possums (because of logistic constraints) were placed in individual metabolism cages (60 × 60 × 45 cm) for 24 hr for urine collection at the School of Pharmacy, University of Tasmania. Possums were fed with their allocated treatment diets of fresh foliage overnight. Urine was collected into containers held in liquid nitrogen and frozen immediately, then stored at −20°C until later analysis. Animals were acclimatized to the metabolism cages for a 24-hr period 1 week before commencement of the feeding trial. Urinary properties of pH, titratable ions, ammonia, and urea were measured for $N = 3$ possums because of low urinary output of one animal during the collection period. Ammonia and urea methods were modified methods of Faulkner and King (1970). The titratable ion variable was based on the concept of titratable acids (Foley, 1992).

pH

Urine samples were thawed and pH was measured with a TPS digital pH meter.

Titratable Ions

Either 0.5 M HCl (for basic urine solutions) or 0.01 M NaOH (for acidic urine solutions) was used to titrate urine samples to pH 7.4 (neutral). Titrations were performed at 20°C, and results are expressed as mmol l^{-1} to pH 7.4.

Ammonia

Tubes were set up with 0.1, 0.4, and 1.0 ml of 1:10 dilutions of urine and made up to 1.0 ml with deionized H_2O. Tubes containing a standard ammonium sulfate solution [800 nmol NH_4^+ ml^{-1}; derived from 53 ml of 0.1% ammonium sulfate (Ajax Chemicals, Sydney, Australia) solution diluted in 1 l deionized H_2O] were also set up. To each tube, 5 ml of phenol–nitroprusside reagent [10.0 g phenol (May & Barker Ltd., Dagenham, England) and 0.05 g sodium nitroprusside (May & Baker Ltd.) dissolved in 1 l deionized H_2O] were added and mixed by vortex. Following this, 5 ml of NaOH–hypochlorate reagent [5.0 g NaOH (Sigma, St. Louis, MO, USA) and 44 ml of 4% sodium hypochlorite (Aldrich, Milwaukee, WI, USA) dissolved in 1 l deionized H_2O] were added to tubes and mixed by vortex. Tubes were incubated at 37°C for 30 min, and absorbance was read at 570 nm against the reagent blank. Results are expressed as μmol d^{-1}.

Urea

Tubes were set up with 0.1, 0.4, and 1.0 ml of 1:500 dilutions of urine and made up to 1.0 ml with deionized H_2O. Tubes containing a standard urea solution [250 nmol ml^{-1}; derived from 30.0 mg urea (AnalaR®, Kilsyth, Australia) diluted in 2 l deionized H_2O] were also set up. To each tube, 0.1 ml of urease suspension {100.0 mg urease [Sigma] dissolved in 100 ml phosphate–EDTA buffer [made from 2.0 g Na_2EDTA (BDH Chemicals Ltd., Poole, England) and 5.7 g anhydrous Na_2HPO_4 (Ajax Chemicals, Auburn, Australia) dissolved in 100 ml deionized H_2O, adjusted to pH 6.5 with 1 M HCl (Ajax Chemicals), then made up to 200 ml with deionized H_2O]}, stored on ice, were added and mixed by vortex, then incubated at 37°C for 15 min. Following this, 5 ml of phenol–nitroprusside reagent were added to tubes and mixed, followed by 5 ml of NaOH–hypochlorate reagent (see *Ammonia* in Methods and Materials), with tubes mixed by vortex. Tubes were incubated at 37°C for 30 min, and absorbance was read at 570 nm against the reagent blank. Results are expressed as μmol d^{-1}.

Feeding Behavior

Feeding behavior was measured for all six possums from nights 1–3. Animals were filmed nightly from 18:00 until 08:00 hr using individual B&W Bullet CCD Cameras (equipped with a Samsung™ sensor, JayCar, Hobart, Tasmania), one camera per cage. Each camera was connected to a Panasonic® Video Cassette Recorder (Series NV-FJ630). An 80-W red flood lamp (Osram Par 38, manufactured in EC) was

positioned in each cage for enhanced visibility. Video footage was recorded directly onto BASF E-300 cassette tapes by using extended long-play recording speed. Data from video footage were summarized using The Observer® (v 4.1, Noldus Information Technology, The Netherlands, 2002). Behavioral variables were calculated using The Observer® and Microsoft® Excel (Microsoft Corporation, 1997). These variables were (1) time from first to last feeding bout (incorporating nonfeeding activity); (2) total feeding time; (3) rate of intake; (4) number of feeding bouts; (5) time per feeding bout; (6) intake per feeding bout; (7) total number of switches; and (8) the frequency of feeding bouts within which switching occurred. A single feeding bout was defined as the time from the possum's first bite to the end of its last chew, with at least 1 min of nonfeeding directly following this last chew (Wiggins et al., 2006). Total number of switches was a count of switches between plant species, which could occur both during and between feeding bouts. The frequency of feeding bouts within which switching occurred was calculated by dividing the total number of "switching bouts" per night by the total number of feeding bouts for that night. Switching behavior could only be measured on the mixed diet. Results for all intake and behavioral variables are expressed as mean values ± standard errors (SE).

Statistical Analyses

The average of 3 d data for each treatment for all six possums was used in the analyses. Dependent variables of intake and aspects of feeding behavior were tested against the independent variables of possum, treatment, period, and carryover, using the general linear model procedure (PROC GLM) in SAS (SAS v6.12, SAS Institute Inc. 1989), following the methods of Ratkowsky et al. (1993). The Wilk-Shapiro statistic, normal probability plots, and standardized residual plots all indicated normality of the data. When an effect was significant, pairwise comparisons of the least-squares means were made by using the Tukey-Kramer adjustment. Data for three possums from d 4 were used for urine analysis. Dependent variables of intake, pH, titratable ions, ammonia, and urea were tested against the independent variables of possum, period, and treatment (PROC GLM, SAS Institute Inc. 1989). Differences between foliar physical and chemical properties of *E. regnans* and *E. globulus* were also tested (PROC GLM, SAS Institute Inc. 1989).

Results

Foliage Chemistry

Leaf percentage DM, leaf toughness, and nitrogen were similar between *E. regnans* and *E. globulus* foliage (Table 1). Of the primary constituents, only fiber differed between the two species (Table 1). Of the secondary metabolites, *E. regnans* contained higher levels of total oils than *E. globulus* (Table 1). Of the 15 major oil compounds identified, only one compound, bicyclogermacrene, was present in both plant species, with amounts similarly low in both species (Fig. 1). There were also major differences in phenolic profiles between plant species. The FPCs peaking between 9 and 11 min in *E. globulus* are not present in *E. regnans* (Fig. 2), whereas differences in the early major peaks indicate the presence of different hydrolyzable

Table 1 Physical and chemical constituents of sapling foliage of *Eucalyptus regnans* and *E. globulus*[a]

Constituent	Units	*E. regnans*	*E. globulus*	*F* value	*P* value
Dry matter	DM	43.1 ± 1.5	39.7 ± 1.5	$F_{1,23} = 2.50$	0.128
Toughness	Grams force	165.9 ± 3.8	171.1 ± 3.8	$F_{1,23} = 0.93$	0.345
Nitrogen	% DM	1.3 ± 0.1	1.2 ± 0.1	$F_{1,23} = 0.83$	0.371
NDF	% DM	32.4 ± 0.9	29.8 ± 0.0	$F_{1,23} = 4.18$	0.053
ADF	% DM	27.4 ± 0.6	21.4 ± 0.6	$F_{1,23} = 45.68$	0.001
Lignin	% DM	15.4 ± 0.8	5.9 ± 0.8	$F_{1,23} = 63.15$	0.001
Total oils	Cineole equivalents (mg g DM^{-1})	36.1 ± 4.7	22.1 ± 4.7	$F_{1,23} = 4.36$	0.049

NDF = neutral detergent fiber, ADF = acid detergent fiber.

[a]$N = 12$ for each species.

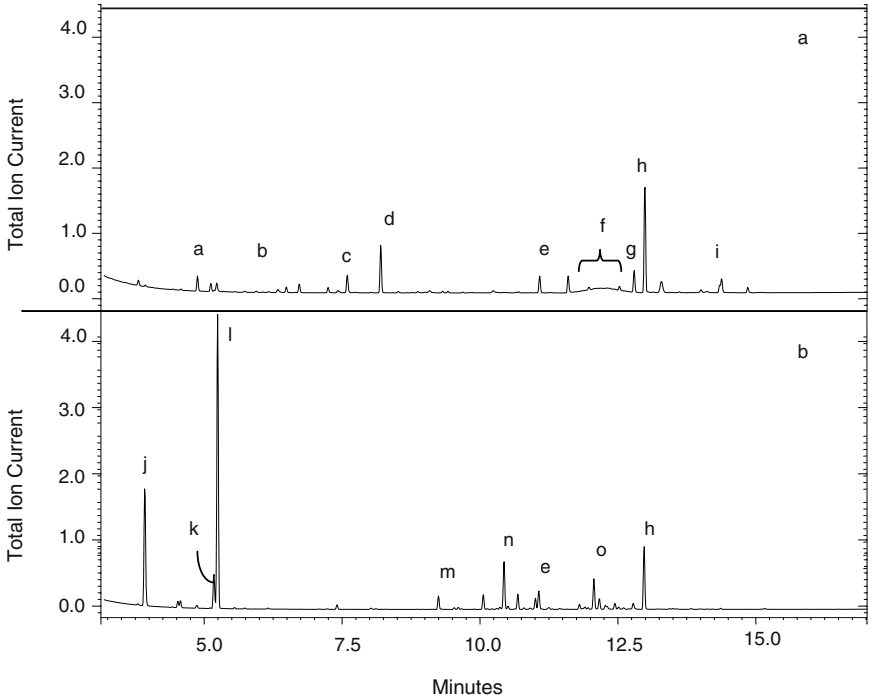

Fig. 1 Representative chromatograms of (a) *E. regnans* and (*b*) *E. globulus* oils derived from combined gas chromatography–mass spectrometry. Compounds are labeled with assigned letters, which stand for (a) α-phellandrene; (b) β-phellandrene; (c) piperitol; (d) piperitone; (e) bicyclogermacrene; (f) hedycaryol/elemol conversion; (g) β-eudesmol; (h) *n*-heptadecane (internal standard); (i) eudesmyl acetate; (j) α-pinene; (k) limonene; (l) 1,8-cineole; (m) terpinyl acetate; (n) aromadendrene; and (o) globulol

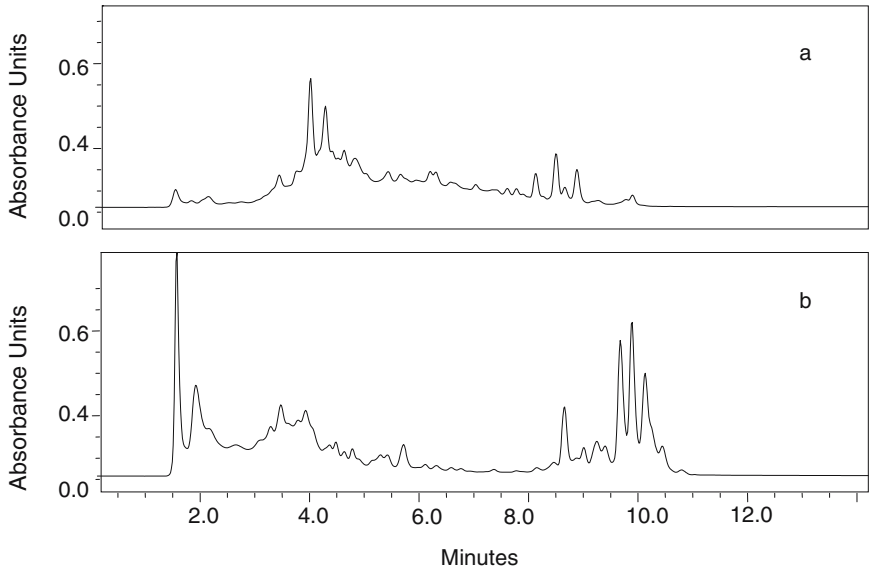

Fig. 2 Representative total phenolic profiles for (a) *E. regnans* and (b) *E. globulus* using high-performance liquid chromatography chromatograms at 280 nm

tannins in each species (peaks substantially differ from 1 to 4 min and from 4 to 6 min between the foliage species).

Intake

Possums consumed substantially more foliage on the mixed- than on the single-species diets ($F_{2,17}$ = 14.99, P = 0.001; Fig. 3).

Urinary Properties

Possums produced a slightly acidic urine on *E. globulus* foliage, alkaline on *E. regnans* foliage, and urine at relatively neutral pH on the mixed diet ($F_{2,8}$ = 9.20, P = 0.032; Fig. 4). Urine from the *E. regnans* single-species diet required the greatest titration to return it to neutral pH ($F_{2,8}$ = 7.68, P = 0.043). Urinary properties of ammonia ($F_{2,8}$ = 0.95, P = 0.458) and urea ($F_{2,8}$ = 1.42, P = 0.343) were not significantly different between diets (Fig. 3).

Feeding Behavior

Possums spent longer feeding on the mixed and *E. globulus* single-species diets than on the *E. regnans* single-species diet ($F_{2,17}$ = 20.15, P = 0.002; Fig. 5a). They ate faster on the mixed diet than the respective single-species diets ($F_{2,17}$ = 4.53, P = 0.049; Fig. 5b). Possums did not alter the number of feeding bouts per night (mean 11.8 ± 1.3, P = 0.209), but their time per feeding bout ($F_{2,17}$ = 7.55, P = 0.014; Fig. 5c) and intake per feeding bout ($F_{2,17}$ = 8.09, P = 0.008; Fig. 5d) were least on *E. regnans* and generally greater on the mixed diet. There was no difference between the time

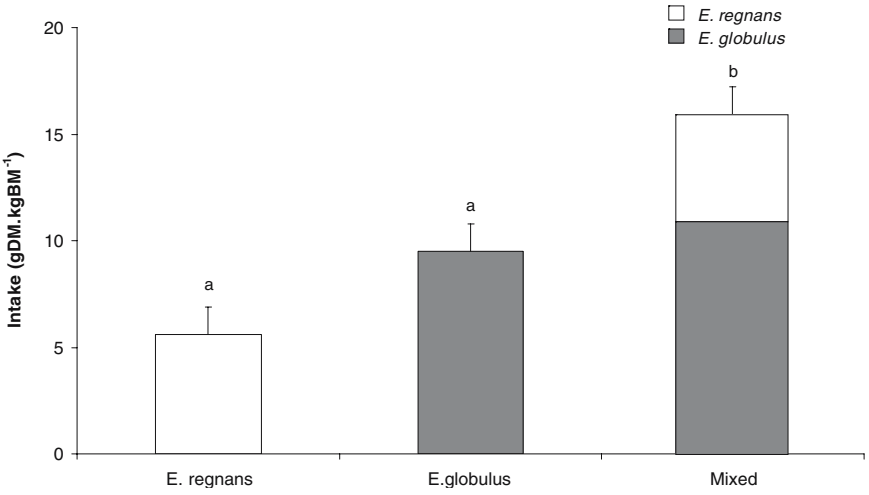

Fig. 3 Total intake [g DM (kg BM)$^{-1}$] of *E. regnans* and *E. globulus* foliage by brushtail possums across diet treatments. Values are least-squares means with SE bars for total intake. Letters that differ are significantly different ($\alpha = 0.05$ after Tukey-Kramer adjustment for multiple comparisons)

from the first to last feeding bout in response to treatment diets (mean 7.0 ± 0.2 hr, P = 0.113).

On the mixed diet, possums switched between foliage both within and between feeding bouts 10.6 ± 0.9 times per night. Of these, 46% of switches (4.4 ± 0.4) occurred within feeding bouts, whereas 54% (6.3 ± 0.4) occurred between feeding

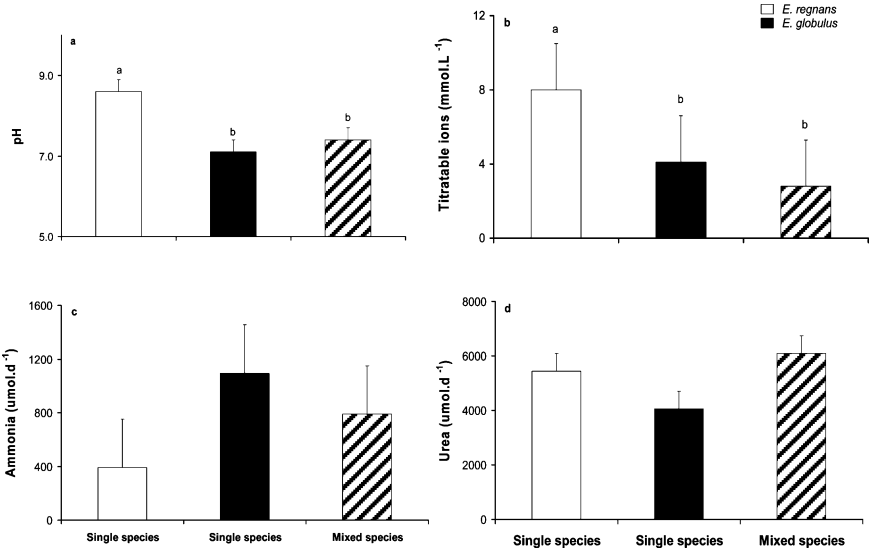

Fig. 4 Urinary properties for brushtail possums fed *E. regnans* and *E. globulus* foliage across treatment diets. (a) pH; (b) titratable ions (mmol l^{-1}); (c) ammonia (μmol d^{-1}); and (d) urea (μmol d^{-1}). Values are least-squares means for three possums with SE bars. Letters that differ are significantly different ($\alpha = 0.05$ after Tukey-Kramer adjustment for multiple comparisons)

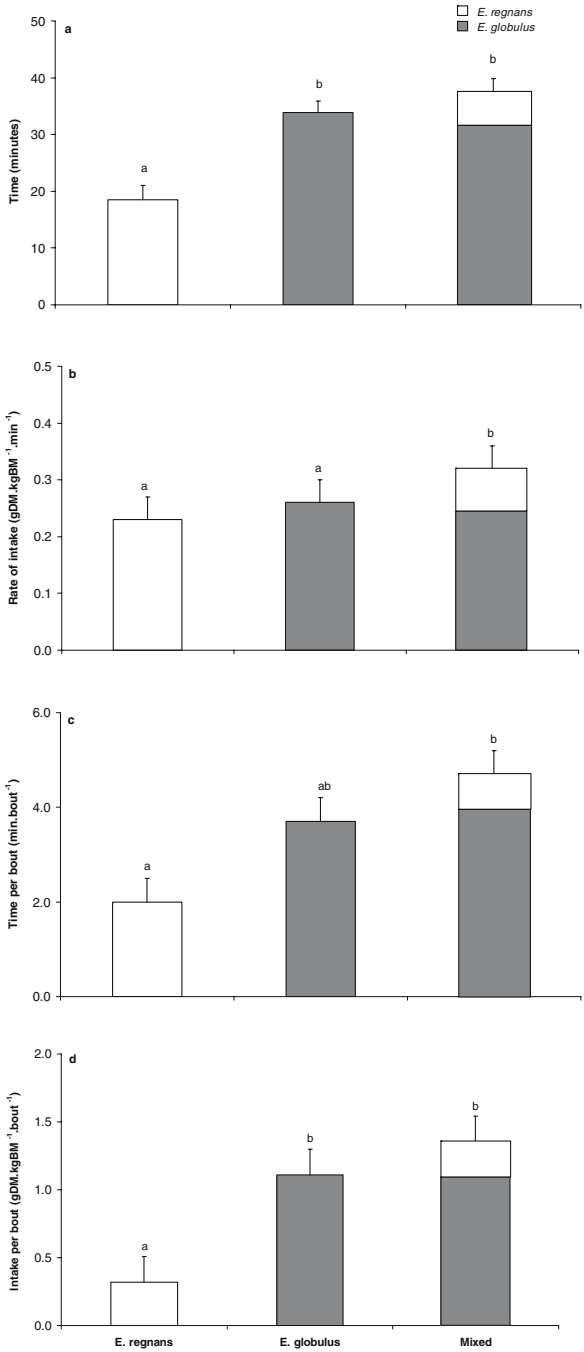

Fig. 5 (a) Total feeding time (minutes); (b) rate of intake [g DM (kg BM)$^{-1}$ min^{-1}]; (c) time per feeding bout (min bout^{-1}); and (d) intake per feeding bout [g DM (kg BM)$^{-1}$ bout^{-1}] of *E. regnans* and *E. globulus* foliage by brushtail possums across diet treatments. Values are least-squares means with SE bars for total intake. Letters that differ are significantly different (α = 0.05 after Tukey-Kramer adjustment for multiple comparisons)

bouts. Of the within-bout switching figure, possums switched more often after 50% of their total feeding bouts had passed (3.0 ± 0.2 times) than before (1.5 ± 0.2 times; mean difference 1.2 ± 0.2, $P = 0.001$).

Discussion

Foliar Chemical Properties

Foliar secondary chemical constituents of total oils and total phenolics differed substantially between *E. regnans* and *E. globulus*, as demonstrated by the minimal overlap in the major oils identified for either species and the absence of known herbivore feeding deterrents, the FPCs (O'Reilly-Wapstra et al., 2004), in *E. regnans*. There were also differences in the types of hydrolyzable tannins present in either species. This result confirms major differences between the foliar secondary chemistry in the two species, as previously demonstrated (Wiggins et al., 2006).

Herbivore Urinary Status

Consumption of each diet treatment generated different urinary characteristics in the common brushtail possum (*T. vulpecula*). Brushtail possums produced highly alkaline urine on the *E. regnans* single-species diet, slightly acidic urine on the *E. globulus* single-species diet, and relatively neutral urine on the mixed diet. Previous studies have shown that the consumption of *Eucalyptus* diets generally creates acidic urine to varying degrees, as a result of high buffering activity (as indicated by levels of ammonia excretion) because of organic acid production from the metabolism of dietary terpenes (Hamm and Simon, 1987; Foley, 1992; Foley et al., 1995). Interestingly, the consumption of *E. regnans* did not generate an acid urine. In fact, it was highly alkaline and showed similarities with urine of both brushtail possums and desert woodrats (*Neotoma stephensi* and *N. albigula*) consuming an artificial maintenance diet of fruit and vegetable matter (Dearing et al., 2000; Wiggins et al., unpublished data). The generation of alkaline urine may explain why the urinary properties of pH, ammonia, and urea indicated minimal disturbances to the acid–base status of possums feeding on *E. regnans*, as these measurements have only previously been used to demonstrate changes in the physiological properties of acid urine (e.g., Foley, 1992). Therefore, with the examination of acid *and* alkaline urine, titratable ions appear to be the most relevant indicator of herbivore physiological status. What the urinary results do suggest, more importantly, is that possums deal with dietary PSMs in *E. regnans* differently from *E. globulus* (confirming our premise for using these two species to test the effects of mixed diets).

Intake and Behavioral Responses

Brushtail possums ate more on a mixed diet of *E. regnans* and *E. globulus* foliage than on their respective single-species diets. This result is consistent with previous research (Dearing and Cork, 1999; Wiggins et al., 2003, 2006) and offers support for the predictions of the detoxification–limitation hypothesis (Freeland and Janzen, 1974). Marsh et al. (2006) recently demonstrated that brushtail possums were only able to eat more on a mixed PSM diet, compared with their respective single-PSM

diets, when individual PSMs were metabolized by using different (major) detoxification pathways. Brushtail possums were unable to eat more on a mixed diet when the PSMs were metabolized with competing detoxification pathways (Marsh et al., 2006). The fact that intake of the mixed diet in our trial was greater than either single-species diet indicates that the chemical properties of *E. regnans* and *E. globulus* foliage were sufficiently diverse to impose different physiological (detoxification) constraints to brushtail possums.

Brushtail possums ate more on the mixed-species than either of the single-species diets by eating faster, while tending to spend more time and eating more per feeding bout. They frequently switched between both species of foliage on the mixed diet, with the number of within-bout switches increasing in the latter half of their feeding bouts. By frequently switching between foliage on the mixed diet, possums may have been able to overcome the short-term intake constraints because of the toxic effects of each species. Wiggins et al. (2006) proposed that diet switching was a direct behavioral response to the detoxification limitations of a PSM-rich diet. That is, when intake on one species is constrained because of saturation of specific detoxification pathways, possums can continue feeding on the other species. They demonstrated that brushtail possums were only able to maximize their intake when able to frequently switch between dietary species during their nightly feeding activities: when foliage time and availability constraints were imposed on possums, they were unable to maintain intake (Wiggins et al., 2006). Results of this current study expand on this hypothesis through a more detailed investigation of the frequency and occurrence of dietary switching.

By switching between foliage more frequently during the latter half of their feeding bouts, we suggest that brushtail possums were responding to the accumulation of PSMs and/or detoxification metabolites over their nightly feeding timeframe. Brushtail possums have been shown to regulate dietary 1,8-cineole ingestion so as not to exceed a critical threshold level over a 3-hr feeding timeframe, but cineole metabolites accumulated throughout that sampling period (Boyle et al., 2005). Cessation of feeding on one plant species may have been a direct response to blood PSM levels that were nearing, or had indeed reached, a specific critical threshold level. By immediately switching to the next available plant species, possums may have been attempting to regulate PSM ingestion of *E. regnans* while continuing to feed on *E. globulus*. Increasing the frequency of this switching behavior during the latter stages of their feeding activities presumably reflects that detoxification limitations were at their most critical stages for both species of foliage. This result strengthens the argument that diet switching in a generalist mammalian folivore is a behavioral response to detoxification limitations of a PSM-rich diet (Wiggins et al., 2006).

The ability to frequently switch between foliage, which was not an option on the single-species diets, enabled possums to feed more efficiently, through a faster feeding rate and greater feeding bout size and length. These behavioral responses to a physiologically constraining diet represent an important facet that helps explain why, and how, generalist mammalian herbivores are able to benefit from a chemically diverse diet.

In summary, the common brushtail possum was able to benefit from the consumption of a chemically diverse diet, even over a short feeding timeframe. Possums fed more efficiently on a mixed- than on a single-species diet and achieved increased intake. We propose that diet switching is a behavioral strategy adopted by *T.*

vulpecula as a direct response to the physiological limitations of detoxification pathways, enabling them to efficiently consume a chemically diverse diet.

This integration of dietary chemical properties, animal physiological, and behavioral data has enabled us to test predictions of the detoxification–limitation hypothesis. Herbivore feeding behavior (e.g., diet switching) may be a useful indicator of the physiological constraints (e.g., detoxification limitations) imposed by a PSM-rich diet. Understanding the underlying processes that govern the intake of generalist herbivores is important: the way in which a herbivore is physiologically constrained by its diet, and the resulting behavioral responses, will directly impact on the herbivore's foraging decisions, and subsequently their foraging energetics.

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