

Inter-population differences in the tolerance of a marsupial folivore to plant secondary metabolites

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Abstract Plant secondary metabolites (PSMs) strongly influence diet selection by mammalian herbivores. Concentrations of PSMs vary within and among plant species, and across landscapes. Therefore, local adaptations may cause different populations of herbivores to differ in their ability to tolerate PSMs. Here, we tested the food intake responses of three populations of a marsupial folivore, the common brushtail possum (*Trichosurus vulpecula* Kerr), from different latitudes and habitat types, to four types of PSMs. We found clear variation in the responses of northern and

southern Australian possums to PSMs. Brushtail possums from southern Australia showed marked decreases in food intake in response to all four PSMs, while the two populations from northern Australia were not as sensitive and their responses did not differ from one another. These results were unexpected, based on our understanding of the experiences of these populations with PSMs in the wild. Our results suggest that geographically separated populations of possums may have evolved differing abilities to cope with PSMs, as a result of local adaptation to their natural environments. Our results provide the basis for future studies to investigate the mechanisms by which populations of mammalian species differ in their ability to tolerate PSMs.

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Introduction

Levels of chemical defence against herbivory vary enormously between plant species, plant communities and biogeographical regions (Palo and Robbins 1991). Many attempts have been made to understand this variation, within the context of the availability of resources for plant growth (Coley et al. 1985), levels of competition and pressure from herbivores (Bryant et al. 1991) and latitude (Andrew and Hughes 2005; Coley and Aide 1991; Hallam and Read 2006). These hypotheses have found varying degrees of empirical support, but it is nevertheless clear that populations of herbivores living in different plant communities are often challenged by different types and amounts of plant secondary metabolites (PSMs). Because the tolerance of herbivores to PSMs is largely the product of co-evolutionary “arms races”, we might expect to find that

herbivore populations have evolved counter-adaptations to PSMs that reflect the challenges they face in their local environment.

Studies of insect herbivores reveal population-level differences in abilities to cope with PSMs (Ayres and Scriber 1994; Fox and Morrow 1981). However, whether vertebrates in different vegetation communities or regions vary in their ability to tolerate PSMs is still unclear. A key exception is the work of Mangione et al. (2000), who showed that a population of desert woodrats that ate creosote (*Larrea tridentata* D.C. Coville) could ingest more creosote resin than another population that ate mainly juniper (*Juniperus osteosperma* Torr. Little). Subsequently, McEachern et al. (2006) reported specialization by woodrats on local species of plants, which they concluded was a result of avoidance of novel chemical compounds. Among the marsupial folivores, common brushtail possums (*Trichosurus vulpecula* Kerr) in Western Australia are much more resistant to naturally occurring fluoroacetate than are their counterparts in eastern Australia, where the occurrence of fluoroacetate in plants is much lower (Twiggs and King 1991). Therefore, like woodrats, brushtail possums may also exhibit inter-population variation when faced with PSMs that vary across the landscape.

Concentrations of PSMs in eucalypt forests vary in response to abiotic factors such as altitude, climate and soil fertility (Moore et al. 2004a). Captive feeding studies have shown that a class of potent PSMs found only in eucalypts, the formylated phloroglucinol compounds (FPCs), largely determines food intake by possums and koalas, although tannins also have an effect (Lawler et al. 1998a; Marsh et al. 2003; Moore et al. 2005; Wallis et al. 2002). The types and concentrations of PSMs in Australian forests may vary from northern to southern Australia due to parallel differences in plant communities. For instance, species from the eucalypt genus *Corymbia*, which tend to contain little or no FPCs (Eschler et al. 2000), dominate the woodlands of northern Australia (Boland et al. 1991). In contrast, the *Eucalyptus* sub-genus *Symphyomyrtus*, which contains FPCs, and *Monocalyptus*, with no FPCs (Eschler et al. 2000) prevail in the southern forests (Brooker and Kleinig 1990). Because PSMs vary greatly within the habitat of marsupial folivores, we might expect local specialization conferring tolerance of specific types of PSMs among marsupial populations.

Our aim was to investigate whether three populations of a generalist folivore from different geographical regions and habitat types differed in their responses to a range of PSMs: 1,8-cineole, the dominant monoterpene in eucalypts, and which also occurs in other plants; quebracho tannin; salicin, a bitter phenolic glycoside; and an FPC, jensenone. Our study included two common brushtail possum populations, one from northeastern and one from southeastern Australia. Common brushtail possums have a wide distribution

and incorporate varying amounts of *Eucalyptus* in their diets, with northern populations eating relatively more eucalypt foliage (Kerle 2001; Tyndale-Biscoe 2005). As a further contrast, we included a population of coppery brushtail possums, which are restricted to fragments of rainforest in north Queensland at altitudes above 600 m (Kanowski et al. 2001). Although previously classified as a separate sub-species of brushtail possum (*Trichosurus vulpecula johnstonii* Ramsay) (Taylor and Foulkes 2004), recent evidence suggests that they are not genetically distinct (S. E. Kerr, unpublished data). Regardless, they occupy a separate dietary niche, eating a mixture of tropical rainforest plant species and plant parts, but no eucalypts (Procter-Gray 1984).

Cineole occurs more commonly, and in higher concentrations in eucalypts from southern Australia, compared to those in the tropics (Boland et al. 1991). It is present in high concentrations in the main eucalypt species known to be commonly eaten by possums in Canberra and is also present in some eucalypts eaten by our northern population (DeGabriel et al. 2009). Tannins are almost ubiquitous in browse, including eucalypts, so all populations would be expected to consume them as part of their natural diets, although the types of tannins may differ. We chose quebracho tannin as standard tannin for our experiments. Although the precise mixture of condensed tannins found in quebracho is novel to brushtail possums, its protein-precipitating activity is representative of condensed tannins. Salicin occurs in many plant families and deters feeding on willows (*Salix* spp.) by common brushtail possums in New Zealand (Edwards 1978). Unlike the other compounds, brushtail possums limit their intake of salicin because of its bitter taste rather than any measurable post-ingestive consequences of feeding (Pass and Foley 2000). Although we do not know whether possums in our study populations had previously encountered salicin, we included it as a representative of bitter compounds. However, we would expect bitter compounds to occur more commonly in the rainforest habitat of coppery brushtail possums.

Coppery brushtail possums do not eat eucalypts. Thus, they would have been naïve to jensenone although they are likely to have encountered various monoterpenes. Similarly, the main tree species eaten by the source population of northern brushtail possums does not contain FPCs (DeGabriel et al. 2009), although they are present in other eucalypts in the area, so these possums would have limited experience with FPCs. In contrast, the southern brushtail possums are likely to encounter cineole and FPCs in locally common eucalypt species. Table 1 summarises the likely experience of each possum population with each of the classes of PSMs used in these experiments. It is likely that the three populations of possums have been separated across both geographic and evolutionary scales and have

Table 1 Summary of assumed prior experiences of the three possum populations with each of the classes of compounds [formylated phloroglucinol compounds; (FPCs)]

	Northern brushtail possums	Southern brushtail possums	Coppery brushtail possums
Monoterpenes	Experienced	Experienced	Probably experienced
Tannins	Experienced	Experienced	Experienced
Bitter compounds	Probably naïve	Probably naïve	Possibly experienced
FPCs	Low level of experience	Experienced	Naïve

different experiences with the PSMs tested. Thus, we might expect them to differ in their tolerance of some classes of PSMs but respond similarly to others.

Materials and methods

Animals

We caught nine adult male common brushtail possums in woodland at Tabletop Station, Hervey Range (19°23'S, 146°27'E) near Townsville (henceforth “northern brushtail possums”; mean body mass = 1,999 g) and six adult male coppery brushtail possums (henceforth “coppery brushtail possums”; mean body mass = 1,981 g) in complex notophyll vine rainforest (type 5b, regional ecosystem 7.8.3; Jones et al. 2006) in the Atherton Tablelands (17°18'S, 145°30'E), both in north Queensland. We caught all of the northern brushtail possums and three of the coppery brushtail possums in wire cage traps, and caught the remaining coppery brushtail possums by immobilizing them (30 mg ml⁻¹ Zoletil, 0.15 ml at 200 mg ml⁻¹; Virbac) using 0.5-ml darts fired from a gas-powered dart gun (Black Wolf; Tranquil Arms, Seymour, Victoria). We transferred all possums to James Cook University, Townsville, and maintained them in large individual wire mesh cages from August to December 2006.

We caught seven common brushtail possums in wire cage traps in woodland around Canberra, in southeastern Australia (35°15'S, 149°08'E) (henceforth “southern brushtail possums”; mean body mass = 2,570 g) and housed them in individual wire mesh cages at the Australian National University from November 2006 to April 2007. In both locations, each cage was fitted with a nest box and climbing perches and was kept in an outdoor enclosure, so possums were exposed to their natural light and temperature conditions. We weighed all possums upon capture and at the start and end of each of the feeding trials to monitor their welfare. At the completion of the study, we released all possums at the capture location.

Diets and feeding protocol

Immediately after capture, we fed possums a mixture of seasonal fruits and vegetables, foliage from local plants and

commercial rabbit pellets. We then gradually introduced them to a basal diet, until this was the only food on offer. We prepared the basal diet as a wet mash consisting of [% wet matter (WM)]: grated apples (39.25), cooked pumpkin (14.0), cooked red sweet potato (14.0), grated carrot (10.0), rolled oats (8.0), ground rice hulls (6.0), ground lucerne (4.2), wheat bran (2.7), vegetable oil (1.25), acid casein (0.25), potassium permanganate (0.254), NaCl (0.03) and vitamin and mineral mix (0.066). The diet contained about 32% dry matter (DM), of which 1.4% was N and 16.3% was neutral detergent fibre.

We recorded the daily amount eaten by each possum for 2 weeks before the start of the first experiment to determine their baseline intakes. We prepared the diet fresh each day and dried a sub-sample in an oven at 80°C for 24 h to determine its DM content. We fed the possums at 1730 hours and removed any uneaten food (henceforth “food refusals”) at 0630 hours. We oven-dried the refusals at 80°C for 24 h and calculated dry matter intake (DMI) by subtracting the DM refused from the DM offered. During experimental periods, we offered a small amount (ca. 50 g WM) of basal diet to possums from 0630 to 0730 hours and recorded the amount they ate. This ensured their welfare, if possums ate little the night before, and reduced the possibility of carry-over effects from one treatment night to the next. Fresh drinking water was always available ad libitum.

We mixed the 1,8-cineole (Felton Grimwade & Bickford, Oakleigh South, Victoria), quebracho tannin (derived from the wood of *Schinopsis lorentzii*; Quebracho MGM-C, Unitan Australia, Narrabeen, New South Wales), or salicin [JS (Tianjin) Chemical and Metallurgical, China] thoroughly with the dry ingredients before adding the fruit and vegetables, and presented the food to the animals immediately. Jensenone was extracted from *Eucalyptus jensenii* foliage as described by Lawler et al. (1998b). We dissolved the jensenone in a minimum volume of acetone before mixing with the dry ingredients then allowed the acetone to evaporate completely. The control diets in this experiment were treated with acetone alone.

Experimental design

We tested each PSM in a separate feeding experiment divided into two parts: a 4-day acclimation period, followed

by a 6-day experiment with a Latin square design, using six possums in each group. For each population, we first tested cineole, followed by quebracho tannin, salicin and finally jensenone.

Acclimation

The purpose of the acclimation period was to eliminate naïve responses to PSMs before the actual experiment and to allow for the induction of any necessary enzymes for detoxification. We selected six possums and for 4 consecutive nights fed them the basal diet containing a moderate concentration of a single PSM (Table 2). We determined the DMI of each possum each night and the amount of additional food eaten in the morning. We then fed the possums the basal diet for 2 nights before the start of the Latin square trial.

Latin square feeding trials

In a no-choice feeding experiment for each PSM, we offered the possums a basal diet to which we added one of six concentrations of the PSM (Table 2) using a digram-balanced 6×6 Latin square design (Ratkowsky et al. 1993), so each treatment was preceded and followed equally often by the other treatments. We selected concentrations that were most likely to elicit a full range of responses, based on previous studies (Lawler et al. 1998b, 1999; Marsh et al. 2006; McArthur and Sanson 1993; Pass and Foley 2000; Stapley et al. 2000). We determined the DMI for each possum each night and the amount of supplementary food eaten in the morning. At the completion of each experiment, we fed the possums the basal diet for a minimum of 3 nights, before commencing the next PSM experiment.

Statistical analyses

We used linear mixed models with DMI as the dependent variable to determine the effects of PSM concentration for each of the four compounds tested. We also tested for

differences in response between populations with PSM concentration and the interaction between concentration and population as the fixed model, and possum as a random term. Possum body mass, previous night's concentration of PSM and the amount of supplementary morning food eaten were also included in the fixed model, but where these had no significant effect on DMI, they were progressively dropped from the final model and are not mentioned further. We also tested the effects of PSM concentration on PSM intake between populations using the same procedure. All analyses were performed in GenStat (10th edition).

Results

Experiment 1: cineole

One possum from each of the northern and southern brush-tail possum populations stopped eating abruptly after cineole was introduced and was removed from the experiment. An increasing concentration of cineole in the diet caused a significant decrease in DMI across all possum populations (Wald = 14.79, $P < 0.001$, $n = 96$; cineole effect \pm SE = -1.91 ± 0.56 ; Fig. 1a), but there was no significant interaction between cineole concentration and population (Wald = 2.75, $P = 0.253$).

Increasing cineole concentration caused a significant increase in cineole intake across all populations (Wald = 300.0, $P < 0.001$, $n = 96$; Fig. 1b). The interaction between cineole concentration and population was also significant (Wald = 15.08, $P < 0.001$) with the slope of the response curve for the northern population significantly steeper than for the southern possums. The cineole intake versus cineole concentration curve for the coppery possums was not significantly different from those of the other populations (effect sizes: northern = 0.51, coppery = 0.34, southern = 0.31, average SE = 0.09).

Experiment 2: quebracho

Again, data from the northern brushtail possum population comprised five animals, as one possum refused to eat the experimental diet. The feeding responses of the three possum populations to increasing concentrations of dietary quebracho differed in direction so that there was no effect of quebracho on DMI (Wald = 1.62, $P = 0.203$, $n = 102$; Fig. 2a) but there was a strong interaction between quebracho concentration and possum population (Wald = 35.90, $P < 0.001$) with respect to DMI. The northern brushtail and coppery brushtail possums both ate more with increasing concentrations of quebracho, whereas the southern population ate less with increasing concentrations (effect sizes: northern = 0.59, coppery = 0.42, southern = -1.48 , average SE = 0.66).

Table 2 Concentrations of each plant secondary metabolite (PSM) used in the acclimation and Latin square experiments to test the responses of three populations of brushtail possums

PSM	Acclimation concentration (% DM)	Latin square concentrations (% DM)
1,8-cineole	5	0, 2, 4, 6, 8, 10
Quebracho	5	0, 3, 6, 9, 12, 15
Salicin	2	0, 1.5, 3, 4.5, 6, 7.5
Jensenone	0.2	0, 0.1, 0.2, 0.4, 0.6, 0.8

DM Dry matter

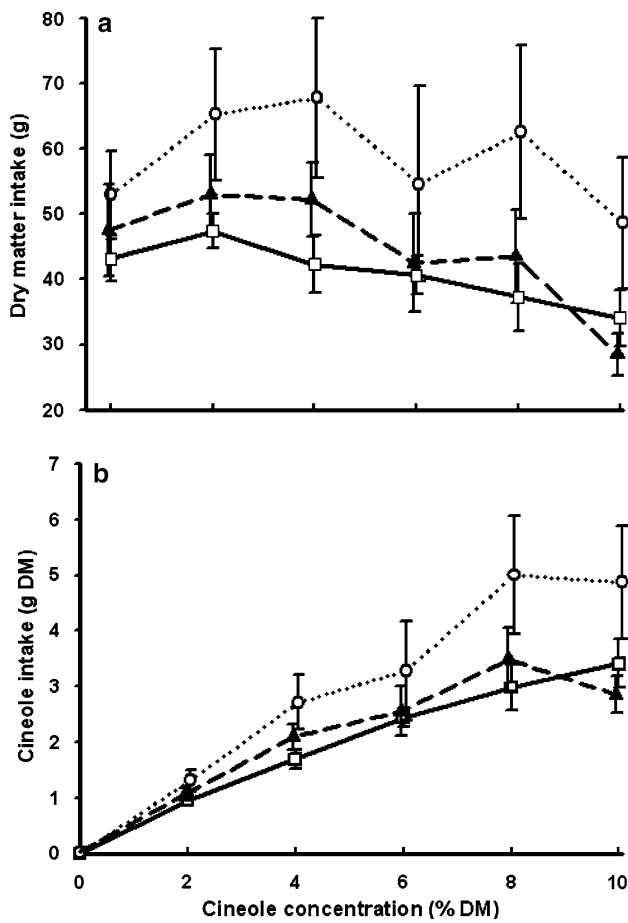


Fig. 1 **a** Mean dry matter (DM) intake (\pm SE) and **b** mean 1,8-cineole intake (\pm SE) of coppery brushtail possums (squares), northern brushtail possums (circles) and southern brushtail possums (triangles) fed a basal diet containing six concentrations of 1,8-cineole

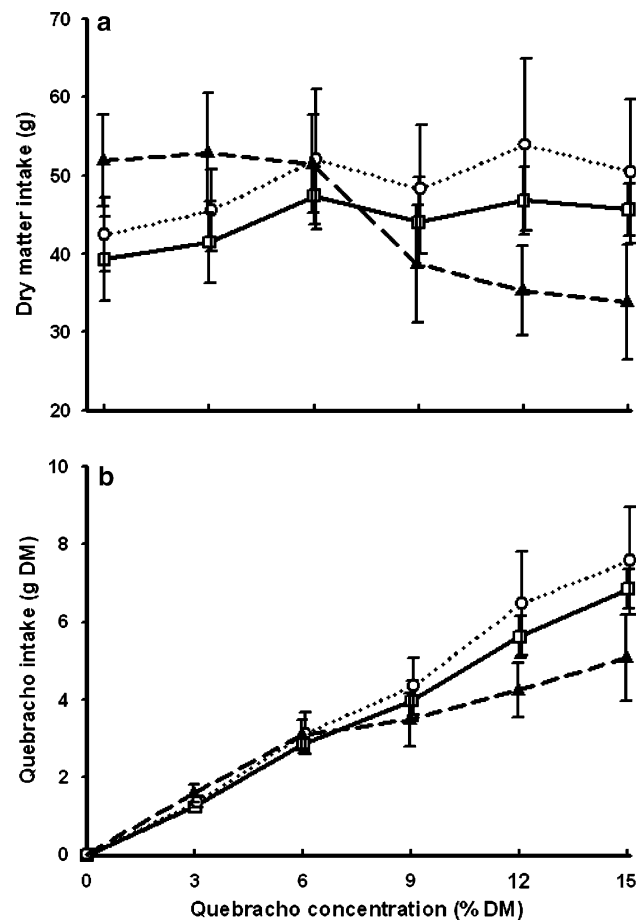


Fig. 2 **a** Mean DM intake (\pm SE) and **b** mean quebracho intake (\pm SE) of coppery brushtail possums (squares), northern brushtail possums (circles) and southern brushtail possums (triangles) fed a basal diet containing six concentrations of quebracho

All populations ingested more quebracho as its concentration in the diet increased (Wald = 554.49, $P < 0.001$, $n = 102$; Fig. 2b), and the interaction between dose and population was again significant due to the different response of the southern population (effect sizes: northern = 0.52, coppery = 0.46, southern = 0.32, average SE = 0.08; Wald = 10.35, $P < 0.001$).

Experiment 3: salicin

Compared to the response curves for cineole and quebracho, that for salicin was more uniform, with declining DMI for all populations with increasing concentrations of salicin (Wald = 139.75, $P < 0.001$, $n = 108$; Fig. 3a). Even so, the interaction of salicin concentration and population on DMI was significant (Wald = 10.15, $P = 0.006$), largely because the northern brushtails were more tolerant of salicin than were the southern brushtails (effect sizes: northern = -2.02, coppery = -2.94, southern = -3.96, average SE = 1.04). The concentration of salicin that possums were exposed to

on the previous night significantly influenced DMI (Wald = 7.14, $P = 0.008$), such that possums showed a smaller reduction in food intake if they had experienced a higher concentration of salicin on the previous night.

Even with the declining DMI, possums still ingested more salicin with increasing dietary concentration (Wald = 207.91, $P < 0.001$, $n = 108$; salicin effect \pm SE = 0.18 ± 0.03 ; Fig. 3b), but there was no interaction between concentration and population on salicin intake (Wald = 5.85, $P = 0.054$). In contrast to the response for food intake, the concentration of salicin that possums experienced on the previous night did not affect salicin intake.

Experiment 4: jensenone

Possums from all three populations ate less with increasing concentrations of dietary jensenone (Wald = 187.52, $P < 0.001$, $n = 108$; Fig. 4a), while the interaction between concentration and population was also significant (Wald = 18.50, $P < 0.001$). The slope of the response curve

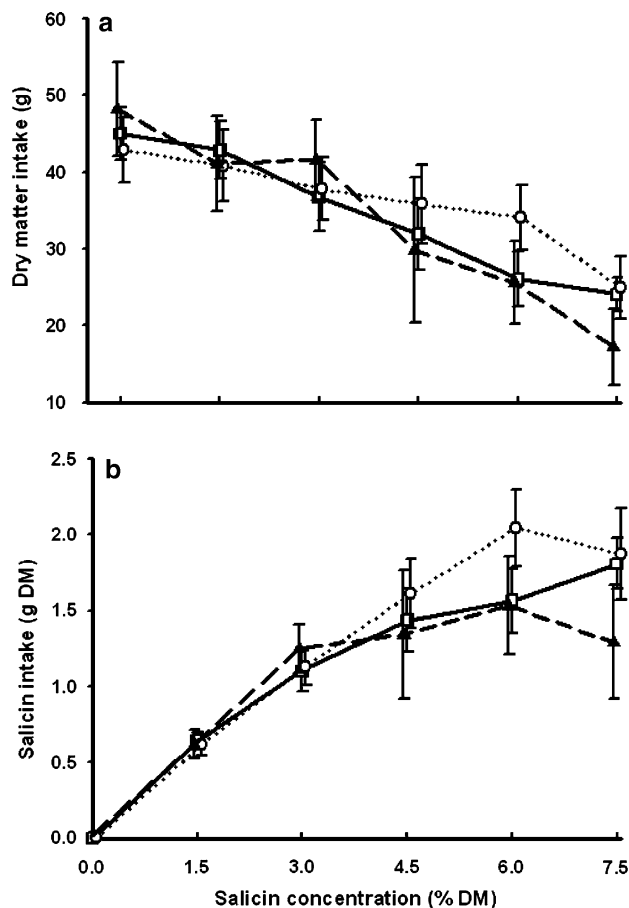


Fig. 3 **a** Mean DM intake (\pm SE) and **b** mean salicin intake (\pm SE) of three coppery brushtail possums (*squares*), northern brushtail possums (*circles*) and southern brushtail possums (*triangles*) fed a basal diet containing six concentrations of salicin

for the southern brushtail possums was significantly steeper than that for the northern population, whereas that for the coppery possums was intermediate and not significantly different from the others (effect sizes: northern = -21.95 , coppery = -30.64 , southern = -47.13 , average SE = 10.15).

Even with the sharp decrease in food intake with increasing dietary jensenone, possums still ingested more jensenone as its concentration in the diet increased (Wald = 161.86, $P < 0.001$, $n = 108$; jensenone effect \pm SE = 0.13 ± 0.02 ; Fig. 4b). Surprisingly, given the marked difference in feeding between populations, the interaction between concentration and population on jensenone intake was not significant (Wald = 2.25, $P = 0.324$).

Discussion

We found clear differences in the responses of the three brushtail possum populations to the PSMs tested. Our key finding was that the southern population showed a marked

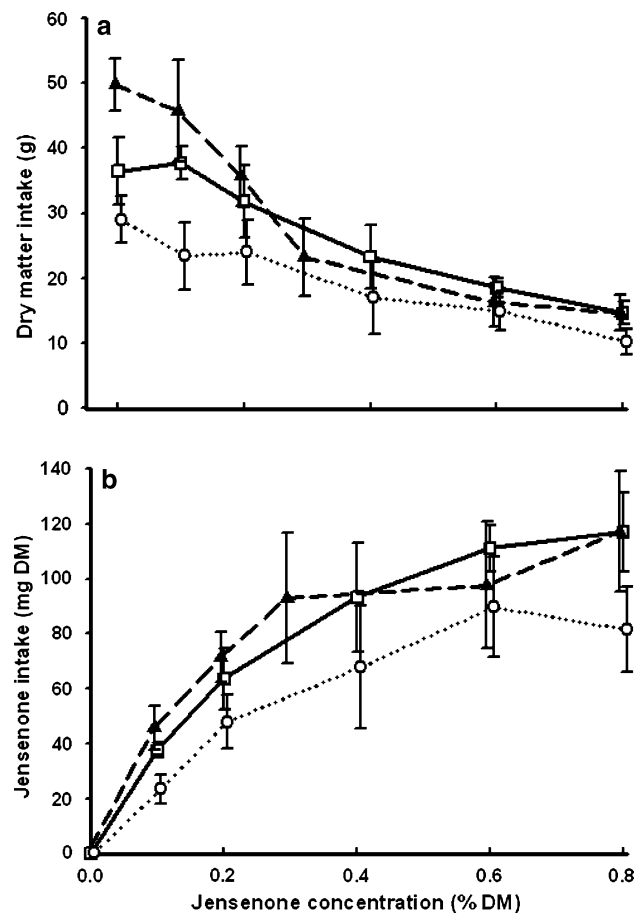


Fig. 4 **a** Mean DM intake (\pm SE) and **b** mean jensenone intake (\pm SE) of coppery brushtail possums (*squares*), northern brushtail possums (*circles*) and southern brushtail possums (*triangles*) fed a basal diet containing six concentrations of jensenone

response to all four PSMs, with clear declines in feeding as the concentration of PSM in the diet increased. In contrast, possums from the two populations in northern Australia proved more resilient to increasing concentrations of dietary PSMs.

These results were unexpected, assuming that a population's tolerance depends on the likelihood of it encountering a particular PSM in its diet (see Table 1). All populations were likely to encounter terpenes and tannins, and possibly bitter compounds in the wild, although there is tremendous diversity among the compounds in these groups. In summary, we might expect all three populations to respond similarly to these compounds, but instead the southern brushtails proved most sensitive. The exception was the FPC, jensenone, because such compounds occur only in eucalypts and are particularly widespread in southeastern Australia. Thus, we might expect the southern brushtails to be the most tolerant of jensenone, northern brushtails to be moderately tolerant and the coppery brushtails, which were presumably naïve to FPCs including

jensenone, to be relatively intolerant. The results, however, were the reverse; southern brushtails ate markedly less with small increases in the dietary concentration of jensenone, while the northern brushtails had a shallower feeding response. The coppery brushtail possums ate less as the jensenone concentration increased but the slope of the response did not differ from those of the other populations. Admittedly, the southern brushtails ate more of the basal diet, so one might argue that they had the greatest potential for decrease. The results of jensenone intake confirm this, as animals from the three populations ingested similar amounts of jensenone. Nevertheless, this was still an unexpected result.

There are many possible and often competing explanations for these seemingly contradictory results. First, our expectations depended on knowing the diets of brushtail possums, which are highly variable, and there is scant information for many populations. Brushtail possums in northern Australia tend to eat more eucalypt foliage (Kerle 2001). Indeed, the northern possums used in these experiments came from a closely studied population that ate a diet comprising 80–90% *Eucalyptus* (DeGabriel et al. 2009). These eucalypt leaves contained negligible FPCs but they exposed possums to a variety of terpenes, including cineole, and to tannins, which limited the availability of N in the diet. Unfortunately, we did not have such detailed knowledge of the diets of the other populations, but that of the southern brushtail possum was likely to be more general, including non-eucalypts as well as fruits, flowers and grasses, the latter of which usually do not contain tannins (Freeland and Winter 1975). Thus, with more foods available, southern possums may be able to switch diets to avoid high concentrations of PSMs, and so may be more cautious of PSM-rich diets when they have no choice. The coppery brushtail contrasts with the northern and southern brushtails in eating no eucalypt foliage. These possums are likely, however, to encounter a wide range of PSMs due to the diversity of plant species in rainforest environments. For instance, although coppery brushtail possums are unlikely to experience 1,8-cineole, some rainforest plant species which they eat in the Atherton Tablelands (e.g. *Flindersia schottiana* F. Muell.; Procter-Gray 1984) contain monoterpenes, such as α -pinene and sabinene (Brophy et al. 2005). Our study investigated short-term effects of PSMs on food intake, but it should also be recognised that persistent effects of PSM exposure on an individual's fitness (e.g. depression of nervous system by terpenes; Torregrossa and Dearing 2009), may influence their willingness to ingest diets containing these compounds.

By focusing on individual PSMs our expectations disregarded the fact that common biochemical pathways detoxify many PSMs or signal their presence. For instance, oxidative enzymes detoxify cineole before its conjugation

with glucuronic acid (Boyle et al. 1999, 2000). Likewise, conjugation with glucuronic acid is part of the metabolism of salicin (McLean et al. 2001), suggesting competition for detoxification (Marsh et al. 2006), but also that an animal consuming one PSM may have enzyme systems expressed should it consume another that it metabolises in the same way. Similarly, we know that FPCs exert strong physiological effects on marsupials, via serotonin 5HT₃ receptors, which likely signal sensations of emesis to the brain and cause the animal to stop eating (Lawler et al. 1998b; Stapley et al. 2000). This is a common response to many PSMs and presumably animals quickly detect PSMs that elicit the response, even if they are unfamiliar with the specific compound (Dearing et al. 2005; Torregrossa and Dearing 2009). This may explain why all populations responded similarly to jensenone, although they do not all encounter it in the wild. This response is argued to occur generally in mammalian browsers (Provenza 1995) but no studies have investigated evolutionary mechanisms for adaptations between populations and compounds eliciting the response.

Complicating this response further is a behavioural link, as animals learn about PSMs through previous encounters. In possums, this seems likely for cineole, whereby an animal's feeding response may depend on it associating cineole with more potent PSMs. Monoterpenes are highly volatile, so possums presumably recognize their presence pre-ingestively (i.e. by taste and smell). Because the concentrations of cineole in eucalypt leaves correlate strongly with those of FPCs (Moore et al. 2004b), southern brushtail possums, at least, can associate cineole with the presence of FPCs and develop a conditioned flavour aversion to cineole (Lawler et al. 1999). If we assume that the coppery and northern possum populations had minimal exposure to FPCs, then they might have no conditioned flavour aversion to cineole, which could explain why they did not respond to this compound.

There are suggestions that body size influences detoxification rates, with smaller animals metabolizing PSMs faster than larger animals due to their higher metabolic rate (Iason 2005). Thus, combined with the higher mass-specific daily food intake of smaller herbivores, we might expect them to ingest proportionally larger amounts of PSMs. Our southern brushtails weighed about 500 g or 25% more than their northern relatives, but this had no influence on the amount eaten, so there was no evidence that body size influenced detoxification or tolerance to PSMs. Thus, the next step may be physiological experiments to investigate differences in detoxification capacities between populations.

Superficially, our results do not appear to fit the concept of local adaptation, because PSM tolerance seems to be independent of exposure. This, however, relies on many assumptions, one being that the southern brushtail possums regularly encounter terpenes, FPCs and tannins. If we

discount any knowledge of possum diets then local adaptation provides a plausible explanation for our results. This may occur on several scales. First, evolution may shape the responses of herbivores to PSMs by producing genetic differences between separated populations. Indeed, Magnanou et al. (2009) demonstrated differences in the expression of biotransformation genes between two populations of woodrats when fed either juniper or creosote bush. Perhaps the best example, in the case of brushtail possums, is the differing ability of possums in different parts of Australia to metabolise fluoroacetate. Possums in western and northern Australia, which have evolved with fluoroacetate-bearing shrubs, ingest 40–50 times more of the compound than can their eastern Australian counterparts (Twigg and King 1991). There is similar scope for genetic differentiation to explain the different responses between the northern and southern possums. Molecular analyses have revealed distinct clades of *T. vulpecula* in southeastern, northeastern and southwestern Australia (Taylor and Foulkes 2004). The evolution of these clades is presumably due to the restriction of gene flow across biogeographic barriers, for example, the Nullarbor and Eyrean plains that divide eastern and western populations (Taylor and Foulkes 2004). As a result, separated populations of animals could evolve to tolerate certain PSMs. For example, there are many ways brushtail possums might counter tannins. These include selection upon the proline-rich protein sequences that bind tannins or the rates of secretion of these proteins in the saliva (McArthur et al. 1995; Shimada 2006), or associations with gut microbes that dissociate tannin–protein complexes (Foley et al. 1999; Osawa and Sly 1992) or that secrete tannase (Sasaki et al. 2005; Shimada et al. 2006). The result would be populations with differing physiological abilities to cope with tannins. Likewise, recent studies identified genes responsible for the activation of bitter taste receptors (Bachmanov and Beauchamp 2007; Shi and Zhang 2006; Shi et al. 2003), suggesting that differences between the ways that individuals or populations react to bitter compounds, such as salicin, may have a genetic basis.

Such an explanation is difficult to separate from the alternative that animals may develop more labile responses through experience with foods in their local environments or by the induction of physiological mechanisms after exposure. This may occur on a relatively short time scale, of several days to a few weeks. For example, by comparing the results of short- (6 days) and long-term (20 days) experiments, Pass and Foley (2000) showed that possums had a substantial ability to acclimate to dietary salicin. When offered increasing doses of salicin, conditioned possums regulated their intake of the PSM at a much higher dose than did naïve animals. Some have suggested that species that regularly encounter bitter foods in the wild should be more tolerant of bitterness (Glendinning 1994; Nolte et al.

1994). The same result occurs when feeding cineole to southern brushtail possums. Common brushtail possums are known to decrease their intake of diets containing cineole at doses above a threshold level (Lawler et al. 1999; Marsh et al. 2006; Wiggins et al. 2003), but they can quickly adjust to increasingly higher concentrations (McLean et al. 2008). In other words, the threshold increases with exposure, just as it does for salicin. The concentrations we used (0–10% DM) were not high enough to elicit large reductions in DMI, but were still sufficient to reveal inter-population differences in cineole intake. Furthermore, these concentrations are higher than would be encountered naturally (Hume and Esson 1993; Lawler et al. 1998a). In contrast, brushtail possums in Tasmania reduced their intake of an artificial diet by more than 50% upon exposure to 2% WM (equivalent to about 6% DM) 1,8-cineole (McLean et al. 2008).

Our finding that southern brushtail possums were the most sensitive to cineole, together with those of McLean et al. (2008), suggests differences between possums in northern and southern Australia in their tolerance of this compound. We can draw the same conclusion for the animals' responses to quebracho. Southern brushtail possums decreased their intake by over 30% across the concentrations measured, while food intake by the northern brushtail populations was unaffected by increasing quebracho concentrations. McArthur and Sanson (1993) found that adding quebracho had no effect on food intake by brushtail possums in southern Australia; however, the "high" treatment used in their study was only 6% (DM), compared to 15% in this study. Marsh et al. (2003) showed that common brushtail possums increased their intake of eucalypt foliage when the effects of tannins were blocked with polyethylene glycol. The chemical structures of tannins, however, are complex and those in eucalypt leaves may differ from quebracho. Thus, our results may not translate directly to wild possums, but they do suggest that different populations of possums respond differently to tannins.

A long-standing hypothesis is that plants growing in tropical forests invest more resources in defence than do plants growing in temperate climes (Coley and Aide 1991). If so, it is reasonable to expect corresponding patterns in herbivores, with those in the tropics more capable of coping with defensive chemistry. Our northern and coppery possums came from woodland and forest at latitudes 19°23'S and 17°18'S, respectively, some 16–18° north of where we caught the southern brushtails, suggesting that a cline in plant defences is a plausible explanation for our results. There is, however, no definitive proof of such a cline, while proving a corresponding gradient in animals would be a logical extension of this work.

Our results clearly demonstrate the existence of broad-scale inter-population differences in the responses of

brush-tail possums to PSMs between northern and southern Australia and highlight the need for more detailed studies to tease apart the mechanisms underlying this effect. Inter-population differences in the ability of browsers to cope with PSMs may have important ecological and evolutionary implications, influencing factors such as habitat selection, dispersal and gene flow (Mangione et al. 2000), as well as the distribution and abundance of species. McEachern et al. (2006) suggested that the lack of dispersal of woodrats across habitat boundaries may be a consequence of local dietary specialization in relation to chemical defences. Similarly, coppery and common brush-tail possums may be isolated across the rainforest-eucalypt boundary in northern Australia, which could influence population structure. Our study provides a strong basis for field studies to investigate adaptations of populations of mammalian herbivores to plant defence across habitats at multiple scales.

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