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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Behavioral archives link the chemistry and clonal structure of trembling aspen to the food choice of North American porcupine

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Abstract Understanding the links among plant genotype, plant chemistry, and food selection by vertebrate herbivores is critical to assess the role of herbivores in the evolution of plant secondary chemistry. Some specialized vertebrate herbivores have been shown to select plants differentially according to plant genotype, but examples from generalists, which constitute the vast majority of vertebrate herbivores, are few, especially in natural conditions. We examined the relationship between the North American porcupine (*Erethizon dorsatum*), a generalist mammalian herbivore, and clonal trembling aspen (*Populus tremuloides*), a preferred food source of porcupines. We determined preference for certain aspen trees through visual examination of porcupine climbing scars left on tree bark, and through a controlled feeding experiment. We used genetic and biochemical analyses to link the behavioral archives (climbing scars) left by porcupines on aspen trunks to the clonal structure and chemical composition of trees. We show that two phenolic glycosides (tremulacin and salicortin), which are under a high degree of genetic control and thus vary in concentration across clones, are the chemical variables that most influence (deter) feeding choices by porcupines. Using behavioral archives left by a wild herbivore on a natural stand of plants thus allowed us to demonstrate that a generalist vertebrate herbivore can choose plants according to their clonal structure and genetically based chemical composition. Our results contribute to extending previous findings obtained with generalist herbivores studied in controlled conditions, and with specialist herbivores studied in the field.

Keywords Phenolic glycoside · Mammalian herbivory · Selective herbivory · Plant–animal interactions · Defensive chemistry

Introduction

Demonstration of the evolutionary role played by herbivores with respect to plant secondary metabolites is a multistep process that involves concepts from genetics, chemistry, ethology, and ecology (Bryant et al. 1991). A number of studies have attempted to link plant genotype, plant chemistry, and food selection by invertebrate herbivores (e.g., Mauricio and Rausher 1997; Fornoni et al. 2004; Donaldson and Lindroth 2007), but parallel efforts with vertebrate herbivores are less abundant (e.g., Snyder 1992; Jia et al. 1997; Pusenius et al. 2002; Vourc'h et al. 2002; Bailey et al. 2004; Laitinen et al. 2004; O'Reilly-Wapstra et al.

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2004). Furthermore, very few of these attempts were made on wild herbivore populations feeding on natural plant populations. For example, Jia et al. (1997), Pusenius et al. (2002), Laitinen et al. (2004) and O'Reilly-Wapstra et al. (2004) used plants removed from their original population to show that vertebrate herbivores can select among plant genotypes differing in phytochemistry. Whereas the experimental approach bears obvious advantages in its ability to detect cause–effect relationships, the ecological and evolutionary relevance of experimental results depends on how the preferences expressed by herbivores translate into natural conditions, where the relative frequency of plant genotypes can differ from experimental situations and where animals face multiple trade-offs as they must avoid predators, parasites, and weather extremes, and engage in social interactions (Moore and Foley 2005).

Interestingly, the most demonstrative studies linking intraspecific variation in plant chemistry to food selection by vertebrate herbivores studied in natural conditions often involve specialist herbivores, such as Abert's squirrels (*Sciurus aberti*; Snyder 1992) or koala (*Phascolarctus cinereus*; Moore and Foley 2005). It is unclear whether or not this reflects an intrinsic difference in the ability of specialists versus generalists to finely tune plant selection at the intraspecific level, although generalists could potentially be under less directional selection to adapt to a particular set of plant secondary metabolites. Understanding food choice by generalist herbivores is important because they are the main consumers of plants in the vast majority of ecosystems (Freeland 1991).

We studied a generalist herbivore, the North American porcupine (*Erethizon dorsatum*), originating from South America and now ranging as far north as the Canadian tundra. Porcupines feed at ground level on a large variety of herbs, forbs, fruits, and flowers, and climb various coniferous and deciduous trees to eat buds, catkins, bark, twigs, and leaves (Roze 1989). Their feeding regime varies widely according to seasons and geographical areas (Roze 1989).

Porcupines feed partly on leaves of trembling aspen (*Populus tremuloides*) in summer (Plate 1; Morin et al. 2005; Berteaux et al. 2007). Aspens have the largest distribution of any North American deciduous tree species and show striking genetic variation (Lindroth and Hwang 1996). This genetic variation is evidenced as visually apparent differences among clones, including variation in morphology, autumn coloration, and timing of bud-break and leaf senescence (Lindroth and Hwang 1996 and references therein). Trembling aspens produce phenolic-based secondary metabolites (phenolic glycosides and condensed tannins) in abundance, and these are well-known for deterring aspen-feeding insects (Lindroth 2000) and some

Plate 1 Variation in utilization of trembling aspen by North American porcupines in Parc National du Bic, Quebec, Canada. *Top* Aspen avoided by porcupines. *Bottom* Aspen heavily used by porcupines. Each panel shows the canopy of a clone (*left*), a close up of a single branch (*center*), and a close up of the bark of a tree trunk (*right*). Note the defoliated branch and cut stems on the lower middle panel, and the scars left by porcupine claws on aspen bark on the lower right panel. Photo credits: Dominique Berteaux

mammals (Basey et al. 1990; Wooley et al. 2008). Variability in phenolic glycosides is potentially high across clones but low within clones, and concentrations of phenolic glycosides are influenced strongly by clonal genetics (Hemming and Lindroth 1995; Osier and Lindroth 2001; Donaldson and Lindroth 2007; Lindroth et al. 2007). Natural aspen stands typically contain multiple trees of the same genotype, as well as multiple units (clones) of differing genetic composition (Barnes 1969a; Wall 1971; Mock et al. 2008).

The hard claws of porcupines leave long-lasting marks on the thin bark of aspens, and the build-up of these marks through the years constitutes a collection of historical records (a natural archive) of past herbivory pressure on individual trees (Plate 1). We used this opportunity to investigate porcupine food selection on trembling aspen. We tested the hypothesis that phytochemical variation among clones would affect food selection by porcupines. More specifically, we predicted that aspen clones would differ in their concentrations of phenolic glycosides, and that porcupines studied in natural conditions would bias their food choice toward clones with lower concentrations of these secondary metabolites.

In supporting these predictions, and based on the known importance of genetics in the phytochemical variation of aspens, we show the ability of a wild generalist herbivore to recognize variation in aspen chemistry (and thus most probably variation in aspen genetics), and to translate this recognition into biased food selection at the individual tree level.

Methods

Study site

We worked from 6 June to 25 August 2002, and from 5 May to 5 July 2003, in Parc National du Bic (48°21′N, 68°45′W), Quebec, Canada, at the southern limit of the boreal forest. Details on the topography, vegetation, and climate of the study area, and on the demography and natural history of the porcupine population are available in Klvana et al. (2004) and Berteaux et al. (2005).

We performed field work in a 2.2 ha patch of forest dominated by trembling aspens, of relatively uniform topography, and heavily used by porcupines. We tagged all aspen trees with a minimum circumference of 20 cm ($n = 577$). We excluded trees smaller than this from the study, due to a distinct absence of climbing scars and to the developmental variation in the phytochemistry of aspens under 10 years of age (basal circumference 10–15 cm) (Donaldson et al. 2006). We measured the circumference at 1.30 m of each tree, and determined its spatial position (±1 m) using a land survey theodolite (Leica TC 605/L, Leica Geosystems, St. Gallen, Switzerland).

Identification of aspen clones

We delineated clones using the field techniques described in Barnes (1969b) and Kemperman (1977). In May 2003, we examined all trees daily for bud break, flowering, and leaf flush. We recorded the phenology of these events and determined the sex of flowers. We also examined tree bark for differences in color, texture, and susceptibility to frost cracks and disease, and characterized stem and branch form (straight, undulated or twisted), branching habit (upwards, downwards or horizontal), and stem fork (presence or absence; if present, height of lowest fork). Variation in these traits is usually sufficient for delineating clones due to the heritable basis of these phenological and morphological characteristics, providing that multiple traits are used in the analysis (Gom and Rood 1999 and references therein). Similar morphometric techniques employed in related research proved accurate in over 90% of clone identifications, when compared with molecular (microsatellite) techniques (R.L.L., unpublished data).

Using these qualitative characteristics of aspen trees, two observers independently clumped trees into clones according to the observed spatial variation in tree characteristics. Observers classified 81% ($n = 467$) of trees into the same set of 16 clones. We excluded from subsequent analyses the remaining 110 trees that were not classified similarly by the two independent observers. Amplification of microsatellite markers (using four to ten loci as necessary for unique characterization of individual clones) from the DNA of 24 trees (three trees taken randomly from each of eight presumed clones) confirmed the field delineation [see Sect. S1 in the electronic supplementary material (ESM) for laboratory techniques].

Determination of tree use by porcupines

We first evaluated climbing scars left by porcupines on aspen bark to quantify the intensity of use of individual aspen trees by porcupines. We then used feeding trials on captive porcupines to verify that the variability in use of trees by wild porcupines corresponded to differential food selection (see section Linking tree use to food selection). Scratches observed on aspen bark were attributed to porcupine climbing based on two criteria: they were oriented diagonally on the tree trunk (due to the position of the forepaws when climbing) and they were clumped in groups (multiple scars are left simultaneously when several nails of a single paw puncture the bark). No other animal in the study area could have generated such climbing scars (see S2, Fig. S2.1, for a picture of a fresh climbing scar).

We quantified the density of climbing scars on individual trees using two 64 cm^2 quadrats located on areas of the trunk considered visually to contain the highest number of porcupine scars (S2, Fig. S2.2). We adopted this stratified sampling strategy (one stratum) rather than a random sampling, because preliminary data showed that it was the most efficient at capturing the among-tree variability in scar density (scars were highly clumped on tree trunks). We calculated the average number of scars per quadrat for each tree. Climbing scars remain on tree trunks for many years (we first observed fresh climbing scars in May 2000, and these scars were still clearly identifiable by April 2009; D.B., unpublished data). Because older trees may have registered porcupine climbing scars for a longer period of time, we performed a regression analysis to remove any effect caused by tree size. We considered residual values as an index of tree use independent of tree age and size (S3). Hereafter, "climbing scar index" refers to the average number of scars per quadrat per tree, while "tree use index" refers to residual values of the climbing scar index once the effect of tree size has been removed. We assumed that these indices reflected long term herbivory pressure, but this could not be demonstrated. Scars were produced by an unknown number of porcupines (but see Discussion). Bark texture was appropriate to register claw marks on 510 trees (frost cracks on the bark of 67 trees may have led to an underestimation of the number of claw marks).

Linking tree use to food selection

We captured five porcupines from 25 May to 10 June 2003 (four adult males and one juvenile female) in the area surrounding the study site, and housed them separately in cages (1.5 m × 1.5 m × 1.5 m) to perform feeding trials. Each cage contained a rubber pipe, which provided shelter to the porcupine. Cages were placed in a forest stand close to the capture locations and porcupines were released at their site of capture immediately after finishing the experiment (on average, 19 days after their initial capture). Capture and handling techniques were approved by the Comité de protection des animaux de l'Université du Québec à Rimouski (permit # CPA12-02-06) and the Société de la Faune et des Parcs, Gouvernement du Québec (permit #20030401-001-01-S-F).

We conducted the experiment over 12 consecutive nights from 16 June to 28 June 2003. Each night, we offered the five porcupines a choice between leaves coming from lightly versus highly scarred aspens. Each evening, we selected one bundle from each of two trees belonging to two clones (trees were thus never compared within clones). Leaves from the two trees did not differ in any obvious characteristic such as density, size, shape or color. The lightly and highly scarred trees had an average tree use index of -7.2 ± 2.2 (range: -10.0 to -2.5) and 19.5 ± 12.8 (range: 5.6–41.3), respectively. We tied three or four 50–80 cm long terminal branches from individual trees into equal size bundles containing a similar quantity of leaves (fresh mass = ca. 250 g). We placed bundles in cages so that they were equally accessible to porcupines. Once bundles were placed in cages, porcupines were continuously observed for 1 h, after which the percentage of leaves eaten in each bundle was estimated visually. Visual estimation was performed without removing bundles form the cage, so as not to disturb the porcupines. Unfortunately visual estimations were not calibrated against a more direct method such as weighing. The observer (B.D.) was blind as to which bundle came from a lightly versus highly scarred aspen. The positions of bundles relative to each other were random. Additional estimates were also performed 1.5, 4, and 24 h after bundles were placed in cages. In addition to the aspen leaves offered for the experiment, porcupines were fed daily with dandelions, apples, grass, clover, and aspen leaves from trees not used in this study, in order to ensure that their nutritional requirements were met. We therefore used 24 trees (one tree per "treatment" × 2 "treatments" × 12 replicates) in the experiment, and each porcupine went simultaneously through the same set of tests.

Chemistry of aspen

In July 2003, we sampled for chemical analysis leaves from 252 aspen trees representing the diversity of genotypes and phenotypes present on our study site. Our sampling included all trees from clones ($n = 13$) containing <20 trees, and 50% (randomly selected) of the trees from clones ($n = 3$) containing ≥20 trees. We measured the content of nitrogen, carbohydrates (starch and sugar), condensed tannins, and phenolic glycosides (tremulacin and salicortin) in order to assess the quality of leaves to porcupines. The leaf sampling methodology and chemical analyses are detailed in S4.

Data analysis

Many trees had never been climbed by porcupines, so that our climbing scar index had numerous null values. We therefore investigated the influence of clonal structure on use of trees by porcupines by comparing the observed to expected (average) proportion of scarred trees in each clone, using a Chi square test.

To analyze data from the feeding experiment, we first calculated the mean value of the four estimates (at 1, 1.5, 4, and 24 h) of leaf consumption obtained every night for each porcupine and each leaf bundle. We then tested the effects of treatment (lightly vs highly scarred trees), tree identity, and individual porcupine on leaf consumption using a three-way mixed-model ANOVA (Sokal and Rohlf 1981).

We lumped salicortin and tremulacin (hereafter called "phenolic glycosides") together in all analyses of aspen chemical composition, because these two constituents are structurally related and were strongly correlated ($r^2 = 0.781$, $n = 252$). We compared concentrations of nitrogen, carbohydrates, condensed tannins, and phenolic glycosides between scarred and unscarred trees using a MANOVA. We then calculated the percentage of variation in our tree use index that could be attributed to concentrations of condensed tannins and phenolic glycosides (the only two significant variables in the MANOVA) using multiple regression analysis. Finally, we investigated the contribution of the clonal structure of the aspen stand to the heterogeneity of the chemical composition of aspen trees using a series of ANOVAs with chemical variables as dependent variables.

We checked normality of data before performing parametric tests. We normalized data using log-transforms when the assumption of normality was not met. We performed analyses using SAS version 9.1 (SAS 2002); results are expressed as mean ± SE.

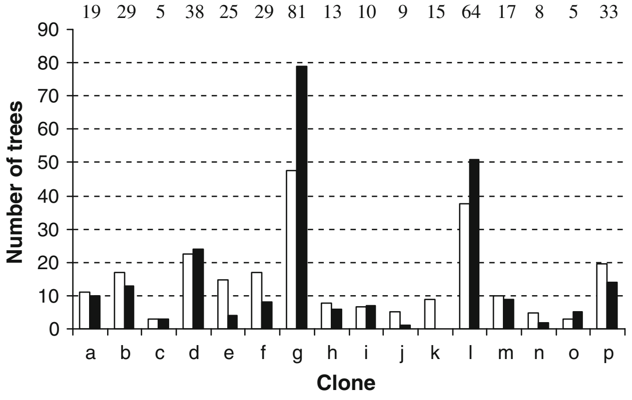

Fig. 1 Observed (*black bars*) and expected (*white bars*) numbers of trees bearing porcupine climbing scars in 16 trembling aspen clones studied in Parc National du Bic, Quebec, Canada. Expected numbers were generated under the null hypothesis that porcupines climbed trees randomly. Observed and expected numbers refer to the 400 trees with readable bark that we identified to the clonal level using field and laboratory techniques. Numbers above bars indicate sample sizes (e.g. number of trees with readable bark that were assigned to each clone)

Results

Does clonal structure of the aspen stand influence visitation of trees by porcupines?

Of the 577 aspen trees identified in our study site, 467 could be assigned to a particular clone and 510 had bark suitable to register porcupine claw marks, while 400 satisfied both conditions. Among these 400 trees, 234 (59%) showed signs of porcupine climbing. The climbing scar index of these 234 visited trees ranged from 1 to 49 and averaged 12.3 ± 10.6.

We assigned the 400 trees described above to 16 different clones. Within clones, the number of trees suitable to register porcupine claw marks varied from 5 to 81 (average = 25.0 ± 20.6 trees). The percentage of trees used by porcupines within clones also varied greatly, from no tree used to 97% used (average = 48.9% ± 7.2). Scarred trees were thus not distributed evenly among clones ($X_2 = 138.03$, $df = 15$, $P < 0.001$, Fig. 1), and the probability of a given aspen tree to be visited by porcupines depended on the clone to which it belonged. We conclude that clonal structure of the aspen stand influenced visitation of trees by porcupines.

Does visitation rate of trees reflect porcupine feeding preferences?

Captive porcupines preferred leaves sampled from highly scarred trees over leaves coming from trees with fewer scars ($F_{1,22} = 8.92$, $P = 0.0068$, Fig. 2). In 10 of 12 feeding trials, porcupines consumed a greater percentage of leaves from highly scarred trees (Fig. 2). Percent leaf matter consumed varied significantly among porcupines ($F_{4,88} = 24{,}55$, $P < 0.0001$) and trees ($F_{22,88} = 5{,}45$, $P < 0.0001$), but no interaction was significant. We conclude that variations in our tree use index did reflect food preferences of porcupines, and thus differential herbivory pressure from porcupines.

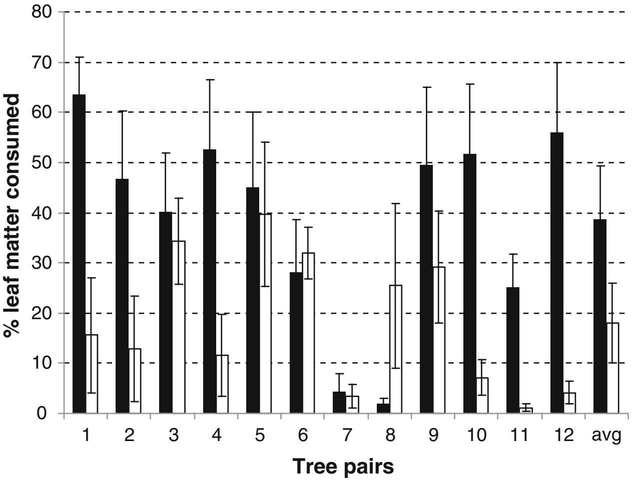

Fig. 2 Average consumption (%) of trembling aspen leaves offered to five captive porcupines during a 12-night feeding experiment in June 2003. Every night, a different pair of trees (numbers 1–12) containing a highly scarred (*black bars*) and a lightly scarred (*white bars*) tree was used as a source of leaves for the experiment. Quantity of leaves offered to porcupines was ca. 250 g fresh material per tree. *Error bars* SE

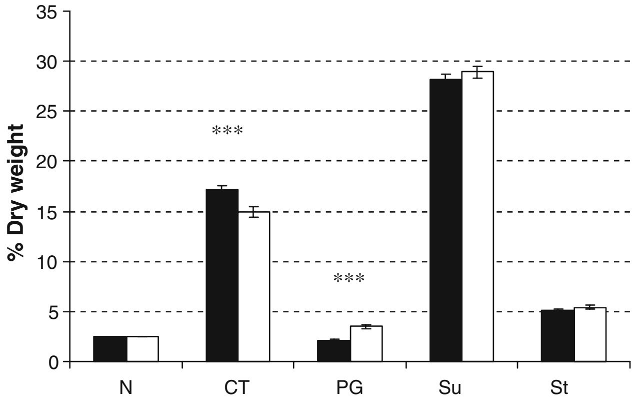

Fig. 3 Average (±SE) content of five chemical variables (*N* Nitrogen, *CT* condensed tannins, *PG* phenolics glycosides, *Su* sugar, *St* starch) measured in trembling aspen leaves from a 2.2-ha study site in Parc National du Bic, Quebec, Canada. Chemical content of 111 aspen trees bearing porcupine scars (*black bars*) is compared to chemical content of 141 trees showing no scars (*white bars*). ***$P < 0.01$

Does chemical composition of a tree influence its preference by porcupines?

There were no significant differences in the concentrations of nitrogen ($F_{1,250} = 0.32$, $P = 0.57$), sugar ($F_{1,250} = 0.84$, $P = 0.36$), and starch ($F_{1,250} = 1.44$, $P = 0.23$) between scarred and unscarred trees (Fig. 3). However, concentrations of condensed tannins were 15% lower in unscarred trees relative to scarred trees ($F_{1,250} = 19.14$, $P < 0.001$), whereas concentrations of phenolic glycosides were 63% greater in unscarred trees relative to scarred trees ($F_{1,250} = 44.93$, $P < 0.001$) (Fig. 3).

A multiple regression analysis, using only tannins and phenolic glycosides shows a significant relation between our tree use index and concentrations of the two classes of compounds ($R^2 = 0.19$, $P < 0.001$, $n = 252$, Tree use index = 1.04 tannins − 7.68 phenolic glycosides + 1.0385). Beta coefficients of the regression equation show that the effect of phenolic glycosides is considerably stronger than that of condensed tannins. We conclude that differential use of aspen trees by porcupines can be attributed partially to phytochemical variation among trees.

Does clonal structure of the aspen stand influence the chemical composition of trees?

Examination of the 252 trees analyzed for chemical composition shows significant differences in chemical variables among clones. The largest variations were found in condensed tannins and phenolic glycosides, which varied across clones by as much as 283% and 392%, respectively. By comparison, maximum differences found between clones for the other compounds were 129% (nitrogen), 163% (sugar), and 221% (starch). Descriptive chemistry of clones (as well as that of the sub-sample of trees used for the feeding experiment) and statistical testing of variations among clones are given in S5, Table S5.1. We conclude that the clonal structure of studied aspen trees did influence their chemical composition.

Discussion

We found that porcupines select aspen leaves with lower concentrations of phenolic glycosides and higher concentrations of condensed tannins. The deterrent effects of tremulacin and salicortin are well established. These chemicals reduce the performance of gypsy moth (*Lymantria dispar* L.) and forest tent caterpillar (*Malacosoma disstria* Hbn) larvae (Hemming and Lindroth 1995; Osier and Lindroth 2004) and high concentrations of phenolic glycosides reduce browsing of aspen by elk (Bailey et al. 2007).

In contrast to our findings, the association between tannins and mammalian herbivory is usually negative (Downs et al. 2003; Marsh et al. 2003; Bailey et al. 2004). Some positive associations have been found between aspen-feeding insects and the foliar concentration of condensed tannins, but they were likely due to the negative covariance between phenolic glycosides and condensed tannins, rather than to a preference for high concentrations of condensed tannins (Hemming and Lindroth 1995). The same chemical covariance likely explains apparent selection for high-tannin trees in this study. Alternatively, the positive correlation between porcupine herbivory and condensed tannins may be a result, rather than a cause, of porcupine food choice. Peters and Constabel (2002) and Osier and Lindroth (2001, 2004) showed an increase in tannin concentration in trembling aspen following wounding or defoliation.

Concentrations of phenolic glycosides in aspen are determined strongly genetically, but weakly environmentally (i.e., genetically canalized; Osier and Lindroth 2001, 2004, 2006; Donaldson and Lindroth 2007). In our study plot, 16 clones grew within a relatively uniform 2.2-ha patch of forest containing no obvious variation in soil characteristics, water availability, or light accessibility. In addition, most clones were intertwined and did not form isolated clumps of trees. Yet concentrations in phenolic glycosides markedly differed among clones. Similarly, Lindroth and Hwang (1996) reported marked variation in foliar concentrations of tremulacin (5.9-fold) and salicortin (10.3-fold) across 31 clones in Michigan. Some strong developmental changes in chemistry do occur in trembling aspen (Donaldson et al. 2006), but ontogenetic variation in chemistry occurs mostly before trees reach the 6- to 10-year age class, and is thus irrelevant to this study (see Methods). Finally, levels of phenolic glycosides were unlikely to have been affected by prior porcupine feeding. Phenolic glycosides are not induced in damaged leaves (Osier and Lindroth 2001) or in leaves produced a year after defoliation (Osier and Lindroth 2004). Levels are elevated in new leaves produced from lateral buds immediately following extreme defoliation (Stevens and Lindroth 2005; Donaldson and Lindroth 2008) but such was not the case for feeding by porcupines.

Our pen experiment involved only five porcupines, and food choice studies performed on wild herbivorous mammals typically face a large inter-individual variability in diet preferences (Berteaux et al. 1998; Bråthen et al. 2004). Although our sample size was sufficient to allow rejection of the null hypothesis of no difference between control and treatment, confidence in our results might have been increased by using more individuals. This, however, must be weighed against the ethical costs of restraining the movements of wild mammals in small pens for several weeks.

We have some evidence that scars were not due to a very small number of individuals. Our study plot is part of a larger study area where a long-term study involving marked individuals is ongoing (Berteaux et al. 2005). From 2000 to 2002, 46 individuals were observed within 200 m of the center of our 2.2 ha study plot (D.B., unpublished data). Many of these individuals probably fed in the study plot. In addition, climbing scars remain on tree trunks for many years (see Methods), so that some unknown individuals that were alive before 2000 could also have produced some scars.

North American porcupines can climb trees to feed, to rest in a safe place, or to escape predators (Roze 1989). The number of scars on aspen trunks would be unrelated to the intensity of herbivory if feeding was not the main reason why porcupines climbed aspens. In our study area, Morin et al. (2005) found that aspen was the main food source of porcupines whereas porcupines used rock dens and eastern white cedars as resting places. Typically they spent the night in one or a few aspens and the day in a rock den or cedar tree. This shows that porcupines climb aspens to feed, not to rest. Porcupines do sometimes climb aspens to escape terrestrial predators (D.B., unpublished data). When doing so, however, they climb the first available tree, so that scars generated by predator avoidance could not have biased use of aspen clones.

Influences of herbivory on plant fitness must be demonstrated before an herbivore is considered to be a selective agent, and a considerable amount of work on plant life history is needed to quantify the selection gradient imposed by herbivory. Heavy defoliation of aspen trees does suppress plant growth (Osier and Lindroth 2004; Stevens et al. 2007), therefore porcupine browsing may have negative effects on individual trees. However, the asexual reproduction of *P. tremuloides* makes it difficult to measure the effects of herbivory on individual fitness, because the growth pattern of clonal units buffers negative impacts, spreads the risk of death, and retards selection (Jelinski and Cheliak 1992). Finding a direct link between single-species herbivory and plant fitness is further confounded by the interacting effects of multiple herbivores and other variables in the ecosystem. For example, in our study plot the effects of aspen-feeding insects need to be separated to obtain an accurate measure of the impacts of porcupine herbivory on trembling aspens.

Our findings echo those of Heiska et al. (2007), who reported that vole (*Microtus agrestis*) feeding seemed to be highly affected by willow (*Salix myrsinifolia*) cultivation method and plant genotype. Similarly, Snyder and Linhart (1997) showed that when porcupines fed on phloem of Ponderosa pine (*Pinus ponderosa*) during winter months, eaten trees differed biochemically (lower concentrations of the monoterpene limonene) and genetically (allele frequencies at two of nine polymorphic loci) from adjacent trees that had not been fed upon.

Interestingly, when Snyder and Linhart (1997) compared the feeding patterns of porcupines with those of Abert's squirrels, a specialist feeding primarily on the phloem of Ponderosa pine, the level of feeding selectivity was found to be far less pronounced in the porcupine. More generally, many studies have reported that generalist feeders show a limited amount of preference in their choice of diet (e.g. Ben-Shahar 1991). This assertion could even appear as a logical tautology. However, our study shows that a generalist species feeding on a large range of plant species and plant parts can still show selectivity at the level of individual plants within a species.

In conclusion, our study suggests that a generalist vertebrate herbivore has adapted the ability to discriminate among individual plants within a clonal species, in a situation where the discriminating factor (leaf concentration in phenolic glycosides) had previously been shown to be largely of genetic origin. Our demonstration would have been stronger if the number of porcupines producing the scars was known, and if we had been able to associate climbing scars directly to herbivory. Yet our findings are particularly valuable given the important ecological impact of generalist vertebrate herbivores on most plant communities. Much work remains, however, to determine whether vertebrate herbivores have been sufficiently important players in the evolutionary past to shape the phytochemistry of plants (Moore and Foley 2005).

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