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Defensive adaptations of *Thuja plicata* to ungulate browsing: a comparative study between mainland and island populations

Abstract Forests on the Haida Gwaii (HG) archipelago (British Columbia, Canada) evolved for about 10,000 years in the absence of large-mammal browsing. The introduction of black-tailed deer (*Odocoileus hemionus sitkensis*) from the mainland prior to 1901 provides an opportunity to evaluate changes in the adaptive defensive responses of plants to herbivory. We compared (1) food choice by deer and (2) chemical defence (terpene concentrations) between HG and mainland red cedars (*Thuja plicata*) using (1) nursery-grown seedlings never exposed to deer, (2) branches from trees that grew before the introduction of deer ("old trees") and (3) saplings exposed to deer herbivory on the mainland and on HG. We used the first two plant categories to test the hypothesis that plants that evolve under low herbivory levels have lower anti-herbivore defences. We used saplings to study the consequences of the dramatic increase in browsing on HG. During food experiments, deer preferred HG seedlings and old tree branches compared to those from the mainland. Total monoterpene concentrations were much higher than diterpene concentrations in all plant categories. Within plant categories, multivariate analysis showed that terpene profiles differed significantly between HG and mainland red cedars: HG seedlings and old trees had lower monoterpene levels. These results suggest that some monoterpenes may be determinants of deer food choice and that the defences of HG plants are less effective than those of mainland plants. The deer used branches from HG and mainland saplings indiscriminately. However, terpene profiles differed significantly between HG and mainland saplings, with multi-variate analysis suggesting a higher defensive response in browsed HG saplings. Monoterpene profiles were different in lightly and heavily browsed saplings from HG, suggesting that under the current browsing regime, individuals with the greatest constitutive defences, or with greatest potential for induced defences, grow better and are selected on HG.

Keywords Herbivory · Terpene · Plant defences · Food choice experiment

Introduction

Herbivores can affect vegetation by modifying plant community structure and composition (Crawley 1989; Huntly 1991), reducing plant growth and reproduction, and altering plant fitness (Crawley 1983). Plants have a variety of defensive characters that can decrease their consumption by herbivores. These can be physical, like thorns, or chemical, like secondary metabolites arising from the shikimic acid, acetogenin or terpenoid pathways (Harborne 1991; Marquis 1991). Two major categories of chemical defences have been distinguished: constitutive defences that are independent of the history of herbivory on the individual plant, and induced defences that appear in response to herbivory (Karban and Baldwin 1997). Herbivores, in turn, have physiological and behavioural adaptations (e.g. diet selection and detoxification) that can overcome these defences (Simms 1990; Robbins et al. 1995; Hanley 1997).

Understanding how plants evolve anti-herbivore defences is important for many aspects of plant and animal ecology. Plant defence theories attempt to explain how plants survive in the presence of herbivores (Ehrlich and Raven 1964; Janzen 1979; McKey 1979; Bryant et al. 1983). Trade-offs among different allocation functions (e.g. reproduction, growth or defence; Bazzaz et al. 1987; Herms and Mattson 1992) or ecological trade-offs (Fritz 1992) and evolutionary constraints are key concepts in these theories (Mole 1994). Trade-offs arise

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from costs (see Simms 1992) such as the production and maintenance of defences against herbivores (Rhoades 1979; Skogsmyr and Fagerström 1992) or the existence of self-toxicity (McKey 1979; Zucker 1983). In practice, however, the existence of costs associated with plant defences is difficult to demonstrate for several reasons (Bergelson and Purrigton 1996). First, many of the chemicals involved in plant defence appear to have other functions within the plant (Seigler and Price 1976; Jones 1979; Langenheim 1994). Defence may thus simply be a pleiotropic effect of genes controlling traits that evolved through selection in response to other environmental stresses. Second, some mechanisms of defence may have low costs. Third, costs could be reduced by selection for individuals producing less costly defence traits (Simms 1992).

Comparing conspecific plant populations growing in similar habitats, but with different histories of herbivory, provides a means of addressing the issues of the costs associated with anti-herbivore defences and the role of herbivores in the evolution and expression of defences. These conspecific populations should differ only in having evolved with or without herbivores during a biologically relevant period of time. If the ancestral state of the two populations was resistant (Simms 1992), then under the hypothesis that plant defences are costly, plants that have evolved in a herbivore-free environment should be less defended than those that have evolved in the presence of herbivores.

Oceanic islands often have an impoverished fauna (MacArthur and Wilson 1967) and lack large mammalian herbivores. Islands may be colonised by mainland plants that have already evolved with herbivores (and that have thus been selected for resistance). Therefore, plant populations from oceanic islands and nearby mainland may share a common ancestor with anti-herbivore defences. Defences between plant populations on herbivore-free islands and on the adjacent mainland can be then compared. Janzen (1973) and Rickson (1977) used this approach to show that in the Caribbean, islands with reduced insect herbivores lack a symbiotic relationship with ants that defended the plant from insect attacks. Bryant et al. (1989) and Bowen and Van Vuren (1997) found that insular plants that had evolved in the absence of browsing mammals were more palatable and less defended compared to those that grew in the presence of browsers. However, whereas Bryant et al. (1989) compared plants growing in different regions (Iceland versus continental boreal forest), Bowen and Van Vuren (1997) compared different endemic subspecies of island and mainland plants rather than populations.

The Haida Gwaii (HG) archipelago, British Columbia, Canada, provides a quasi-experimental situation to test the prediction that island plants growing in the absence of large browsers are characterised by lower defences. The ecosystems of HG have evolved without the mammalian forest browser community typical of the adjacent mainland since the end of the last glaciation (16,000 years BP) (Cowan 1989). The only large terrestrial herbivore, the extinct Dawson caribou (*Rangifer tarandus dawsonii*), was restricted to parts of the northern island (Cowan 1989). HG is covered by a temperate rainforest that is similar to the coastal mainland forest (Pojar and MacKinnon 1994). Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) from the adjacent mainland were introduced to HG prior to 1901 (Carl and Guiguet 1972). Deer have since colonised most of the archipelago and their impact on the vegetation has been both extensive and dramatic (Pojar et al. 1980), thus providing a natural experiment on the response of "naive" plant populations to ungulate herbivory. If defence potential is variable among HG plants, deer should preferentially eat less defended plants, and thus act as a selective pressure that favours the survival of the most defended individuals.

We chose to study western red cedar (*Thuja plicata* Donn, Cupressaceae) because it is one of the dominant conifers in British Columbia coastal forests (Pojar and MacKinnon 1994) and is a preferred food of black-tailed deer (Pojar et al. 1980; Coates et al. 1985). Black-tailed deer inhibit red cedar regeneration on HG by browsing seedlings (Baltzinger 1997).

We used an experimental and an analytical approach to first investigate the long-term effects of reduced browsing. We asked the following questions. (1) Do deer prefer HG red cedars never exposed to deer compared to mainland red cedars that evolved with deer? (2) Do HG red cedars have reduced chemical defence compared to those from the mainland? We then investigated the short-term consequences of the introduction of deer to HG by asking the following questions. (3) Do deer prefer HG red cedars that grew after the introduction of deer compared to mainland trees of the same age? (4) Do these HG and mainland red cedars differ in their chemical defences? (5) On HG, do heavily browsed red cedars have lower chemical defences than lightly browsed red cedars?

Materials and methods

Haida Gwaii

HG (Fig. 1) is an oceanic archipelago about 300 km long and about 80 km from mainland Canada. There are two major islands (Graham and Moresby), both with a central range of mountains (maximum elevation 1,148 m above sea level), and several hundred smaller islands. HG belongs to the same climatic zone as the adjacent mainland and was probably briefly connected to the mainland during the last glaciation (30,000–16,000 years BP).

Western red cedar

The western red cedar grows to a maximum height of 70 m and a diameter of 4.3 m (Hebda and Mathewes 1984). During the last glaciation, it spread northward along the coast from California to British Columbia (Clague 1989). Red cedar pollen first appears in palaeobotanical records in British Columbia about 10,000 years BP. About 6,000 years BP, red cedar began to increase along the coastal mainland and on HG (Hebda and Mathewes 1984). It is endemic to the moist coastal regions of northwestern North America, growing
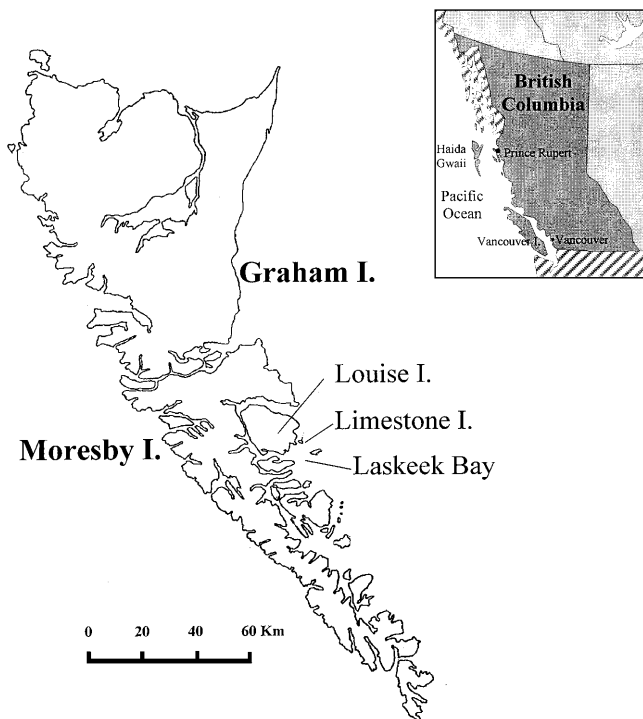
Fig. 1 Location of the study areas in Haida Gwaii, British Columbia, Canada

in coastal southern Alaska, the coastal ranges of British Columbia, western Washington, Oregon, and northern California (Hebda and Mathewes 1984). The tree is rather shade tolerant and can grow in wet and boggy areas (Pojar and MacKinnon 1994). Red cedars can produce cones at <20 years of age (Fowells 1965) and have a low outcrossing rate (32% on Vancouver Island; El-Kassaby et al. 1994). Yeh (1988) noted low genic heterozygosity in western red cedar. von Rudloff and Lapp (1979) found that, among North American conifer species, the western red cedar has very low intra- and inter-population variation in the composition of leaf oil terpenes, which could be due to a bottleneck in population size some 10,000 years ago, at the end of the last glaciation (Yeh 1988). Seeds of red cedar are small and wind dispersed but the relatively small wing surface limits the range of dispersal (Fowells 1965). The distance between HG and the mainland (80 km) is therefore likely to have been a barrier for gene flow between the two populations.

Sitka black-tailed deer

Sitka black-tailed deer were introduced to HG prior to 1901 (Carl and Guiguet 1972). The deer have colonised most islands in the archipelago and occur at higher densities on HG than on the adjacent mainland, probably due to the absence of predators (S. Sharpe, personal communication).

Plant samples

Long-term effects of reduced browsing

Plants on HG have evolved with an impoverished browser community. To test the long-term consequences of this history, we selected two plant categories in which individuals have no recent exposure to deer browse on both HG and the mainland: nursery grown seedlings and branches from old trees.

The seedlings were grown from HG and mainland seeds under standardised conditions. HG seedlings came from seeds collected

from adults that grew before the deer were introduced. All seedlings were <1 year old and about 30 cm high. The seedlings were removed from cold chambers on the same day and stored in cardboard boxes until used in experiments. The old trees were ≥80 cm in diameter and estimated to be ≥150 years old (Fowells 1965), and so on HG, germinated at least 50 years before the introduction of deer.

On the same day, we collected leaves and branches (about 50 cm long) that were inaccessible to deer (≥2.5 m off the ground) from 90 HG and 90 mainland old trees. The mainland branches were brought overnight to HG. The trees were in first-growth forest and were ≥30 m apart. To reduce the influence of site-specific biases on plant defences (Duncan et al. 1994; Langenheim 1994; Iason et al. 1996), we were careful to minimise variation in climatic and edaphic conditions between sampling localities on Graham Island and in the Prince Rupert region (Fig. 1).

Short-term consequences of ungulate introduction

Sapling branches and leaves were sampled at sites logged about 15 years previously. Although the ancestors of these saplings had grown in an environment with reduced herbivory on HG, these saplings had been exposed to deer for their entire lifetime on both the mainland and on HG. Deer density is higher on HG compared to the mainland (S. Sharpe, personal communication), and some saplings on HG are very heavily browsed (not higher than 0.5 m and browse scars on more than 70% of the leaves). These saplings grow on the same sites and could be the same age (G. Wiggins, personal communication) as tall lightly browsed saplings (1.5–2 m high, less than 30% of the leaves with browse scars, and estimated to be about 10 years old).

We collected sapling branches (about 50 cm long) from 90 HG and 90 mainland individuals that had been lightly browsed; the saplings were ≥20 m apart in three different localities on Graham Island and Prince Rupert. Saplings were collected on the same day and mainland saplings were brought to HG overnight. Heavily browsed individuals on HG did not provide branches long enough for food choice experiments, but their numerous browse scars and their growth form strongly suggested that deer browsing was more intense compared to lightly browsed saplings. To test whether chemical defence was correlated with deer choice among HG saplings we sampled leaves from 15 lightly and 15 heavily browsed saplings. All these individuals were easily accessible to deer and were sampled on the same day on central Graham Island.

Food choice experiments

Field protocol

To test the preference of deer for HG and mainland red cedars, we conducted food choice experiments within the three red cedar categories (seedlings, old trees and saplings). These experiments were performed with free-ranging black-tailed deer on islands in Laskeek Bay, HG (Fig. 1). This area was selected because of its high density of relatively tame deer. For seedlings, two trials were performed at each of the following three sites: Limestone Island, Vertical Point (on Louise Island) and Crescent Bay (on Louise Island, 2 km from Vertical Point). For old trees and saplings, we conducted one feeding trial in three different sites: Limestone Island, Vertical Point and Teepee Cove (on Louise Island, 2 km from Vertical Point, because deer at this site became more active than deer at Crescent Bay).

Feeding trials lasted 1–5 days, except for two seedling trials that lasted 21 days. We alternated 30 HG and 30 mainland individuals of the same plant category in a 7.5×13.5 m plot. Adjacent samples were 1.5 m apart and planted into the ground. We checked each plot every day and stopped the experiment when at least 15 individuals (50%) of either HG or the mainland were browsed. Browsing was assessed by the presence of at least one leaf tip clipped (with a jagged edge typical of deer browse, as deer lack upper incisors). We counted the number of HG and mainland individuals that had been browsed.

Statistical analyses

We analysed separately the number of individuals browsed within each plant category and separated the two seedling sets. We used "origin" (HG versus mainland) and "site" as qualitative explanatory variables. The number of individuals browsed was modelled with a binomial error according to a survival model (Aitkin et al. 1989). We used general linear modelling (GLIM software; Aitkin et al. 1989) to estimate the probability that a plant was browsed in relation to the different parameters, using the logit as a linking function. The backwards procedure was used, sequentially removing the interactions and the main effects from the complete model (Aitkin et al. 1989). The difference in deviance between two models follows a χ^2 law, and that test was used to examine the effects of the different variables and their interactions.

Plant chemical composition

Protocol

We chose to analyse concentrations of terpenes because these compounds are abundant in red cedar leaves (Lewinsohn et al. 1991b) and are known to decrease food consumption by cervids (Elliott and Loudon 1987; Danell et al. 1990; Duncan et al. 1994).

For the HG/mainland comparisons, we analysed leaf extracts from 30 individuals per plant category per origin. Leaves of old trees and saplings were collected on a second branch taken at the same time from one-third of the trees that were sampled for food choice experiments. We took leaf samples from seedlings belonging to the same set as those used for the feeding trials. Three grams of fresh leaf material from each of the 180 plant samples was extracted with 30 ml methanol for 48 h. After filtration, the extracts were stored at –20°C until analysed for terpene content by gas chromatography. Extracts were analysed on a Hewlett Packard (model 6890) gas chromatograph equipped with a flame ionisation detector and a 30 m×0.25 mm 5% phenyl methyl siloxane column (Hewlett Packard); He flow=2.5 ml/min (38 cm/s); injector and detector temperatures=200°C and 300°C, respectively; the temperature program was 50°C for 2 min, ramped to 280°C at 10°C/min and then held at 280°C for 5 min. Assignments of chromatogra-phic peaks were made by similar chromatographic analyses using a Hewlett Packard (model 5972) mass selective detector and subsequent computer matching of the mass spectra with library spectra. We confirmed the identification of monoterpenes by comparing pure compounds to extract peaks.

We used the same protocol to determine the terpene composition of the leaf material from the 15 lightly and 15 heavily browsed HG saplings.

The red cedar sampling and food choice experiments were conducted in March and April 1998, and the terpene analyses in May 1998.

Statistical analyses

For the HG/mainland comparisons, we considered "plant category" (seedlings, old trees and saplings) and "origin" (HG/mainland) as qualitative explanatory variables. We first calculated the total concentration of the monoterpene fraction and then the diterpene fraction per individual. We analysed these data using ANOVA (SAS 1996) and compared the means by the LSMEANS procedure (SAS 1996). We then considered every terpene concentration as a quantitative variable and used a MANOVA (SAS 1996) with the same explanatory variables. To obtain an overall description of the HG/mainland differences, we used principal component analyses (PCAs; PROC FACTOR; SAS 1996).

For the lightly/heavily browsed HG saplings comparison, we considered "browse intensity" (light/heavy) as the qualitative explanatory variable. We followed the same procedure by first using one-way ANOVA on the total concentration of mono- and diter-penes, followed by MANOVA and PCA on the concentrations of all individual terpenes.

Results

Deer food choice between HG and mainland red cedar

Long-term effects of reduced browsing

HG individuals were browsed more often than the mainland individuals in both the seedling and the old-tree categories (Fig. 2; "origin" effect, $df=1$; first set of seedlings: $\chi^2=13.34$, $P<0.001$; second set of seedlings: $\chi^2=4.73$, $P=0.03$; old trees $\chi^2=11.00$, $P<0.001$). The site of the experiments had a significant effect on the probability of browsing for both plant categories ($df=2$; first set of seedlings: $\chi^2=16.60$, $P<0.001$; second set of seedlings: $\chi^2=55.84$, $P<0.001$; old trees $\chi^2=10.86$, $P=0.004$). The origin×site interactions were not significant in either category.

Short-term consequences of ungulate introduction

"Origin" had no significant effect on the incidence of browsing for saplings (Fig. 2; $df=1$, $\chi^2=0.65$, $P=0.42$), while the site of the experiments again had a significant effect ($df=2$, $\chi^2=22.06$, $P<0.001$). The origin×site interaction was not significant.

Total monoterpene and diterpene concentration in red cedar

We focused on the 11 terpene peaks (eight monoterpenes and three diterpenes) which were present in more than 75% of the samples (Table 1). Six of the eight monoterpenes were identified and confirmed with pure compounds obtained commercially. The identification of two of the three diterpenes could not be confirmed because the pure compounds were not available (Table 1).

Monoterpene concentrations were much higher than diterpene concentrations in all plant categories (Figs. 3, 4). The "plant category" effect was significant in the ANOVA of the total monoterpene concentrations ($F_{2,174}=139.12$, $P<0.001$). The monoterpene concentration was highest in saplings (at least double those of the other categories) and lowest in seedlings (Fig. 3). The plant category×origin interaction was not significant ($P=0.73$).

The total diterpene concentrations also varied significantly between plant categories ($F_{2,174}=44.80$, $P<0.001$), being lowest in seedlings and highest in old trees (i.e. increased with plant age; Fig. 3). The plant category×origin interaction was significant (Fig. 3; $F_{2,174}=6.45$, $P=0.002$).

Long-term effects of reduced browsing

The concentration of the total monoterpene fraction was not significantly different between HG and mainland

Fig. 2 Probability of browsing
of red cedar seedlings, branches
of old trees and lightly browsed
saplings in food choice experi-
ments with black-tailed deer at
three sites in relation to the
geographic origin of samples.
Two independent sets of trials
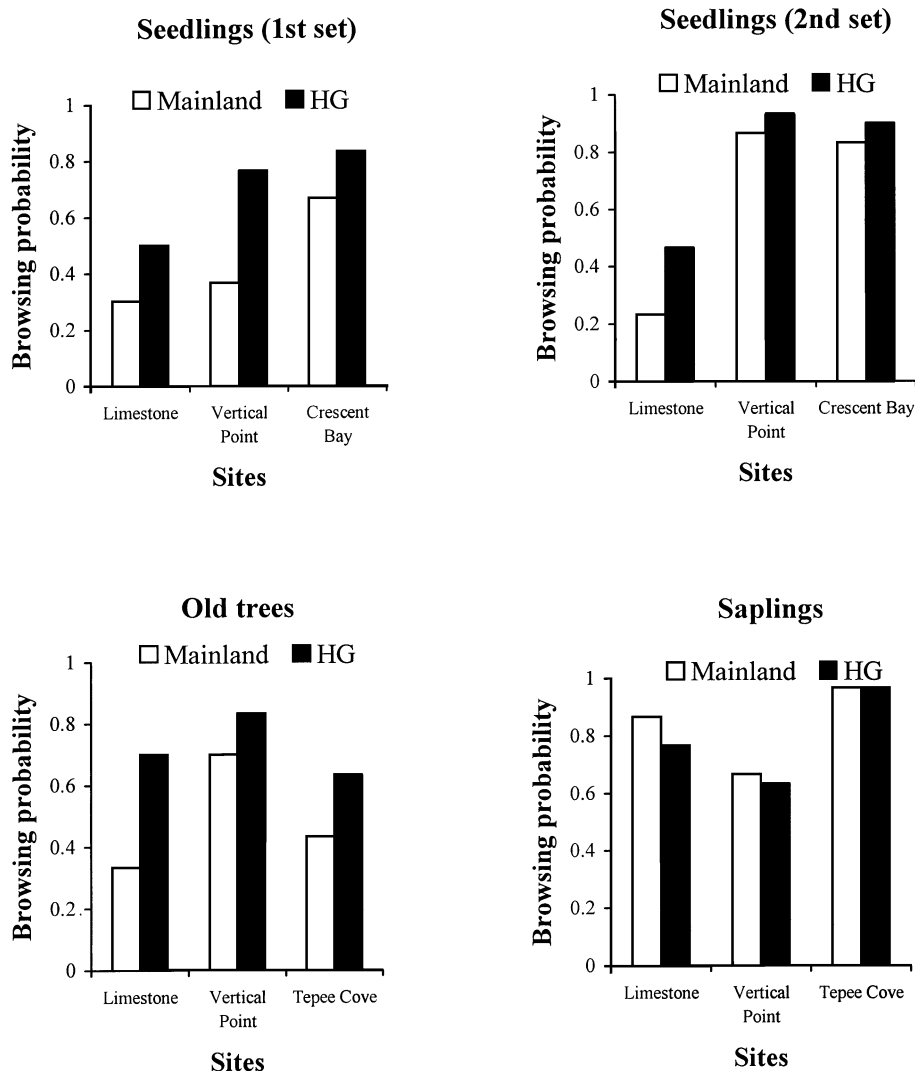were conducted for seedlings

Table 1 Terpenes isolated in the red cedar leaf extracts; retention
times (*RT*) correspond to terpene peaks observed with gas chroma-
tography with a flame ionisation detector. The codes are those
used in this paper to symbolise the different terpenes. Some terp-
enes were identified by gas chromatography with a mass selective
detector. The identities of the monoterpenes were confirmed with
pure commercial compounds

RT (min)	Code	Terpene type	Identification
8.80	F	Monoterpene	?
8.89	G	Monoterpene	α-Pinene
9.53	H	Monoterpene	Sabinene
9.78	I	Monoterpene	β-Myrcene
10.45	L	Monoterpene	*dl*-Limonene
11.48	N	Monoterpene	?
11.66	O	Oxydate monoterpene	α-Thujone, isomer 1
11.83	P	Oxydate monoterpene	β-Thujone, isomer 2
24.66	T	Oxydate diterpene	?
25.04	U	Oxydate diterpene	Totarol?
25.27	V	Oxydate diterpene	Ferruginol?

seedlings and old trees (Fig. 3; test LSMEANS: seed-
lings $P=0.75$, old trees $P=0.19$). The concentration of the
total diterpene fraction in branches from old trees was
significantly higher on the mainland than on HG (Fig. 3;
test LSMEANS: $P=0.01$) but the concentration in seed-
lings did not differ between HG and mainland plants
(Fig. 3; test LSMEANS: $P=0.39$).

Short-term consequences of ungulate introduction

The concentrations of the total monoterpene and diter-
pene fractions were not significantly different between
lightly browsed HG and mainland saplings (Fig. 3; test
LSMEANS: monoterpene $P=0.71$, diterpene $P=0.07$).
Lightly and heavily browsed HG saplings did not dif-
fer in their total monoterpene concentrations either
(Fig. 4; test LSMEANS: $P=0.13$), but heavily browsed
saplings had significantly higher total diterpene con-
centration than lightly browsed ones (Fig. 4; test
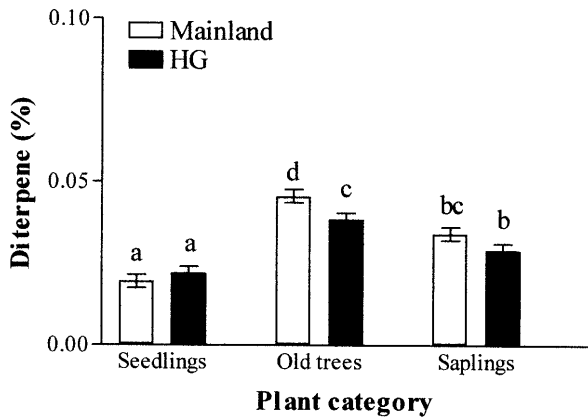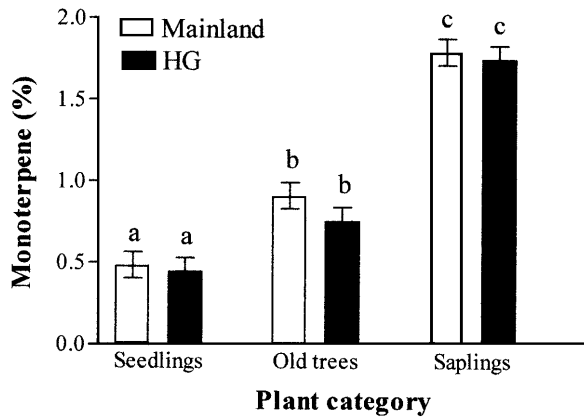LSMEANS: $P=0.01$).

Fig. 3 Mean total concentration of monoterpenes and diterpenes (percentage of fresh leaf weight) in leaves of red cedar seedlings, old trees and saplings in relation to their geographic origin. Means with different letters are statistically different (LSMEANS, $P<0.05$)
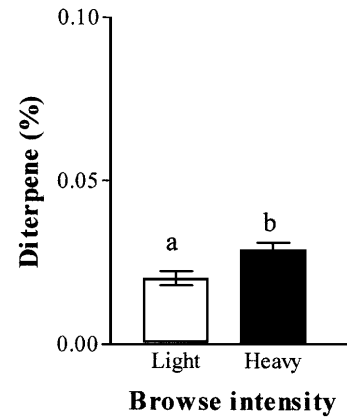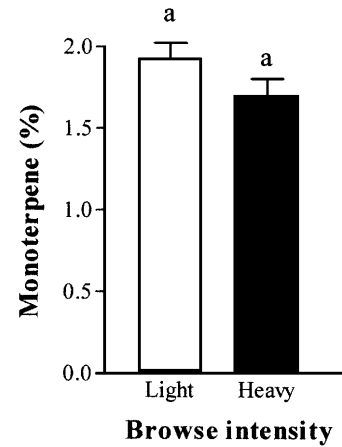
Fig. 4 Mean total concentrations of monoterpenes and diterpenes (percentage of fresh leaf weight) in red cedar leaves of heavily browsed and lightly browsed Haida Gwaii saplings. Means with different letters are statistically different (LSMEANS, $P<0.05$)

Table 2 Correlation coefficients of the different terpenes (F–V; Table 1) with the first two components of principal component analyses (PCAs). Only significant coefficients ($P<0.05$) are shown (*HG* Haida Gwaii, *ML* mainland, *Light* lightly browsed, *Heavy* heavily browsed)

| | Seedlings | | Old trees | | Saplings | | | |
| | HG vs ML | | HG vs ML | | HG vs ML | | Light vs Heavy | |
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Monoterpenes								
F		−0.31	0.42		0.74			−0.52
G	0.54		0.89		0.96		0.68	0.52
H	0.61		0.86		0.78		0.73	
I	0.86		0.90		0.97		0.91	
L	0.92		0.99		0.96		0.90	
N	0.91		0.97		0.83		0.94	
O	0.88		0.92		0.89		0.86	
P	0.88		0.90		0.85		0.81	
Diterpenes								
T		0.64		0.45	−0.32	0.67	−0.64	
U		0.88		0.90	−0.45	0.83		0.84
V		0.78		0.76	−0.36	0.83		0.88

Multivariate analyses of terpenes

A MANOVA on the terpene composition of all individual plant samples of the HG/mainland comparisons revealed a significant origin×plant category interaction (Roy's greatest root=0.18, $F_{11,165}$=2.66, P=0.004).

Since MANOVAs within each plant category showed that terpene profiles differed (see below), we explored the sources of these differences by PCA within each category. In all the analyses, there was a sharp drop in eigenvalues from the first to the second component, indicating that the first PC explained most of the variability in the data. For all PCAs, the monoterpenes were significantly ($P<0.05$) and positively correlated to the first component, except monoterpene F (Table 2). The next most important source of variance, PC2, expressed the amounts of the three diterpenes. However, in each case, the scores of individual plants on the PC1×PC2 plane showed considerable overlap between samples of different origins (e.g. Fig. 5).
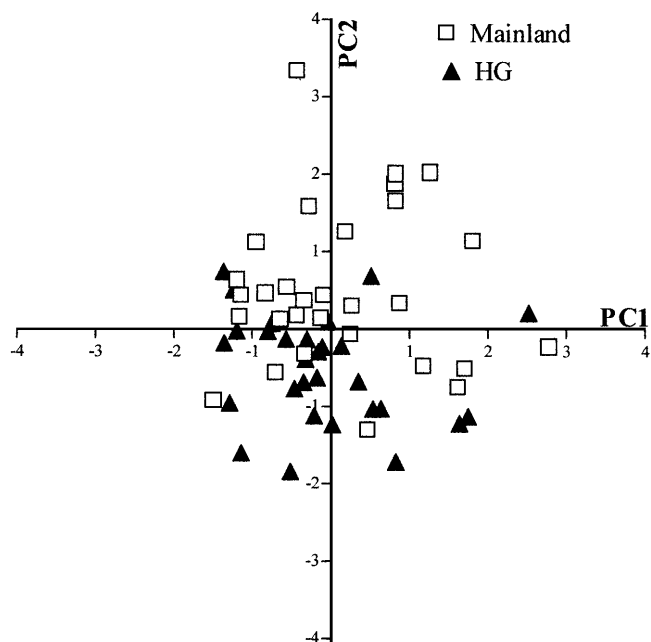
Fig. 5 Scores of mainland and Haida Gwaii individuals on axes 1 and 2 of the principal components (PC) analysis of terpene concentrations in fresh leaves of old red cedar trees. *PC1* reflects concentrations of most of the monoterpenes (see Tables 1, 2) and *PC2* mainly reflects diterpene concentrations

Table 3 Results of experiments on food choice by deer in the Haida Gwaii/mainland comparisons (+ more browsed, – less browsed) and browsing impact in HG saplings (+ heavily browsed, – lightly browsed) compared with the means of individual scores along the first and second PCA axes. Scores along PC1 increase with concentrations of monoterpenes G–P, scores along PC2 increase with diterpenes (an *asterisk* indicates a significant difference ($P<0.05$) in the incidence of browsing in the food choice experiments; see Fig. 2)

Category	Comparison	Browsing	PC1	PC2
Seedlings	Haida Gwaii*	+	−0.07	0.11
	Mainland*	−	0.07	−0.11
Old trees	Haida Gwaii*	+	−0.13	−0.48
	Mainland*	−	0.13	0.48
Saplings	Haida Gwaii lightly	−	0.04	−0.19
	Mainland lightly	+	−0.04	0.19
	Haida Gwaii heavily	+	−0.12	0.25
	Haida Gwaii lightly	−	0.12	−0.25

sity" variable (Roy's greatest root=3.59, $F_{11,18}=5.88$, $P<0.001$). The first two axes of the PCA accounted for 50% and 20%, respectively, of the variance. The heavily browsed HG saplings had lower average PC1 scores compared to lightly browsed saplings.

Discussion

Plant choice by deer and its relation to chemical composition

For red cedar seedlings and old trees, both of which had not been exposed to deer before the experiments, deer preferred HG to mainland plants. HG red cedars, which evolved in the absence of large herbivores, were therefore more palatable than mainland red cedars. This result is consistent with the studies of Bryant et al. (1989) and Bowen and Van Vuren (1997), who showed that species that had evolved without mammalian herbivores were more palatable than those that evolved with herbivores.

In contrast, HG and mainland saplings, which had both been exposed to deer prior to the experiments, were browsed similarly by deer. Since total monoterpene concentration in saplings was much higher than in other plant categories, both HG and mainland saplings may have defence levels above the threshold at which deer can discriminate between individuals. However, such an interpretation seems unlikely because the large differences in browsing observed on adjacent saplings in the field indicate that deer do choose among saplings. An alternative explanation is that the difference between the two populations is too small for deer to detect.

The significant effect of experimental site on deer feeding behaviour is a common feature of feeding trials and reflects differences in consumption level between sites explained by differences in the number and identity of deer (Duncan et al. 1994). However, despite this variation, there was no significant origin×site interaction and within a plant category, deer choice was similar on every site.

Long-term effects of reduced browsing

The MANOVA indicated that "origin" had a significant effect in both seedling and old-tree categories (seedlings: Roy's greatest root=1.04, $F_{11,48}=4.53$, $P<0.001$; old trees: Roy's greatest root=0.61, $F_{11,48}=2.67$, $P=0.009$).

The first two axes of the PCAs accounted for 44% and 18%, respectively, of the variance for the seedlings, and 56% and 15%, respectively, for the old trees. Within these two categories, the choices of the deer in the food choice trials were consistent with the average score of the samples on PC1 (Table 3). Plants with high incidences of browsing (i.e. HG seedlings and HG old trees) had lower scores on PC1 than the mainland plants which had been less browsed. There is no such consistency for the second component.

Short-term consequences of ungulate introduction

In the MANOVA, "origin" had a significant effect in the HG/mainland saplings comparison (Roy's greatest root=0.69, $F_{11,48}=3.02$, $P=0.004$). The first two axes of the PCA accounted for 60% and 18%, respectively, of the variance. The lower average score of the mainland samples on PC1 (Table 3) was consistent with the results of the food choice experiments: although the difference was not statistically significant, deer tended to eat more mainland saplings (Fig. 2).

The MANOVA of lightly/heavily browsed HG saplings showed a significant effect of the "browse intensity"

The monoterpenes that we identified (α-pinene, sabinene, β-myrcene, *dl*-limonene and α- and β-thujone) have previously been recorded in red cedar leaves (von Rudloff and Lapp 1979). The high concentrations observed in saplings are consistent with other studies showing that juveniles, which have a relatively higher risk of herbivory, are the most defended age class; seedlings generally do not have enough resources to defend themselves fully (Sinclair and Smith 1984; Bryant et al. 1991).

Total mono- and diterpene concentrations did not vary significantly among origins, except for diterpenes in HG/mainland old trees and in lightly/heavily browsed HG saplings. However, it is not necessarily the differences in total concentration that influence food choice. Differences in profiles of individual secondary compounds in plants or combinations of compounds can be more important (Connolly et al. 1980). Such a situation requires a multivariate approach. In this study, the MANOVA showed significant differences in terpene profiles in relation to plant origin (HG/mainland) and natural browsing pattern (lightly/heavily browsed). The PCAs of terpene concentrations indicate that despite large variation among individual samples and a large overlap in the range of chemical profiles between samples, the significant segregation disclosed by the MANOVAs was due principally to variations in monoterpene profiles between plants of different origins.

Several studies have already demonstrated that monoterpenes can affect cervid food choice (mule deer: Longhurst et al. 1968; Welch et al. 1981; red deer, *Cervus elaphus*: Elliott and Loudon 1987; Duncan et al. 1994), and in vitro experiments have shown that monoterpenes can be toxic to mammalian herbivores by inhibiting rumen microbial activity (Oh et al. 1967; Schwartz et al. 1980). Monoterpenes are volatile molecules and in addition to being toxic could serve as olfactive signals (Elliott and Loudon 1987) and influence food choice before browsing by deer. The possibility exists that some monoterpenes are inactive as feeding deterrents but act as predictors of plant quality. The two roles are not mutually exclusive. In contrast, diterpenes, which occurred at much lower concentrations, are essentially nonvolatile and few studies have shown that they can be repulsive to cervids (Danell et al. 1990). Future work needs to test the response of deer to the individual monoterpene molecules that were identified in this study.

Evolutionary and adaptive significance

Long-term effects of reduced browsing

The lower PC1 scores for terpene profiles recorded for HG seedlings and old trees, and the preference of deer for these plants, is consistent with our hypothesis of lower anti-herbivore defence in plant populations which do not coexist with mammalian herbivores. As both seedlings and old trees had not been exposed to deer pri-

or to the experiments, the differences we observed must reflect a reduction in constitutive defences as opposed to induced defences. The trend towards a reduction in defence in the absence of herbivores could be interpreted as evidence for costs associated with defences against herbivory. This interpretation should, however, be considered cautiously as, in contrast to our observations in chemical profiles, we did not find a significant difference due to origin in the total terpene content, the latter being likely to be a better measure of defence investment.

Since the seedlings were grown in standardised conditions, and because herbivore defence (Berenbaum and Zangerl 1992) and monoterpene production are principally under genetic control in conifers (Hanover 1966; Merk et al. 1988), there may be genetic differences underlying the phenotypic differences observed in this study. The work of Mopper et al. (1991) on another long-lived conifer, the Pinyon pine (*Pinus edulis*) on the Sunset Crater (Arizona, USA), supports this interpretation. They showed that under strong differences in selection regime caused by herbivory and environmental stress across a narrow geographic range, genetic differentiation occurred between pine populations in less than 800 years. Similarly, Daehler and Strong (1997) compared resistance to insect herbivory (planthoppers, *Prokelisia marginata*) in two introduced smooth cordgrass (*Spartina alterniflora*) populations differing in their history of herbivory: one population has spread in the absence of herbivores for more than a century, the other was introduced 20 years previously in a area with herbivores. Daehler and Strong (1997) also compared these two populations to the resistant source population. They found that the herbivore-free population had reduced planthopper resistance and suggested that factors including a founder effect, loss of herbivore tolerance, and herbivore preference could explain this difference. In red cedar, the low outcrossing rate observed by El-Kassaby et al. (1994) could actually favour relatively rapid genetic evolution (Linhart and Grant 1996) since the arrival of the tree on HG around 10,000 years ago (Hebda and Mathewes 1984).

Short-term consequences of ungulate introduction

The higher PC1 score of lightly browsed HG saplings when compared to heavily browsed saplings suggests that deer were choosing HG plants with lower defence potential. This is consistent with other studies showing that, within the same population, the most intensively eaten trees are those with the lowest levels of secondary compounds (moose, *Alces alces*: Danell et al. 1990; Abert's squirrels, *Sciurus aberti*: Snyder 1993).

These results could explain why there was no difference in browsing by deer on HG and mainland saplings. Selective browsing could conceivably give rise to an HG sub-sample of little-browsed saplings with monoterpene profiles similar to or higher than the mainland plants.

There are two non-exclusive mechanisms that could explain why HG saplings had similar or higher average PC1 values: (1) deer avoid HG seedlings and saplings that had the strongest induced defences to deer attack and/or (2) deer avoid the few young plants with unusually high constitutive defences. Lerdau et al. (1994), Marpeau et al. (1989) (in wood) and Litvak and Monson (1998) (in leaves) have shown that monoterpenes can be involved in both induced and constitutive defences. Furthermore, Lewinsohn et al. (1991a) observed an increase in the activity of the monoterpene cyclase in red cedar following wounding. Hence, there may be a trade-off between constitutive and induced defences. Low resource availability and low herbivory risk should favour mechanisms of induced responses because of the higher cost of constitutive defences (Rhoades 1979; Berenbaum et al. 1986; Zangerl and Berenbaum 1990; Simms 1992).

Conclusions

HG plants that evolved in the absence of ungulate browsing (seedlings and old trees) are preferred by deer and show a trend towards reduction of constitutive chemical defences compared to mainland plants that evolved with ungulate browsing. Deer did not show significant discrimination between saplings of different origins, but tended to prefer mainland ones. The monoterpene profiles differed, with HG saplings tending to have higher scores on the monoterpene axis. Furthermore, heavily browsed saplings on HG had lower scores on the monoterpene axis than adjacent lightly browsed ones. Deer thus appear to create a strong selective pressure by favouring the persistence of individual seedlings with high concentrations of chemical defences. The effects of deer introduction on a long-lived tree population can be observed within a century.

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