Can plant resistance to specialist herbivores be explained by plant chemistry or resource use strategy?

Kirk, H.; Vrieling, K.; Pelser, P.B.; Schaffner, U.

Oecologia (2012) 168:1043–1055
DOI 10.1007/s00442-011-2179-6

PLANT-ANIMAL INTERACTIONS - ORIGINAL RESEARCH PAPER

Can plant resistance to specialist herbivores be explained by plant chemistry or resource use strategy?

Heather Kirk · Klaas Vrieling · Pieter B. Pelser · Urs Schaffner

Received: 24 May 2010 / Accepted: 1 October 2011 / Published online: 5 November 2011

Abstract At both a macro- and micro-evolutionary level, selection of and performance on host plants by specialist herbivores are thought to be governed partially by host plant chemistry. Thus far, there is little evidence to suggest that specialists can detect small structural differences in secondary metabolites of their hosts, or that such differences affect host choice or performance of specialists. We tested whether phytochemical differences between closely related plant species are correlated with specialist host choice. We conducted no-choice feeding trials using 17 plant species of three genera of tribe Senecioneae (*Jacobaea*, *Packera*, and *Senecio*; Asteraceae) and a more distantly related species (*Cynoglossum officinale*; Boraginaceae) containing pyrrolizidine alkaloids (PAs), and four PA-sequestering specialist herbivores of the genus *Longitarsus* (Chrysomelidae). We also assessed whether variation in feeding by specialist herbivores is attributable to different resource use strategies of the tested plant species. Plant resource use strategy was quantified by measuring leaf dry matter content, which is related to both plant nutritive value and to plant investment in quantitative defences. We found no evidence that intra-generic differences in PA profiles affect feeding by specialist herbivores. Instead, our results indicate that decisions to begin feeding are related to plant resource use strategy, while decisions to continue feeding are not based on any plant characteristics measured in this study. These findings imply that PA composition does not significantly affect host choice by these specialist herbivores. Leaf dry matter content is somewhat phylogenetically conserved, indicating that plants may have difficulty altering resource use strategy in response to selection pressure by herbivores and other environmental factors on an evolutionary time scale.

Keywords Phytochemical evolution · Host selection · Pyrrolizidine alkaloids · *Longitarsus* (Chrysomelidae) · Asteraceae (*Senecio*, *Packera*, *Jacobaea*)

Communicated by Diethart Matthies.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-011-2179-6) contains supplementary material, which is available to authorized users.

H. Kirk · K. Vrieling
Institute of Biology, Plant Ecology and Phytochemistry, Leiden University, 9505, 2300 RA Leiden, The Netherlands
e-mail: k.vrieling@biology.leidenuniv.nl

Present Address:
H. Kirk (✉)
ETH Zurich, Applied Entomology, Institute of Agricultural Sciences, Schmelzbergstrasse 9, 8092 Zurich, Switzerland
e-mail: kirkh@ethz.ch

P. B. Pelser
Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand
e-mail: pieter.pelser@canterbury.ac.nz

U. Schaffner
CABI Europe-Switzerland, Rue des Grillons 1, 2800 Delémont, Switzerland
e-mail: u.schaffner@cabi.org

Introduction

A majority of phytophagous insects are restricted to a small selection of host plant species (Schoonhoven et al. 2005), and many of these specialist herbivores retain their association with their hosts over millions of years (Janz and Nylin 1998). These narrow plant–herbivore associations can be constrained by, for example, the secondary

chemistry of the host plant and the ability of herbivores to adapt to varying suites of phytochemicals (Becerra et al. 2009; Futuyma and Agrawal 2009), the biogeographic distribution of related plant species and their associated herbivores (Futuyma and Mitter 1996), or the distribution of plant-associated parasites and predators (Singer and Stireman 2005). There has been recent interest in understanding macroevolutionary patterns that facilitate diversification of plant species and their associated herbivores at the levels of phenotypic, phytochemical, and species diversity (Becerra et al. 2009; Futuyma and Agrawal 2009; Nyman 2010). In particular, mechanisms by which specialists make hosts shift are interesting case studies of coevolution (Ehrlich and Raven 1964; Becerra et al. 2009), species radiations (Futuyma and Agrawal 2009; Nyman 2010), and the evolution of ecological niches (reviewed by Nyman 2010).

Interest in the relationship between phytochemical diversity and the host-specificity of specialist phytophages is not just a recent insurgence; the staggering diversity of secondary metabolites in plants has been a central theme in evolutionary ecology for at least 45 years (e.g. Ehrlich and Raven 1964; Berenbaum and Feeny 1981; Berenbaum et al. 1986; Becerra et al. 2009). A variety of major structural groups of secondary metabolites have been described from the plant kingdom, but the countless closely related structures within some of these major groups (e.g. alkaloids and terpenoids) makes this diversity even more astonishing (Hartmann and Witte 1995; Hartmann 1996; Wink 2003; Pelser et al. 2005; Langel et al. 2011). Yet, except for a few well-known cases, interactions between specialist herbivores and the array of secondary metabolites in their host plants is not well understood. In some cases, the presence or absence of major structural groups of toxic metabolites in plants is known to affect fitness and host choice in specialist insects (Futuyma et al. 1994; Bernays 1998 and references therein). However, little evidence suggests that specialists generally can or do distinguish between closely related chemical structures in potential host plants, or that such compounds differ significantly regarding effects on specialist fitness or host choice (Lindroth et al. 1988; Moyes et al. 2000; Macel et al. 2002; Macel and Vrieling 2003; but see Berenbaum et al. 1986; Becerra et al. 2009).

Some authors have also emphasised the importance of nutritive value and digestibility of food plants in herbivore host choice (Feeny 1976; Rhoades and Cates 1976). These traits can be correlated with nitrogen to carbon ratio, toughness (mediated by quantitative defences including lignin and cellulose), water content, and specific leaf area (Coley et al. 1985; Choong et al. 1992; Hendriks et al. 1999; Elger and Willby 2003; Schädler et al. 2003; Clissold et al. 2009). Plant traits that reflect plant resource use strategy (which encompasses growth rate, resource allocation patterns, and nutrient uptake and retention) are generally correlated with each other, such that herbivores prefer leaves that are high in nitrogen and water content and low in toughness (e.g. Clissold et al. 2009). From among these characters, a good overall indicator of plant resource use strategy is leaf dry matter content (or leaf water content; Wilson et al. 1999). Although the effects of plant physical characters on insect feeding choices have been well studied (Coley et al. 1985; Elger and Willby 2003; Schädler et al. 2003; Clissold et al. 2009), impacts of plant resource characteristics on specialist herbivores have been generally ignored in recent discussions about specialist host choice (Cornell and Hawkins 2003; Becerra et al. 2009). Such characters may play a large role in host selection for specialists when structurally similar secondary metabolites do not have very different impacts on herbivores.

Longitarsus flea beetles (Chrysomelidae) are an illustrative example of a genus that is pre-adapted to specialisation on plant species containing highly toxic defence chemicals (Dobler 2001). *Longitarsus* species specialise to varying degrees on members of pyrrolizidine alkaloid (PA)- and iridoid glycoside (IG)-containing plant families (Dobler et al. 2000). The ability to sequester PAs and IGs has been adopted multiple times independently within the genus (Dobler 2001). Of the approximately 60 *Longitarsus* species occurring in western Europe, at least 10 are known to feed on *Senecio* and *Jacobaea* (tribe Senecioneae, family Asteraceae; Windig and Vrieling 1996), plant genera that are notorious for the production of PAs. Adult beetles feed on the foliage of host species, while larvae generally mine roots, root crowns, and petioles (Windig and Vrieling 1996).

PAs are a diverse group of secondary metabolites that encompass approximately 360 known structures (Hartmann and Witte 1995), found among a number of plant families including Asteraceae, Boraginaceae, Fabaceae, and Orchidaceae (Hartmann 1999). Variation in PA concentrations is genetically determined in at least one *Jacobaea* species (*J. vulgaris*; Vrieling and van Wijk 1994) and PA composition is generally species specific (Hartmann and Dierich 1998). It has been suggested that PA diversification has resulted from selection pressure by herbivores (Hartmann and Dierich 1998; Ober and Hartmann 1999).

Here, we adopt a comparative strategy to test whether inter-specific differences in plant alkaloid profiles affect adult feeding of four PA-adapted *Longitarsus* species. If PA profiles in the host plants have a significant effect on host acceptability, we expect that expression of PAs by plants will impose a restriction on the host range and niche breadth of their specialist herbivores. In addition, we explore whether host choice of adult beetles can be explained by variation in plant resource use strategies, or by other phylogenetically conserved factors.

Materials and methods

Plant species

We collected or germinated 17 species of annual and/or perennial herbs from the genera *Jacobaea* (formerly assigned to the genus *Senecio*), *Senecio*, and *Packera* (tribe Senecioneae, family Asteraceae; Pelser et al. 2002, 2007, 2010) and one species of *Cynoglossum* (C. *officinale*; Boraginaceae) for use in feeding tests (Appendix 1 in ESM). The Senecioneae species included in our study were selected to represent both different evolutionary lineages within the tribe as well as closely related species. *Cynoglossum officinale* is distantly related to the Senecioneae and contains PAs of a different type than those found in the Senecioneae. We included *C. officinale* in this study to examine the possibility that PAs stimulate feeding in *Longitarsus* regardless of PA structure. Whenever possible, we included two or more populations of each test plant species in the experiments. Individuals grown from seed were germinated in a climate chamber (16/8 h day/night, 22/18°C) in February 2003, and were transplanted to an outdoor garden covered by a nylon mesh cage (0.6 mm) to exclude herbivores in May, 2003. Individuals collected from natural habitats were transplanted to the same garden in June 2003, and all plants were grown in common conditions for at least 3 weeks before the initiation of feeding trials.

Longitarsus species

Beetles were collected from *J. vulgaris* plants at several field sites in England, Germany, and Switzerland between July and September 2003, and all feeding tests were conducted between July and October 2003. All *Longitarsus* species included in this study are able to sequester PAs (Dobler 2001). Adult *Longitarsus* individuals feed on the foliage of their host plants, leaving characteristic feeding punctures (hereafter called 'shot-holes'), and lay eggs in the soil around the root crown of host plant rosettes (Frick 1970). Larvae feed on the roots and root crown of host plants. Therefore, besides adult feeding, host use by herbivores is also affected by female oviposition and larval feeding behaviour. However, in the case of *L. jacobaeae*, the number of larvae tunnelling the roots of *J. vulgaris* in spring is significantly correlated with the number of shot-holes recorded in the previous autumn on the same plants (Rapo et al. 2010), indicating that adult feeding is a good indicator of host use by at least *L. jacobaeae*.

Longitarsus jacobaeae is generally considered to be monophagous on *J. vulgaris* (Frick 1970) under field conditions, but experimental host-specificity testing revealed that larvae and adults can also feed and develop on other *Jacobaea* species (Frick 1970; Puliafico, Kirk and Schaffner, unpublished results; this paper). *Longitarsus jacobaeae* individuals used in this study were collected from a number of sites in the Swiss Jura during the month of July, during which time abundance in the field remained high.

Of the four species tested, *L. suturellus* and *L. succineus* are least specific. *Longitarsus suturellus* feeds on *Jacobaea* and *Petasites* species (Dobler 2001), another genus belonging to the tribe Senecioneae (Asteraceae) that is more distantly related to the three Senecioneae genera included in this study (Pelser et al. 2007, 2010). One record also indicates that this species feeds on *Eupatorium cannabinum* (Eupatorieae; Asteraceae) in the field (Dobler et al. 2000). Individuals used in this study were collected in Delémont (Swiss Jura) in July. *Longitarsus succineus* feeds on *Achillea* species (Anthemideae; Asteraceae) and *Jacobaea* species (Dobler 2001). We collected adults in the Rhine Valley (Germany) from the end of July until the end of September.

Longitarsus flavicornis individuals were collected during July and August from a population in Silwood Park (England), and their taxonomic identity was confirmed by molecular means (Puliafico 2003). According to Windig and Vrieling (1996), differences between host ranges of *L. jacobaeae* and *L. flavicornis* have never been properly tested. To our knowledge, no published studies regarding host range of *L. flavicornis* are available.

Leaf dry matter content and PA content measurements

All plant populations (Appendix 1 in ESM) were analysed for leaf dry matter and PA content. Between 2 and 17 individuals from each population were selected based on availability. One to four fresh fully expanded leaves were harvested from each analysed plant on the day that feeding trails were initiated (more leaves were harvested from species with smaller leaves) and were immediately weighed to determine fresh weight. Leaves were then transferred to an oven and dried for 3 days at 50°C, after which they were were re-weighed to determine dry weight. Leaf dry matter content was calculated as the decimal fraction (dry weight)/(fresh weight). Leaf material was not always harvested concurrently for use in PA extractions and feeding trials, due to the duration over which feeding trials were conducted. However, variation in PA concentration and composition is genetically controlled in at least *J. vulgaris* (Vrieling et al. 1993), and PA composition is considered to be species specific (Hartmann and Dierich 1998; Macel et al. 2002; Pelser et al. 2005).

Dried leaves were stored in a freezer (−20°C) until PA extraction, at which time leaves were ground to a fine powder using a mortar and pestle. Leaf samples of 20 mg

from individuals within species were pooled such that at least three replicate mixtures were analysed per species. When three or more populations were available per species, we combined (20 mg) leaf samples from all leaves within each population, and analysed each population separately. When two populations were available, we analysed two leaf mixtures per population (half the individuals pooled for one replicate, and the other half pooled for the second replicate). When one population was available, we divided individuals into three replicates. Replicates therefore contained pooled samples from between three and seven individuals.

Fifteen mg of each plant mixture was extracted according to a modified version (de Boer 1999) of the acid–base extraction method (Hartmann and Zimmer 1986). Extractions were dissolved in methanol containing heliotrine (Latoxan, France) as an internal standard and analysed using gas chromotography (GC). Conditions (injector 250°C, temperature program 220–250°C 5°C min^{-1}, split mode 1:30, carrier gas N_2 0.9 ml min^{-1}, pressure 56 kPa; detector NPD) were controlled by a Hewlett Packard gas chromatographer (model 6890). Concentration was measured by integrating the area under GC peaks, and standardised according to the known concentration of heliotrine. GC traces were compared with known references to identify sample composition. PAs that could not be identified from known references were identified using GC–MS according to Witte et al. (1992).

Experimental setup of feeding trials

All beetles were stored at 10°C and were offered *J. vulgaris* leaves for feeding before and between feeding trials. After a feeding trial, beetles were stored at 10°C and offered *J. vulgaris* leaves for several days before reuse in feeding trials. All beetles were starved for 48 h before the initiation of feeding trials, during which time they were given moist filter paper to prevent dehydration, and were maintained under the conditions to be used in feeding trials (16/8 h day/night, 22/18°C).

No-choice experiments were conducted using all test plant species from all available populations (Appendix 1 in ESM). For each combination of plant and beetle species, the experimental setup was replicated between six and ten times (depending on beetle availability). For each replicate, four leaf disks (diameter 2 cm) were removed from fully expanded, undamaged leaves of experimental plants using a hole-punch, and placed in square formation within Petri dishes (diameter 9 cm) lined with moist filter paper. In cases where leaves were not broad enough to obtain complete leaf disks, we used leaf fragments of approximately the same surface area as leaf disks (estimated by eye). Plant genotypes within a Petri dish were randomly combined, and represented as many available populations within a plant species as possible in order to reduce variation based on individual and population genotypic or environmental effects. We did not measure intra-specific differences in feeding (i.e. the number or size of shot-holes from different populations within species), since we were interested only in inter-specific patterns of host acceptability. In order to control for intraspecific differences in PA composition and dry matter concentration (resulting from different growing conditions or genetic variation), we included leaf disks from several populations (when possible) in each Petri dish that was used in no-choice trials and multiple choice trials. Beetles were therefore able to feed from the most acceptable population within each species.

Two beetles were released into each Petri dish for a period of 48 h, after which they were removed and the number of shot-holes were counted on each leaf fragment. Leaf area removed was estimated by holding leaves/fragment up to 1-mm graph paper and tracing shot-holes using a pencil. Total leaf area removed (total feeding) measured in experiments is the product of both number of shot-holes, and the mean area of shot-holes. Number of shot-holes gives an indication of number of feeding bout initiations, while area of shot-holes is an indication of feeding bout duration.

All *L. jacobaeae* and *L. suturellus* feeding trials were conducted within a 25-day period, between 23 July and 18 August. All replicates for each species were tested at the same time, such that three or four randomly selected plant species were tested per day. Feeding trials with *L. succineus* were conducted between 29 July and 5 October, and with *L. flavicornis* between 5 September and 5 October. Different plant species were randomised throughout the trial period with regard to dry matter content, PA composition, and phylogeny.

Multiple choice experiments

We conducted multiple choice experiments using whole leaves and whole plants to ensure that no-choice data reflected ranking of host preferences in ecologically relevant, multiple-choice situations. First, all species listed in Table 1 with the exception of *S. doronicum*, which was no longer available, were included in an artificial garden arrangement. Four leaves from each species, selected from four different plants from a maximal number of populations, were included in each replicate. Leaves from all species were selected to be approximately equal in size (surface area). Sixty-eight holes (diameter 2 cm) were drilled out of a 3-cm-thick styrofoam sheet, at a distance of 5 cm apart in a rectangular grid formation of 8 × 9 holes (corner holes were excluded, because only 68 leaves were

Table 1 Correlations between PA concentration and leaf dry matter content, and *Longitarsus* feeding characteristics (number and area of shotholes, and total feeding)

	L. jacobaea			*L. suturellus*			*L. succineus*			*L. flavicornis*		
	n	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*
PA concentration: before phylogenetic correction												
No. shotholes	17	0.271	0.370	17	**0.634**	**0.020**	17	0.137	0.601	13	−0.009	0.997
Area shotholes	17	0.251	0.408	17	0.306	0.309	17	−0.067	0.799	13	−0.198	0.517
Feeding area	17	0.464	0.110	17	0.388	0.190	17	0.068	0.795	13	−0.119	0.699
PA concentration: after phylogenetic correction												
No. shotholes	13	−0.110	0.721	13	0.280	0.354	13	0.231	0.448	10	0.358	0.310
Area shotholes	13	0.236	0.437	13	0.341	0.255	13	−0.187	0.541	10	−0.285	0.425
Feeding area	13	0.082	0.789	13	0.258	0.394	13	0.077	0.803	10	0.212	0.556
Dry matter content: before phylogenetic correction												
No. shotholes	17	**−0.509**	**0.037**	17	**−0.677**	**0.003**	17	**−0.678**	**0.003**	13	**−0.718**	**0.006**
Area shotholes	17	0.197	0.449	17	0.028	0.915	17	−0.085	0.745	13	0.564	0.045
Feeding area	17	−0.376	0.137	17	**−0.583**	**0.014**	17	**−0.489**	**0.046**	13	−0.406	0.169
Dry matter content: after phylogenetic correction												
No. shotholes	13	−0.462	0.112	13	**−0.560**	**0.046**	13	**−0.681**	**0.010**	10	−0.600	0.067
Area shotholes	13	0.407	0.168	13	0.055	0.859	13	−0.038	0.901	10	0.176	0.627
Feeding area	13	−0.401	0.174	13	−0.445	0.128	13	−0.423	0.150	10	−0.345	0.328

Results obtained before (Pearson correlation) and after (Spearman rank correlation) phylogenetic correction are presented

Significant correlations shown in bold

used). Cylinders equal in size to holes were bored from green florist's foam, and were inserted into the styrofoam sheet after 30 min of soaking in water. Leaves were inserted in random positions into foam cylinders within the grid and the entire setup was encased in a mesh cage of 80 × 80 cm. We released 15 starved (48 h) beetles into each cage and allowed feeding to proceed for a period of 2 days. Herbivory was measured as number of shot-holes and leaf surface area consumed (mm^2). The experiment was replicated five times for each of *L. jacobaeae* and *L. suturellus*. Only two of the four beetle species were used due to limitations in the number of beetles and the amount of plant material available. Multiple- and no-choice feeding preferences agreed well for both *L. jacobaeae* (Spearman rank correlation; $r = 0.779$, $n = 17$, $P < 0.001$) and *L. suturellus* (Spearman rank correlation; $r = 0.600$, $n = 17$, $P = 0.011$) indicating that no-choice data are robust and ecologically relevant, and that no-choice feeding was not the result of compensatory feeding on normally unacceptable species.

Next, a subset of plant species (Appendix 1 in ESM) included in the artificial garden setup was selected to be placed in outdoor mesh cages measuring 2 m × 2 m × 2 m. Cages were located in an experimental garden and were designed to prevent insects from entering or exiting. Three potted plants of each species from a variety of populations were buried in sawdust (so that soil surface in pots was flush with ground surface) in random orientation in a 5 × 5 grid formation. Because only 24 plants were used in the experiment, no plant was placed in the middle position of the first row. On 10 September, 50 beetles from each of *L. jacobaeae* and *L. suturellus* were released separately into each of two such cages, so that the experiment was conducted once for each of the two *Longitarsus* species. The experiment was continued for 3 weeks, and beetles were recollected on 1 October. The number of shotholes and total feeding area were measured from all plants in the cage. Ranking from cage tests (data not show) agreed with that from no-choice and experimental garden experiments, though we did not apply statistical tests due to lack of replication for cage tests. We therefore present only data from the no-choice dataset below, which was most comprehensive.

Data analysis

All statistical tests were carried out using SPSS 8.0 (SPSS, 1998).

Leaf dry matter content

We used a nested ANOVA, nesting plant population (random factor) within plant species (fixed factor), to determine whether there were significant differences in leaf dry matter content among species.

PA content

Total PA concentration data were log-transformed to achieve a normal distribution. A univariate ANOVA was applied to test for differences in total PA concentration according to species. We used mean concentration per species in Pearson correlations with no-choice data (see below; No-choice data).

To evaluate the effects of PA composition on feeding, we used principle component analysis (PCA) to reduce the number of variables in the dataset, which was a matrix of concentrations of each PA present in the analysed plant samples. We retained variables that explained at least 5% of the variation. We also constructed correlation matrices of beetle feeding against individual PA concentrations; however, P values were never close to significant after correction for multiple testing ($P > 0.5$ in all cases). Before correction for multiple testing, only 7 of 176 tests were significant, which is less than would be expected based on chance alone (i.e. at the 5% level, approximately 8.8 significant correlations would be expected based on chance alone). The results of these tests are therefore not shown.

To determine whether feeding characteristics were correlated with PA composition, we calculated pair-wise dissimilarity of each plant species from *J. vulgaris* (in squared Euclidean distance) based on (1) PCA variables derived from PA data, and (2) feeding characteristics of each beetle species (total feeding, number and size of shot-holes). For each *Longitarsus* species, we tested for relationships between PA composition and beetle feeding using Pearson correlations between (1) and (2) above.

No-choice data

Cynoglossum officinale was excluded from analysis because, in almost all cases, no feeding occurred on this species. PAs from *C. officinale* were structurally different from those of all other species included in the study (see "Results"), and lack of feeding by the beetle species on *C. officinale* may have resulted from large phytochemical differences between that species and the natural host species, and/or physical plant characteristics, which would be impossible to disentangle given the current experimental design.

We used ANOVA to test if total feeding differed between both beetle and plant species. Total feeding data were not normally distributed due to the presence of several outliers. Outliers resulted from extreme variations in beetle feeding, usually due to occasional non-feeding on plant species that were normally accepted by beetles. We therefore performed a rank transformation (Zar 1999) of total feeding (dependent factors), and conducted a two-way ANOVA on rank values using plant and beetle species as fixed factors. To check for consistency of ANOVA results, we also employed ANOVA on standardised feeding after log transformation (which improved normality). Furthermore, we employed ANOVA as outlined above but after removal of outliers, in which case the data were normally distributed. Data remained heteroscedastic after transformation, but this is not considered serious for overall tests of significance (Sokal and Rohlf 1995).

To determine whether variation in shot-hole number or variation in shot-hole size contributed most to variation in total feeding, we used a variance ratio test (Zar 1999) to investigate differences between coefficients of variation for number and size of shot-holes for each *Longitarsus* species. The variance ratio test is described by:

$$F = s^2_{\log \#s} \Big/ s^2_{\log area}$$

where the numerator equals the variance of logarithms of shot-hole numbers, and the denominator equals the variance of logarithms of shot-hole area.

Using Pearson correlations before, and Spearman rank correlations after phylogenetic corrections (see below), we investigated (1) the relationship between number of shot-holes and size of shot-holes for each species (referred to below as feeding traits), and (2) the relationship of feeding characteristics (total feeding, and number and size of shot-holes) to leaf dry matter content and PA concentration per plant species.

Phylogenetic correction

Because phylogenetic relationships create inter-specific dependence of plant traits such as dry matter and PA content, we applied phylogenetically independent contrasts to dry weight, PA concentration, and beetle feeding trait correlations, as described by Harvey and Pagel (1991). In short, we calculated the difference in trait values (PA concentration, dry weight, and feeding traits) between branches diverging from a node that represents a common ancestor (nodes are defined in Fig. 1). Contrasts were calculated by subtracting trait values to the right of the nodes from trait values to the left of nodes. Node differences were then used in correlative analyses.

We constructed a cladogram (Fig. 1) presenting the evolutionary relationships between the Senecioneae species included in this study based on the results of previous and ongoing studies into the phylogeny of Senecioneae. The relationships between the *Jacobaea* species (groups one to five, Fig. 1) were taken from Pelser et al. (2004). Because these studies were not conclusive about the relationships among *J. vulgaris* and its closest relatives,

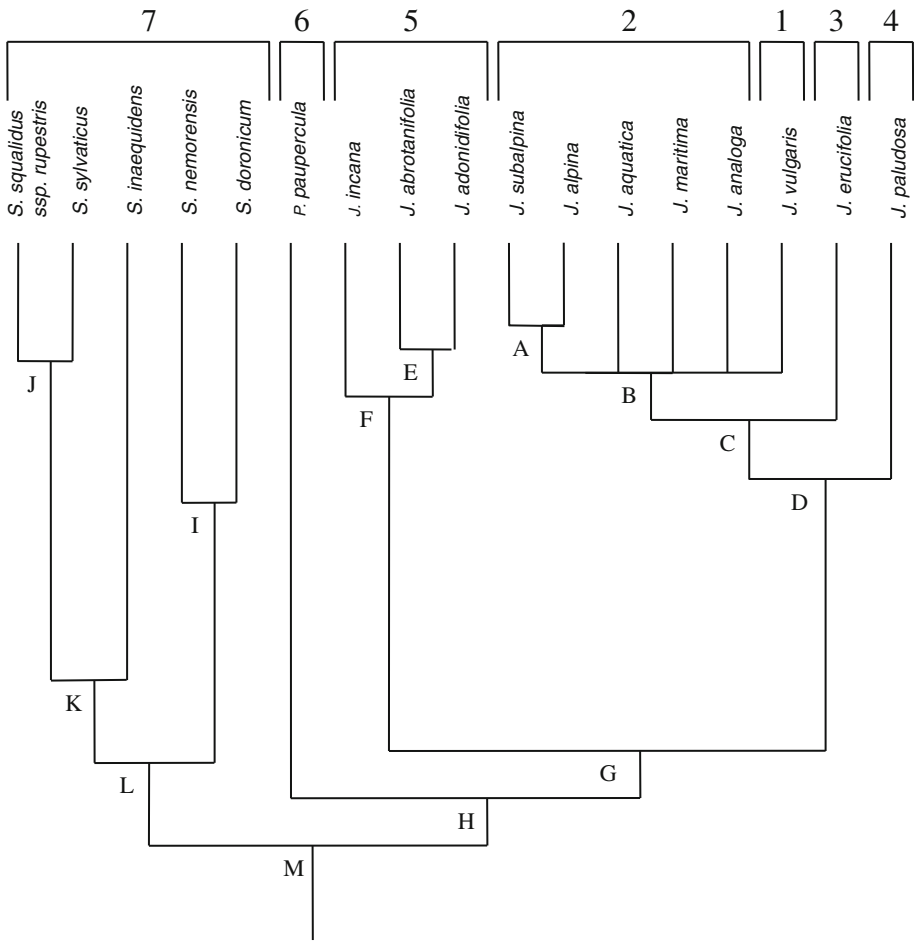

Fig. 1 Composite cladogram based on DNA sequence data (Pelser et al. 2002, 2004, 2007, 2010). *Letters* assigned to nodes of tree represent divisions subjected to independent contrasts in phylogenetically corrected correlation analyses. Groupings above clades were used as phylogenetic values in correlations with feeding and for GLM analysis

we present the phylogenetic relationships between the *J. alpina*/*J. subalpina* clade, *J. aquatica*, *J. maritima*, *J. analoga*, and *J. vulgaris* as a polytomy. Pelser et al. (2002, 2007, 2010) show that *Jacobaea* is more closely related to species of the North American genus *Packera* (represented in this study by *Packera paupercula*; group 6) than to species currently assigned to *Senecio*. Group 7 holds members of *Senecio* sections *Senecio* (*S. squalidus* ssp. *rupestris* and *S. sylvaticus*), *Fruticulosi* (*S. inaequidens*), and *Doria* (*S. nemorenis* and *S. doronicum*). The relationships between these species were taken from Pelser et al. (2007).

Relationships between feeding and phylogeny

Specialist insects often accept host plants that are closely related to natural hosts, which likely reflects chemical or other similarities between closely related species (Futuyma et al. 1994; Hendriks et al. 1999). In order to test whether host acceptability for *Longitarsus* species was mediated by phylogenetically conserved factors other than PA composition or resource use, we split our composite cladogram (Fig. 1) into seven groups as illustrated. *Jacobaea vulgaris* was designated as group one, because all *Longitarsus* species used in bioassays were collected from this natural host species. Groups two to seven are increasingly distantly related to *J. vulgaris*. Group numbering was used for Spearman rank correlations with total feeding, number of shot-holes, and size of shot-holes. Furthermore, we used the general linear model (GLM) function (SPSS) defining plant grouping (phylogeny) as a fixed factor and PA concentration and dry matter content as covariates to determine significance of these factors in determination of feeding by beetle species.

Results

Leaf dry matter content

Leaf dry matter content (Fig. 2) differed both among species ($F = 5.269$; $df = 17, 11$; $P < 0.05$; Fig. 2) and among populations within species ($F = 3.409$; $df = 15, 201$; $P < 0.001$; data not shown).

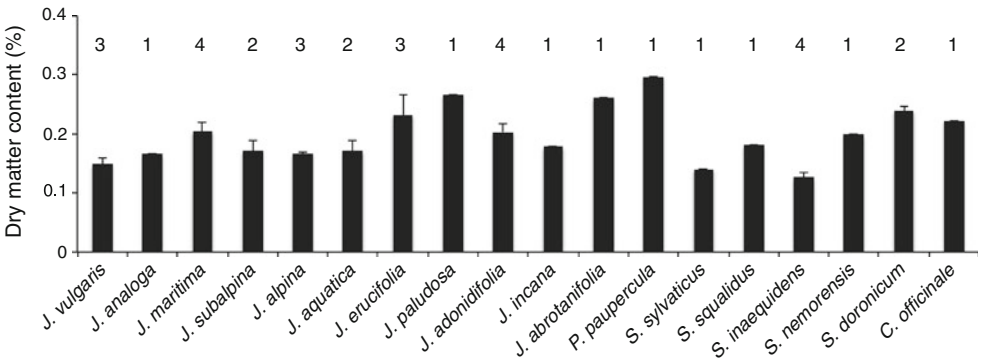

Fig. 2 Leaf dry matter content of plant species. *Bars* represent means of population means, and *error bars* represent standard error of population means. *Numbers above bars* indicate the number of populations analyzed per species. Plant species are listed according to their phylogenetic distance from *J. vulgaris* (see Fig. 1)

PA content

Total PA concentrations differed among plant species ($F = 32.036$; $df = 17, 42$; $P < 0.001$; Fig. 3). Mean PA concentration per species was uncorrelated with mean leaf dry matter content per species ($r = -0.129$, $n = 18$, NS; after phylogenetic correction: $r = -0.079$, $n = 10$, NS).

In total, we identified 52 peaks from GC traces that represent PAs. Of these, eight were specific to *C. officinale*. Because there was no overlap between PAs in *C. officinale* and Senecioneae species, we excluded *C. officinale* from further analysis, as effects of PAs were likely confounded with other effects resulting from the large phylogenetic distance between *Cynoglossum* and the Senecioneae. Therefore, we included 44 Senecioneae-specific PAs in our data analysis, and different species contained different subsets of these PAs (data not shown). PCA allowed us to reduce the number of axes to six. Six PCA axes cumulatively accounted for 82.1% of the total variation in Senecioneae PA composition. Hierarchical cluster analysis based on PCA variables (data not shown) revealed that in some cases closely related species have relatively similar PA profiles (e.g. *J. alpina* and *J. subalpina*; *J. aquatica* and *J. vulgaris*; *J. incana* and *J. abrotanifolia*), while in other cases, PA profiles differ broadly between closely related species (e.g. *J. analoga* and *J. vulgaris*). There was also a large cluster which was poorly resolved, indicating that six species (closely and distantly related; *S. squalidus* ssp. *rupestrus*, *P. paupercula*, *J. paludosa*, *J. erucifolia*, *J. maritima*, *J. adonidifolia*) could not be clearly differentiated according to PA profiles.

No-choice tests

Total feeding (Fig. 4) was determined by plant species ($F = 18.938$; $df = 16{,}516$; $P = <0.001$) and an interaction between plant species and beetle species ($F = 3.676$; $df = 44{,}516$; $P = <0.001$) according to rank-transformed ANOVA. Beetle species alone had no significant effect on total feeding ($F = 0.217$; $df = 3{,}516$; $P = 0.885$). This significant interaction was well supported by additional ANOVAs using log-transformed total feeding with ($F = 3.902$; $df = 44{,}516$; $P < 0.001$) and without ($F = 5.491$; $df = 44{,}500$; $P < 0.001$) outliers. These results indicate that host plant use differed among the four beetle species.

Number of shot-holes was uncorrelated with size of shot-holes for all *Longitarsus* species, implying independence between feeding initiation and continuation of feeding. Furthermore, number of shot-holes was more variable than size of shot-holes for each of *L. jacobaeae* ($F = 11.964$; $df = 1{,}313$; $P < 0.001$), *L. suturellus* ($F = 36.904$; $df = 17, 16$; $P < 0.001$), *L. succineus* ($F = 2.445$;

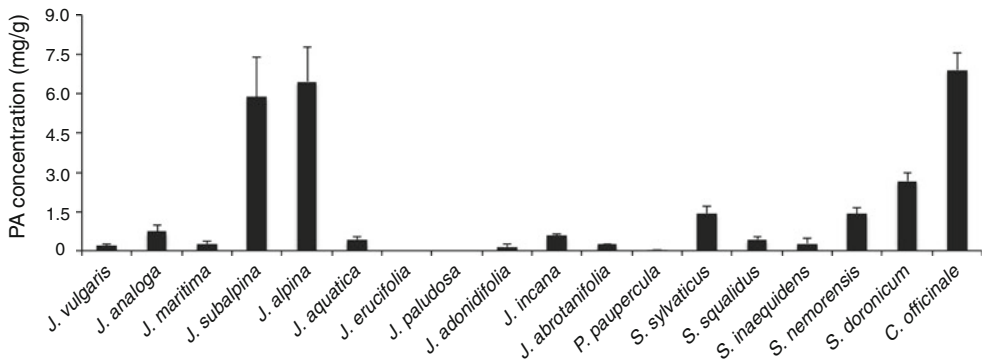

Fig. 3 Mean pyrrolizidine alkaloid concentrations (mg PA/g plant dry biomass) according to plant species. *Error bars* represent standard error. Plant species are listed according to their phylogenetic distance from *J. vulgaris* (see Fig. 1)

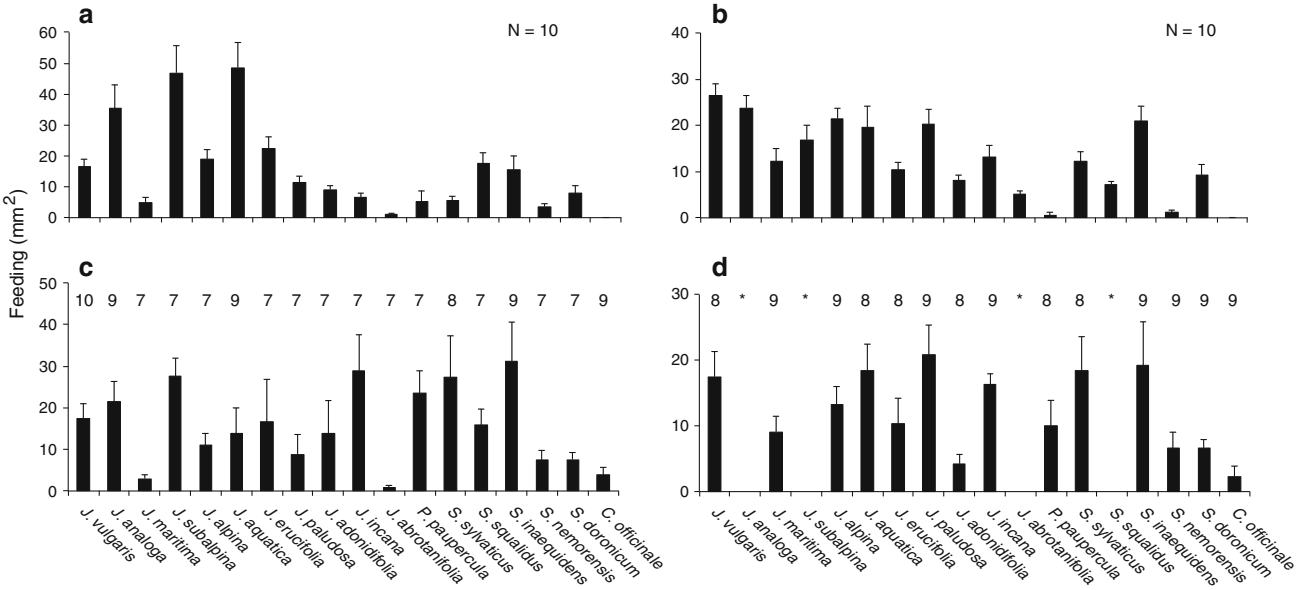

Fig. 4 Non-choice feeding of *L. jacobaeae* (**a**), *L. suturellus* (**b**), *L. succineus* (**c**), and *L. flavicornis* (**d**) measured as mm^2 leaf surface removed during non-choice feeding tests. Number of replicates (*N*) is ten for (**a**) and (**b**), while *numbers above bars* represent *N* for (**c**) and (**d**) (*asterik* indicates that plant species was not tested). *Error bars* represent standard error. Plant species are listed according to their phylogenetic distance to *J. vulgaris* (see Fig. 1)

$df = 1{,}717$; $P < 0.05$), and *L. flavicornis* ($F = 3.095$; $df = 1{,}313$; $P < 0.05$), suggesting that variation in number of shot-holes contributed more to total feeding variation than did variation in size of shot-holes.

Number of shot-holes produced by all beetle species was negatively correlated with leaf dry matter content of plants (Table 1), demonstrating that all beetles initiated feeding more frequently when leaf dry matter content was low. This correlation became weaker for all *Longitarsus* species, and became insignificant for *L. jacobaeae* and *L. flavicornis* after phylogenetic correction (Table 1), which suggests that leaf dry matter content is somewhat conserved among related plant species. Size of shot-holes was not correlated with leaf dry matter content in all cases, implying that decisions to continue feeding are not related to leaf dry matter content.

Of the four *Longitarsus* species tested, total feeding per plant species (Fig. 4) was negatively correlated with leaf dry matter content for *L. suturellus* and *L. succineus*, but not for *L. jacobaeae* and *L. flavicornis* (Table 1).

PA concentration (Table 1) and composition (Table 2) were never correlated with total feeding, number of shot-holes, or area of shot-holes, which suggests that feeding by *Longitarsus* is not correlated with PA profiles in plant species.

Relationships between feeding and phylogeny

Some evidence exists for negative correlation between phylogeny and beetle feeding (Table 3). Phylogeny was negatively correlated with number of shot-holes made by *L. suturellus*, and with total feeding by *L. jacobaeae and L. suturellus*. GLM analysis (Table 4) demonstrates that for total feeding, phylogeny is generally not significant (except for *L. suturellus*), while leaf dry matter content is a significant covariate for three of the four *Longitarsus* species (*L. flavicornis*, *L. suturellus* and *L. succineus*), but not for *L. jacobaeae*.

Table 2 Pearson correlations between squared Euclidean distance from *J. vulgaris* based on PA composition (six PCA axes) and squared Euclidean distance from *J. vulgaris* based on (1) mean total feeding area, (2) mean number of shot-holes, and (3) mean size of shot-holes

	L. jacobaeae	*L. suturellus*	*L. succineus*	*L. flavicornis*
No. shotholes				
n	17	17	17	13
r	−0.095	0.019	−0.331	0.056
P	0.727	0.943	0.211	0.862
Area shotholes				
n	16	16	17	13
r	0.023	−0.330	0.014	−0.377
P	0.933	0.212	0.958	0.227
Total area				
n	17	17	17	13
r	−0.025	0.100	0.189	0.089
P	0.927	0.927	0.484	0.783

Table 3 Spearman rank correlations between phylogeny of plants and feeding characteristics (number and area of shotholes, and total feeding) for each of *L. jacobaeae*, *L. suturellus*, *L. succineus*, and *L. flavicornis*

	L. jacobaeae			*L. suturellus*			*L. succineus*			*L. flavicornis*		
	n	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*	*n*	*r*	*P*
No. shotholes	17	−0.408	0.104	17	**−0.542**	**0.025**	17	−0.154	0.556	13	−0.129	0.674
Area shotholes	17	−0.280	0.277	17	−0.373	0.140	17	0.229	0.376	13	−0.356	0.232
Total feeding	17	**−0.509**	**0.037**	17	**−0.638**	**0.006**	17	0.043	0.870	13	−0.150	0.624

Significant correlations shown in bold

Table 4 ANOVA results of GLM univariate procedure for total feeding including phylogeny as a fixed factor and PA concentration (PA) and leaf dry matter (DM) content as covariates

	df	MS	*F*	*P*
L. jacobaeae				
Phylogeny	6	212.634	1.195	0.396
DM content	1	168.490	0.947	0.359
PA	1	1.182	0.007	0.937
Error	8	177.949		
L. suturellus				
Phylogeny	6	80.195	4.095	**0.035**
DM content	1	163.360	8.343	**0.020**
PA	1	0.255	0.013	0.912
Error	8	19.581		
L. succineus				
Phylogeny	6	125.579	3.462	0.054
DM content	1	938.790	25.880	**0.001**
PA	1	22.972	0.633	0.449
Error	8	36.274		
L. flavicornis				
Phylogeny	4	37.973	2.060	0.252
DM content	1	186.965	10.144	**0.033**
PA	1	2.357	0.128	0.739
Error	7	18.430		

Discussion

Our study demonstrates that PA concentration is highly variable between and within phylogenetic groups, and that PA profiles do not always reflect phylogenetic relationships between species. These findings corroborate data presented by Pelser et al. (2005), who showed that individual PAs were frequently gained and lost in the evolutionary history of *Jacobaea*, and that PAs are therefore often not conserved in closely related species.

We did not find any evidence that composition or concentration of PAs are correlated with adult feeding by the PA-adapted specialist herbivores tested here, although we cannot rule out that one or a small number of uncommon PAs are more effective deterrents than others. The relationships between total PA concentration and feeding were positive for all four beetle species after phylogenetic correction (although these correlations were not significant), indicating that PAs are not deterrent. However, only adult feeding behaviour was measured in this study, and PAs may affect feeding efficiency, pupal weights, fitness, or development time of specialist herbivores, although another study showed that larval density of *L. jacobaeae* in the roots of *J. vulgaris* is significantly correlated with adult feeding (Rapo et al. 2010). An absence of a strong effect of PA composition on host choice by *Longitarsus* spp. suggests that the latter may have overcome PAs in general during their evolutionary history, and plant species are not able to deter these specialist herbivores to any great extent by altering their PA profiles. An earlier study on another Senecioneae-specialised herbivore, the moth *Tyria jacobaeae*, demonstrated that larval performance and oviposition preference were also uncorrelated with PA composition (Macel et al. 2002), which, combined with the results presented here, suggests that differing PA profiles do not affect host choice by specialists that are broadly adapted to PAs. Since varying PA profiles among closely related species appears to have no detectable effect on host choice of PA-adapted herbivores, it is unlikely that small structural changes in PAs are adaptive from the perspective of defence against leaf-feeding specialist herbivores. Similar results have been found with regard to other chemical classes and their effects on adapted herbivores (e.g. glucosinolates, Moyes et al. 2000; Ratzka et al. 2002; and phenolic glycosides, Lindroth et al. 1988). There is, however, also evidence that specialist herbivores can distinguish among structurally similar secondary metabolites. Berenbaum et al. (1986) demonstrated that damage by parsnip webworm, *Depressaria pastinacella*, depended on the furanocoumarine profile of its host plant, *Pastinaca sativa*, and that the concentration of the furanocoumarine bergapten, but not that of xanthotoxin, is positively correlated with host-plant resistance to parsnip webworm. Clearly, more case studies are necessary in order to elucidate how often and under which conditions specialist herbivores are sensitive to minor structural variation among plant secondary metabolites of the same chemical class.

Instead, our results indicate that leaf dry matter content of plant species is a factor contributing to susceptibility for specialist attack. We found that feeding initiation and continuation of feeding are independently regulated in *Longitarsus* species. Number of feeding initiations (measured as number of shot-holes) was negatively correlated with leaf dry matter content, indicating that beetles initiated more feeding bouts when leaf dry matter content was low. Previous studies show that herbivory by generalist invertebrates is also well correlated with leaf dry matter content of host plants (Elger and Willby 2003; Clissold et al. 2009). Leaf dry matter content is a predictor of plant resource use strategy (Wilson et al. 1999), such that plants which grow quickly generally have low dry matter content and invest little in quantitative defences, and vice versa. Herbivores may therefore be deterred by plants with high leaf dry matter content because these species generally possess stronger quantitative defences. These results imply that to understand host plant selection by specialists, we may need to revisit theories proposed by Feeny (1976) and Rhoades and Cates (1976), who suggested that specialists easily adapt to plant toxins (including alkaloids), and are most effectively deterred by digestibility-reducing plant traits. In further studies, it would be useful to carry out detailed analyses of other plant traits (e.g. nitrogen concentration, terpene concentrations, and flavonoid concentrations), in order to better understand the relationship between herbivore preferences, plant nutrient concentrations, and quantitative defenses.

Size of shot-holes was uncorrelated with any plant characteristics measured in this study (including PA profiles and dry matter content), indicating that some other factor regulates continuation of feeding after feeding initiation. One possible factor that we ignored in this study is the presence and composition of other metabolite classes in the Senecioneae. The Senecioneae species contain a number of other secondary metabolites that are known to be important for defense against herbivores, including chlorogenic acid (Kirk et al. 2005), flavonoids (Kirk et al. 2005), and sesquiterpene (Reina et al. 2001, 2002) and monoterpene hydrocarbons (De Feo et al. 2003). Herbivores adapted to PAs may be far more sensitive to differences in other metabolite classes than to structural differences in PAs among Senecioneae species.

In total, number of shot-holes accounts for a larger proportion of variation in total feeding than does area eaten per feeding bout for all *Longitarsus* species. Since number of shot-holes was negatively correlated with leaf dry matter content, a good plant strategy for avoiding aboveground feeding by PA-adapted herbivores may thus be to increase dry matter content (or correlated characters) in leaves. However, the significance of the negative correlation between total feeding area and leaf dry matter content disappeared after phylogenetic correction, although the trend remained. It would be useful to test for similar results using other sets of taxonomically independent species. Another study involving the invasive plant species *Ageratina adenophora* showed that a decrease in specialist herbivore pressure led to decreased dry matter content, increased photosynthetic capacity, and increased growth in its invasive range compared to its native range (Feng et al. 2009).

Yet, regulating leaf dry matter content as a defence strategy may present difficulties for plants because leaf dry matter content is inter-related with a number of other plant functions, including photosynthesis (Marcelis et al. 1998; Feng et al. 2009), nitrogen allocation to cell walls (Feng et al. 2009), and regulation of salt and nutrient concentrations (Loudet et al. 2003). Furthermore, Cunningham et al. (1999) showed that, in a number of different genera, leaf dry matter content (or potential hydration) across species and within genera is generally correlated with environmental resource gradients including rainfall and soil nutrients. In contrast, our analysis showed that correlations between leaf dry matter content and feeding are reduced after phylogenetic correction, indicating that dry matter content is somewhat phylogenetically conserved, even in cases where closely related species differ substantially in ecological range (e.g. *J. vulgaris* and *J. aquatica*). Phylogenetic conservation of leaf dry matter content in the Senecioneae may reflect slow adaptation to environmental conditions. It is, therefore, likely that both environmental and phylogenetic constraints interact with leaf dry matter content, and, therefore, plant susceptibility to specialist herbivores.

It remains unclear why numerous PAs are expressed both within and among Senecioneae species. Previous studies have shown that structurally different PAs from Senecioneae species may have differential effects on generalist herbivores (Macel 2003; Leiss et al. 2009; Cheng et al. 2011), and root-associated fungal species (Hol and van Veen 2002). Other studies have also shown that closely related metabolites can have differential effects on generalist herbivores (Miller and Feeny 1983). Further studies are needed to assess whether generalist herbivores or microbial interactions may at least partly explain the 'raison d'être' of the high structural diversity of PAs in the genus the Senecioneae.

Acknowledgments We thank Prof T. Hartmann for performing GC–MS analyses. Thanks to three anonymous reviewers for their comments on the manuscript.

References

Becerra JX, Noge K, Venable DL (2009) Macroevolutionary chemical escalation in an ancient plant-herbivore arms race. Proc Nat Acad Sc USA 106:18062–18066

Berenbaum M, Feeny P (1981) Toxicity of angular furanocoumarins to swallowtail butterflies: escalation in a coevolutionary arms race? Science 212:927–929

Berenbaum M, Zangerl AR, Nitao JK (1986) Constraints on chemical coevolution: wild parsnip and the parsnip webworm. Evolution 40:1215–1228

Bernays EA (1998) Evolution of feeding behavior in insect herbivores. Bioscience 48:35–44

Cheng D, Kirk H, Vrieling K, Mulder PPJ, Klinkhamer PGL The relationship between structurally different pyrrolizidine alkaloids and western flower thrips resistance in F_2 hybrids of *Jacobaea vulgaris* and *Jacobaea aquatica*. J Chem Ecol 37:1071–1080

Choong MF, Lucas PW, Ong JSW, Pereira B, Tan HTW, Turner IM (1992) Leaf fracture-toughness and sclerophylly—their correlations and ecological implications. New Phytol 121:597–610

Clissold FJ, Sanson GD, Read J, Simpson SJ (2009) Gross vs. net income: how plant toughness affects performance of an insect herbivore. Ecology 90:3393–3405

Coley PD, Bryant JP, Chapin FS (1985) Resource availability and plant anti-herbivore defence. Science 230:895–899

Cornell HV, Hawkins BA (2003) Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. Am Nat 161:507–522

Cunningham SA, Summerhayes B, Westoby M (1999) Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. Ecol Monogr 69:569–588

De Boer NJ (1999) Pyrrolizidine alkaloid distribution in *Senecio jacobaea* minimizes losses to generalist feeding. Entomol Exp Appl 91:169–173

De Feo V, Soria EU, Soria RU, Senatore F (2003) Chemical composition of essential oils of Senecio nutans Sch.–Bip. (Asteracaeae). Flavour Frag J 18:234–236

Dobler S (2001) Evolutionary aspects of defense by recycled plant compounds in herbivorous insects. Basic Appl Ecol 2:15–26

Dobler S, Haberer W, Witte L, Hartmann T (2000) Selective sequestration of pyrrolizidine alkaloids from diverse host plants by *Longitarsus* flea beetles. J Chem Ecol 26:1281–1298

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Evolution 18:586–608

Elger A, Willby NJ (2003) Leaf dry matter content as an integrative expression of plant palatability: the case of freshwater macrophytes. Funct Ecol 17:58–65

Feeny P (1976) Plant apparency and chemical defense. In: Wallace JW, Mansell RL (eds) Biochemical interactions between plants and insects recent advances in phytochemistry. Plenum, New York, pp 1–40

Feng YL, Lei YB, Wang RF, Callaway RM, Valiente-Banuet A, Inderjitd LiYP, Zheng YL (2009) Evolutionary tradeoffs for nitrogen allocation to photosynthesis versus cell walls in an invasive plant. Proc Nat Acad Sci USA 106:1853–1856

Frick KE (1970) *Longitarsus jacobaea*, a flea beetle for the biological control of tansy ragwort. 1. Host plant specificity studies. Ann Entomol Soc Am 63:284–296

Futuyma DJ, Agrawal AA (2009) Macroevolution and the biological diversity of plants and herbivores. Proc Nat Acad Sci USA 106:18054–18061

Futuyma DJ, Mitter C (1996) Insect–plant interactions: the evolution of component communities. Philos Trans R Soc Lond B 351: 1361–1366

Futuyma DJ, Walsh JS Jr, Morton T, Funk DJ, Keese MC (1994) Genetic variation in a phylogenetic context: responses of two specialized leaf beetles (*Coleoptera*: Chrysomelidae) to host plants of their congeners. J Evol Biol 7:127–146

Hartmann T (1996) Diversity and variability of plant secondary metabolism: a mechanistic view. Entomol Exp Appl 80:177–188

Hartmann T (1999) Chemical ecology of pyrrolizidine alkaloids. Planta 207:483–495

Hartmann T, Dierich B (1998) Chemical diversity and variation of pyrrolizidine alkaloids of the senecionine type: biological need or coincidence? Planta 206:443–451

Hartmann T, Witte L (1995) Chemistry, biology and chemoecology of the pyrrolizidine alkaloids. In: Pelletier SW (ed) Alkaloids: chemical and biological perspectives, vol 9. Pergamon, New York, pp 156–233

Hartmann T, Zimmer M (1986) Organ specific distribution and accumulation of pyrrolizidine alkaloids during the life history of two annual *Senecio* species. Plant Phys 112:67–80

Harvey PH, Pagel MD (1991) The comparative method in evolutionary biology. Oxford University Press, New York

Hendriks RJJ, de Boer NJ, Groenendael JM (1999) Comparing the preferences of three herbivore species with resistance traits of 15 perennial dicots: the effects of phylogenetic constraints. Plant Ecol 143:141–152

Hol WHG, van Veen JA (2002) Pyrrolizidine alkaloids from *Senecio jacobaea* affect fungal growth. J Chem Ecol 28:1763–1772

Janz N, Nylin S (1998) Butterflies and plants: a phylogenetic study. Evolution 52:486–502

Kirk H, Choi YH, Kim HK, Verpoorte R, Van der Meijden E (2005) Comparing metabolomes: the chemical consequences of hybridization in plants. New Phytol 167:613–622

Langel D, Ober D, Pelser PB (2011) The evolution of pyrrolizidine alkaloid biosynthesis and diversity in the Senecioneae. Phytochem Rev 10:3–74

Leiss KA, Choi YH, Abdel-Farid IB, Verpoorte R, Klinkhamer PGL (2009) NMR Metabolomics of thrips (*Frankliniella occidentalis*) resistance in *Senecio* hybrids. J Chem Ecol 35:219–229

Lindroth RL, Scriber JM, Hsia SMT (1988) Chemical ecology of the tiger swallowtail: mediation of host use by phenolic glycosides. Ecology 69:814–822

Loudet O, Chaillou S, Krapp A, Daniel-Vedele F (2003) Quantitative trait loci analysis of water and anion contents in interaction with nitrogen availability in *Arabidopsis thaliana*. Genetics 161:711–722

Macel M (2003) On the evolution of the diversity of pyrrolizidine alkaloids: the role of insects as selective forces. Phd dissertation, Leiden University, Leiden

Macel M, Vrieling K (2003) Pyrrolizidine alkaloids as oviposition stimulants for the cinnabar moth, *Tyria jacobaea*. J Chem Ecol 29:1435–1446

Macel M, Klinkhamer PGL, Vrieling K, Van der Meijden E (2002) Diversity of pyrrolizidine alkaloids in *Senecio* species does not affect the specialist herbivore *Tyria jacobaea*. Oecologia 133:541–550

Marcelis LFM, Heuvelink E, Goudriaan J (1998) Modelling biomass production and yield of horticultural crops: a review. Sci Hortic 74:83–111

Miller JS, Feeny P (1983) Effects of benzylisoquinoline alkaloids on the larvae of polyphagous Lepidoptera. Oecologia 58:332–339

Moyes CL, Collin HA, Britton G, Raybould AF (2000) Glucosinolates and differential herbivory in wild populations of *Brassica oleracea*. J Chem Ecol 26:2625–2641

Nyman T (2010) To speciate, or not to speciate? Resource heterogeneity, the subjectivity of similarity, and the macroevolutionary consequences of niche-width shifts in plant-feeding insects. Biol Rev 85:393–411

Ober D, Hartmann T (1999) Hme synthase, the first pathway-specific enzyme of pyrrolizidine alkaloid biosynthesis, evolved from deoxyhypusine synthase. Proc Nat Acad Sc USA 96:14777–14782

Pelser PB, Gravendeel B, van der Meijden R (2002) Tackling speciose genera: species composition and phylogenetic position

of *Senecio* sect. *Jacobaea* (Asteraceae) based on plastid and nrDNA sequences. Am J Bot 89:929–939

Pelser PB, van den Hof K, Gravendeel B, van der Meijden R (2004) The systematic value of morphological characters in *Senecio* sect. *Jacobaea* (Asteraceae) as compared to DNA sequences. Syst Bot 29:790–805

Pelser PB, De Vos H, Theuring C, Beuerle T, Vrieling K, Hartmann T (2005) Frequent gain and loss of pyrrolizidine alkaloids in the evolution of *Senecio* section *Jacobaea* (Asteraceae). Phytochemistry 66:1285–1295

Pelser PB, Nordenstam B, Kadereit JW, Watson LE (2007) An ITS phylogeny of tribe Senecioneae (Asteraceae) and a new delimitation of *Senecio* L. Taxon 56:1077–1104

Pelser PB, Kennedy AH, Tepe EJ, Shidler JB, Nordenstam B, Kadereit JW, Watson LE (2010) Patterns and causes of incongruence between plastid and nuclear Senecioneae (Asteraceae) phylogenies. Am J Bot 97:856–873

Puliafico KP (2003) Molecular taxonomy, bionomics and host specificity of *Longitarsus jacobaeae* (Waterhouse) (Coleoptera: Chrysomelidae): The Swiss population revisited. Master's of Science, Montana State University, Bozeman

Rapo C, Müller-Schärer H, Vrieling K, Schaffner U (2010) Is there rapid evolutionary response in introduced populations of tansy ragwort, *Jacobaea vulgaris*, when exposed to biological control? Evol Ecol 24:1081–1099

Ratzka A, Vogel H, Kliebenstein DJ, Mitchell-Olds T, Kroymann J (2002) Disarming the mustard oil bomb. Proc Nat Acad Sc USA 99:11223–11228

Reina M, Gonzalez-Coloma A, Gutierrez C, Cabrera R, Rodrigues ML, Fajardo V, Villarroel L (2001) Defensive chemistry of *Senecio miser*. J Nat Prod 64:6–11

Reina M, Nold M, Santana O, Orihuela JC, Gonzalex-Coloma A (2002) C-5-substituted antifeedant silphinene sesquiterpenes from *Senecio palmensis*. J Nat Prod 65:448–453

Rhoades DF, Cates RG (1976) Toward a general theory of plant antiherbivore chemistry. Rec Adv Phytochem 19:68–213

Schädler M, Jung G, Augh H, Brandl R (2003) Palatability, decomposition, and insect herbivory: patterns in a successional old-field plant community. Oikos 103:121–132

Schoonhoven L, van Loon J, Dicke M (2005) Insect–plant biology, 2nd edn. Oxford University Press, Oxford

Singer MS, Stireman JO (2005) The tri-trophic niche concept and adaptive radiation of phytophagous insects. Ecol Lett 8:1247–1255

Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. Freeman, New York

Vrieling K, van Wijk CAM (1994) Estimating costs and benefits of the pyrrolizidine alkaloids of *Senecio jacobaea* under natural conditions. Oikos 70:449–454

Vrieling K, de Vos H, van Wijk CAM (1993) Genetic analysis of the concentration of pyrrolizidine alkaloids of *Senecio jacobaea*. Phytochemistry 32:1141–1144

Wilson PJ, Thompson K, Hodgson JG (1999) Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. New Phytol 143:155–162

Windig JJ, Vrieling K (1996) Biology and ecology of *Longitarsus jacobaea* and other *Longitarsus* species feeding on *Senecio jacobaea*. In: Jolivet PHA, Cox ML (eds) Chrysomelidae biology vol. 3: General studies. Kluwer, Dordrecht, pp 315–326

Wink M (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perpective. Phytochemistry 64:3–19

Witte L, Ernst L, Adam H, Hartmann T (1992) Chemotypes of two pyrrolizidine alkaloid-containing *Senecio* species. Phytochemistry 31:559–565

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall, New Jersey