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Diversity of pyrrolizidine alkaloids in *Senecio* species does not affect the specialist herbivore *Tyria jacobaeae*

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Abstract The evolution of the diversity of related secondary metabolites in plants is still poorly understood. It is often thought that the evolution of plant secondary metabolites is driven by specialist insect herbivores and under this coevolutionary model it is expected that related compounds differ in their effects on specialist herbivores. Here we focus on the diversity of pyrrolizidine alkaloids (PAs) in *Senecio* species and their effects on *Tyria jacobaeae*, a specialist moth on *Senecio jacobaea*. As a first step to determine the effects of related PAs on *T. jacobaeae*, we studied larval performance on plants from 11 *S. jacobaea* populations and eight *Senecio* species with different PA compositions. Although the populations of *S. jacobaea* differed in their PA compositions, there was no difference in larval performance among the populations. Larval performance differed among the eight species but we could not show a correlation with PA composition. Oviposition choice experiments showed a strong correlation between oviposition preference and larval performance on the eight species but oviposition preference did not seem to be correlated with PAs. We found no indications that related PAs differ in effects on the specialist *T. jacobaeae*; therefore it seems unlikely that *T. jacobaeae* is a driving force behind the evolution of the diversity of PAs. Alternatively, we propose that the evolution of the diversity of PAs is driven by selection pressure from generalist herbivores or that the diversity of PAs may even be selectively neutral.

Keywords Coevolution · Larval performance · Oviposition choice · *Senecio jacobaea* · Chemical diversity

Introduction

The evolution and maintenance of diversity is one of the central themes in evolutionary ecology. Secondary metabolites in plants present a very intriguing but still poorly understood example of biological diversity. Within each group of secondary metabolites a large variety of compounds can be found, there are for example 5,500 different alkaloids (Harborne 1982). Secondary metabolites can act as defense chemistry against herbivores (Fraenkel 1959) and it is often assumed that specialist insect herbivores play an important role in the evolution of these compounds (Ehrlich and Raven 1964; Rhoades and Cates 1976). Although this assumption is under dispute (Strong et al. 1984; Bernays and Graham 1988; Jermy 1993), it has been shown that plant chemistry can be under selection by insect herbivores (Mauricio and Rausher 1997; Shonle and Bergelson 2000).

One possible explanation for the diversity of structurally related secondary metabolites is that new compounds evolve in a continuous evolutionary arms race between a plant and its specialist insect herbivores, in which a plant that synthesizes new compounds is able to escape herbivory and the insect herbivores, in turn, adapt to these compounds. Under this coevolutionary model it is expected that structurally related compounds differ in their toxic or deterrent effects. There are very few examples of differential effects on specialist herbivores of structurally related compounds present in one plant species. Berenbaum et al. (1986, 1989) showed that in wild parsnip (*Pastinaca sativa*) the toxicity of the angular furanocoumarins differed from the toxicity of structurally related linear furanocoumarins to the oligophagous parsnip webworm. Lindroth et al. (1988) investigated the effects of related phenolic glycosides on the larvae of two subspecies of *Papilio glaucus*. They found that related glycosides only had different effects on the non-adapted subspecies. In contrast, Moyes et al. (2000) found no link between different glucosinolate profiles of wild *Brassica oleracea* and herbivory in the field by *Pieris* spp., slugs and snails, flea beetles or aphids. The authors

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Table 1 Sites where seeds were collected and taxonomic section of the nine selected *Senecio* species

<i>Senecio</i> species	Site	Taxonomic section
<i>S. jacobaea</i>	Meijndel (The Netherlands)	Jacobaea
<i>S. aquaticus</i>	Zwanenwater (The Netherlands)	Jacobaea
<i>S. alpinus</i>	Vanille Noir (Switzerland)	Jacobaea
<i>S. erucifolius</i>	Stockem (The Netherlands)	Jacobaea
<i>S. adonidifolius</i>	Gavernie (France)	Jacobaea
<i>S. rupestris</i>	Pontresina (Switzerland)	Senecio
<i>S. sylvaticus</i>	Scherwille (France)	Senecio
<i>S. inaequidens</i>	Duelmen (Germany)	Fruticulosi
<i>S. viscosus</i>	Leipzig (Germany)	Senecio

concluded that it is unlikely that differences in glucosinolate profiles between plant populations of *B. oleracea* are due to differential selection pressures from herbivores feeding from the plants at the moment of study.

An example of the overwhelming diversity in related plant secondary metabolites are the pyrrolizidine alkaloids (PAs) with about 360 known structures (Hartmann and Witte 1995). One of the most diverse classes of PAs is the group of the macrocyclic senecionine type PAs with over 100 structures. Senecionine type PAs are abundant in the genus *Senecio* (Asteraceae). Each *Senecio* species has a species-specific PA composition (Hartmann and Witte 1995; this paper) and one species, such as *Senecio jacobaea*, can contain more than ten different senecionine type alkaloids. Moreover, the PA composition within one species can vary considerably (Vrieling and de Boer 1999; this paper). PA composition in *S. jacobaea* is at least partly genetically determined (Vrieling et al. 1993), thus providing a basis upon which natural selection may act. In this paper, we focus on the diversity within the senecionine type alkaloids and their effects on the specialist herbivore *Tyria jacobaeae* (Lepidoptera; Arc-tiidae).

T. jacobaeae (cinnabar moth) is a specialist herbivore on *S. jacobaea* that can sequester the PAs from its host plant (Rothschild et al. 1979). The cinnabar moth can have a major impact on the population dynamics of *S. jacobaea*. The caterpillars periodically completely defoliate their host plant and in certain years cause extinction of *S. jacobaea* on a local scale (van der Meijden and van Wijk 1997). If the cinnabar moth has been a selective force in the evolution of different PAs of its host plant *S. jacobaea*, we expect that structurally related PAs differ in their effects on the moth. In particular, related alkaloids not found in the food plant but new to the cinnabar moth should negatively affect larval performance and/or oviposition choice. In turn, the host range of *T. jacobaeae* should be related to PA composition of the host plant species. As a first step to determine the effects of related PAs on the cinnabar moth, we studied larval performance on plants from 11 *S. jacobaea* populations and eight *Senecio* species that differed in their PA composition, and studied oviposition choice between the eight *Senecio* species. We tested if larval performance and oviposition preference were correlated with PA composition.

Materials and methods

Plants and insects

The selection of the *Senecio* species was based on their phylogenetic relatedness (Tutin 1976) and the alkaloid pattern of the species (Hartmann and Witte 1995). Seeds of all *Senecio* species and populations of *S. jacobaea* were collected in the wild. Table 1 shows where the seeds of the *Senecio* species were collected and their taxonomic section.

All plants, except for *S. aquaticus*, were grown from seed for 5 months in a growth chamber under short day conditions to prevent flowering (photoperiod light 8 h: dark 16 h, 20°C day/15°C night, relative humidity 70%) in 11-cm-diameter pots containing a mixture of 50/50 dune sand/peat. *S. aquaticus* plants were collected from the field in May 1999 because no fertile seeds were available of this species. The plants were put in 11-cm-diameter pots containing peat and placed in the growth chamber. *S. aquaticus* and *S. sylvaticus* started flowering during the experiments whereas all other species remained vegetative.

Leaves of *S. jacobaea* with egg batches of *T. jacobaeae* were collected in the Meijndel dunes at the beginning of June 1999. The larvae emerging from these egg batches were used for the larval performance experiment. The adult moths used in the oviposition experiments were reared in the laboratory. Fifth (last) instar caterpillars were collected from Meijndel in the summer of 1998. Each caterpillar was put in a glass tube without food until pupation. The pupae were stored in a cold growth chamber (L8: D16, 4°C, r.h. 70%) for hibernation. In October 1999 the pupae were placed in another growth chamber (L16: D8, 20°C/15°C, r.h. 70%) for emergence. Prior to the oviposition experiments, male and female moths were kept together for at least 1 week to mate. All experiments with *T. jacobaeae*, larval performance and oviposition choice were performed in a growth chamber (L12: D12, 20°C/15°C, r.h. 70%).

PA analysis

One leaf of each plant was harvested prior to the larval performance experiments to determine the PA composition. The leaves were dried at 50°C for 3 days and then stored at -20°C. PAs were extracted by acid-base extraction (Hartmann and Zimmer 1986). PA composition of the plants was determined with GC-FID (Vrieling and de Boer 1999) and GC-PND/FID modified after Hartmann and Dierich (1998) with a DB-1 and a D-17 column. Further analysis of the PA composition was done with GC-MS (Witte et al. 1992). Total PA concentration was determined spectrophotometrically, modified after Mattocks (1967). The concentration of individual PAs was calculated by total PA concentration × fraction of individual PA measured by GC analysis.

Larval performance experiment

Larvae from 25 egg batches were used in the experiment on larval performance. Within 12 h after hatching, the larvae were placed on the plants of eight different *Senecio* species and 11 populations of

S. jacobaea. For each of the *Senecio* species/populations, five plants were used and five larvae were placed on each plant. Per egg batch we used one larva for each *Senecio* species/population to uncouple the effect of genetic differences between egg batches and the effect of plant quality on larval performance (Soldaat and Vrieling 1992). No more than four small plants were available of *S. adonidifolius* and just one larva, instead of five, was placed on each plant because the plants were too small to supply enough food for five larvae. Plants with larvae were placed in transparent plastic cylinders (30 cm diameter, 50 cm high) with tops and bottoms covered with gauze. Larvae were supplied with a new food plant when needed. For each larva the number of days until pupation (development time) and pupal weight were recorded.

Oviposition choice experiments

In the oviposition experiments we used the same *Senecio* species as in the larval performance experiment except for *S. adonidifolius* of which no plants were available. We did two experiments, one with leaves (similar in size) and one with plants (dissimilar in size).

Experiment A, choice experiment with leaves

For this choice experiment we used transparent plastic cages (42 cm diameter, 50 cm high). The bottom of the cage consisted of a dish filled with dune sand and the top of the cage was covered with gauze. In each cage eight glass tubes (2.5 cm diameter) were placed in a circle, 10 cm apart from each other. The tubes were filled with water and in each tube a leaf of one of the *Senecio* species was placed. The top of the tube was covered with Parafilm to avoid evaporation. The tubes with leaves of the eight species were arranged randomly. One female adult and one male adult were placed in a cage. The number of egg batches and number of eggs per batch on each leaf were recorded every 24 h. The tests lasted 72 h and for every replicate a new female was used (males were used more than once). The experiment was replicated 70 times.

Experiment B, choice experiment with whole plants

One plant of each of the eight species was placed in a cage (87 cm diameter); the plant species differed in size. The plants were placed in a circle 20 cm apart. The experiment was repeated twice with 11 females/2 males and 9 females/3 males. The experiment lasted 22 days and after the experiment had finished the number of egg batches and number of eggs on each plant were recorded.

Statistical analysis

Data were analyzed with SPSS (SPSS, Chicago, USA). Differences in larval survival among *Senecio* species and *S. jacobaea* populations were tested with a Kruskal-Wallis test. To test which *Senecio* species are different from each other, a post hoc test for multiple comparison between treatments was done (Siegel and Castellan 1988). Pupal weight and development time of the larvae were tested with a one-way nested ANOVA, post hoc tests for differences between the *Senecio* species were done with a least significant difference test. Total larval performance was measured as [(percentage survival × pupal weight)/development time]. Comparison of PA composition of the plants was done with hierarchical cluster analysis. With the squared euclidean distance the difference between PA composition of a plant and the averaged PA composition of *S. jacobaea* plants from Meijndel was measured. The more different the PA composition of a plant compared to the plants from Meijndel, the bigger the euclidean distance would be. *S. jacobaea* from Meijndel was taken as 'standard' because *T. jacobaeae* used in these experiments were from the same population in Meijndel. Oviposition preference was tested with a Friedman two-way analysis of variance by ranks; to test which *Senecio* species differed

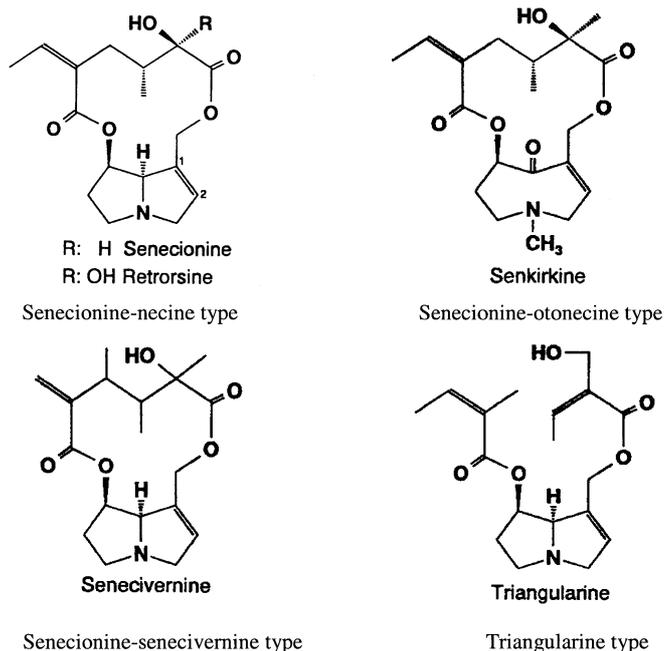


Fig. 1 Different types of pyrrolizidine alkaloids (PAs). Senecionine type PAs are macrocyclic diesters that are derived from or structurally related to senecionine. Triangularine type PAs are monoesters or diesters with C5 acids and their hydroxylated derivatives. From: Hartmann and Witte (1995)

from each other in oviposition preference we used a multiple comparisons test for related samples (Siegel and Castellan 1988). For the correlation between PA concentration/composition and oviposition choice, we averaged the concentration and composition of the plants used in the larval performance experiment.

Results

PA analysis

The alkaloids found in the different *Senecio* species and populations could be classified into four structural types according to Hartmann and Witte (1995) (Fig. 1). These structural types are the triangularine type and the senecionine type with three subgroups: (1) senecionine-necine type, (2) the senecionine-otonecine type and (3) the senecionine-senecivernine type. Figure 2 shows the PA composition of the different populations of *S. jacobaea*. The populations clearly differed in their relative amounts of senecionine-necine type PAs.

Table 2 shows the results of the PA analysis of the nine *Senecio* species. All species, except for *S. sylvaticus*, mainly contained alkaloids from senecionine-necine type. Senecivernine was present in all species. The otonecine esters were found in *S. inaequidens* and in small amounts in *S. aquaticus*. *S. aquaticus* contained an unknown PA of a different structural type and eight unknown alkaloids were found in *S. inaequidens*, most of them in small quantities. Most alkaloids in *S. sylvaticus* were of the triangularine type.

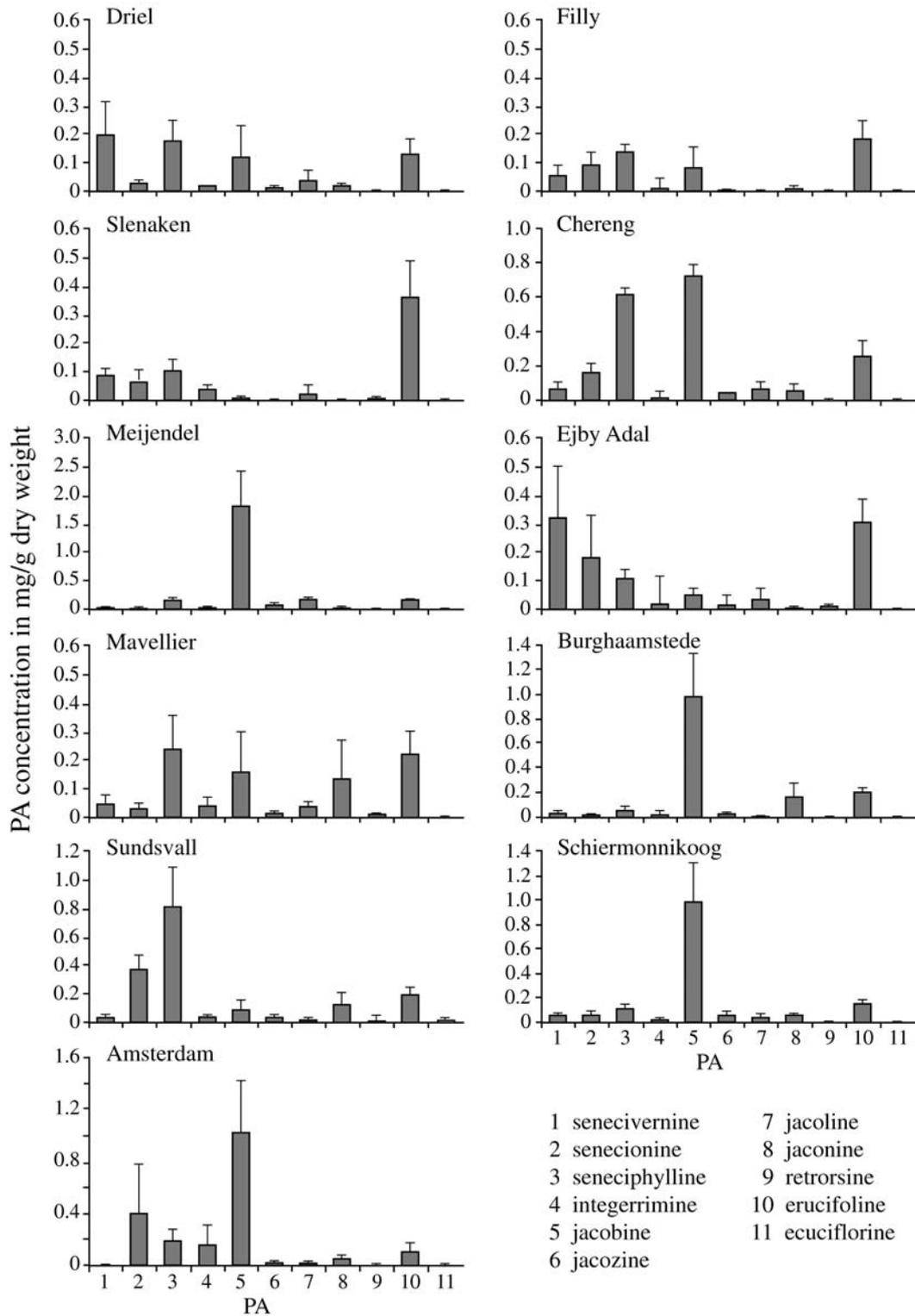


Fig. 2 PA compositions of the *S. jacobaea* populations. Error bars indicate standard error of the mean

Larval performance

The survival of the larvae did not differ significantly among the populations of *S. jacobaea* (Table 3 : Kruskal-Wallis: $\chi^2=7.87$, $df=10$, $P=0.64$). Although pupal weight

of the larvae was significantly different among plants from the same population, there was no difference in pupal weight between the populations (Table 3, 4). Development time also did not differ among the populations (Table 3, 4).

Table 2 PA composition of the nine *Senecio* species. Average concentration (mg/g dry weight) per PA is given

PA type	PA	Retention time	<i>Senecio</i> species								
			<i>jacobaea</i>	<i>alpinus</i>	<i>aquaticus</i>	<i>rupestris</i>	<i>erucifolius</i>	<i>viscosus</i>	<i>sylvaticus</i>	<i>inaequidens</i>	<i>adonidifolius</i>
Senecionine- Senecivermine	Senecivermine	10.89	0.04	0.07	0.04	0.30	0.02	0.07	0.11	0.21	0.05
Senecionine-necine	Senecionine	11.04	0.02	0.43	0.24	0.03	0.02	1.07	0.02	0.03	0.01
Senecionine-necine	Seneciphylline	11.23	0.16	0.81	0.34	0.23	0.07	0.10	0.06	0.03	0.06
Senecionine-necine	Intergerrimine	11.84	0.03	0.09	0.06	0.91	tr	0.36	0.02	0.16	
Senecionine-necine	Jacobine	12.91	1.82	0.07	tr	0.44	tr	0.02	0.04	0.01	
Senecionine-necine	Jacozine	13.25	0.10	0.40	tr	tr	0.03	tr			tr
Senecionine-necine	AcetylSeneciphylline	13.35		0.01							
Senecionine-necine	Jacoline	13.49	0.16	0.05	tr	tr	tr	0.01			
Senecionine-necine	Retrorsine	13.94		0.02		0.02	0.12	tr		0.44	
Senecionine-necine	Erucifoline	13.95	0.15		0.24	0.04	0.10				
Senecionine-necine	Jaconine	14.03	0.02	0.08		tr	tr				
Senecionine-necine	Adonidifoline	14.13									0.08
Senecionine-necine	Usaramine	15.13								0.02	
Senecionine-otonecine	Senkirkine	13.36								0.16	
Senecionine-otonecine	Orosenine	15.05		0.09							0.10
Triangularine	Platynecine diester	8.82							0.17		
Triangularine	Platynecine diester	9.20							0.04		
Triangularine	Platynecine diester	9.50							0.05		
Triangularine	Platynecine diester	1.016							0.06		
Triangularine	Triangularine	12.27		tr					0.40		
Triangularine	Sarracine	12.55		0.01					1.46		
Triangularine	Sarracimine	12.73							0.03		
Unknown	Unknown	4.92			0.25						
Unknown	Unknown	11.75		0.02							
Unknown	Unknown	12.48			0.05					0.03	
Unknown	Unknown	14.03								tr	
Unknown	Unknown	14.63								tr	
Unknown	Unknown	15.02								0.03	
Unknown	Unknown	15.60								0.01	
Unknown	Unknown	16.43								0.01	
Unknown	Unknown	16.87								0.01	
Unknown	Unknown	17.33								0.01	
	Total PA conc.	2.49	2.05	1.31	1.99	0.36	1.63	2.70	1.12	0.30	

tr traces (amount <0.01 mg/g)

Retention times of the DB-1 column on GC-PND/FID

Table 3 Survival, pupal weight (SE) and development time (SE) of *T. jacobaeae* larvae (A) on 11 populations of *S. jacobaea* and (B) on eight *Senecio* species. Different letters indicate significant differences (survival: multiple comparisons between treatments by

ranks; pupal weight and development time: LSD). Survival, pupal weight and development time did not differ among the *S. jacobaea* populations

	Population/species	Survival (%)	Pupal weight (mg)	Development time (days)
A <i>S. jacobaea</i> populations	Driel (The Netherlands)	76	153.7 (3.4)	33.9 (0.6)
	Filly (Belgium)	80	147.7 (4.9)	33.3 (0.9)
	Slenaken (Neth.)	80	148.9 (4.0)	31.8 (0.5)
	Chereng (France)	84	152.3 (5.6)	31.9 (0.5)
	Meijendel (Neth.)	92	142.3 (2.5)	32.3 (0.7)
	Ejby Adal (Denmark)	92	151.3 (3.7)	32.7 (0.7)
	Mavellier (Switzerland)	72	144.2 (4.3)	32.8 (0.4)
	Burghaamstede (Neth.)	92	162.9 (3.3)	31.9 (0.6)
	Sundsvall (Sweden)	80	144.2 (4.2)	33.1 (0.6)
	Schiermonnikoog (Neth.)	76	143.2 (4.1)	31.7 (0.5)
	Amsterdam (Neth.)	72	143.1 (5.2)	31.7 (0.7)
B <i>Senecio</i> species	<i>S. jacobaea</i>	92 ^b	142.3 (2.5) ^c	32.3 (0.7) ^c
	<i>S. alpinus</i>	72 ^{ab}	155.5 (4.6) ^d	31.9 (0.5) ^c
	<i>S. aquaticus</i>	72 ^{ab}	157.2 (3.2) ^d	31.4 (0.5) ^c
	<i>S. rupestris</i>	88 ^b	129.4 (4.9) ^b	36.1 (0.6) ^b
	<i>S. erucifolius</i>	56 ^{ab}	133.4 (6.6) ^{bc}	35.6 (1.1) ^b
	<i>S. sylvaticus</i>	28 ^{ab}	99.4 (6.3) ^a	49.0 (3.5) ^a
	<i>S. viscosus</i>	0 ^a	-	-
	<i>S. inaequidens</i>	0 ^a	-	-

Table 4 One way nested ANOVA (model I) on pupal weight and development time of *T.*

jacobaeae on 11 populations of *S. jacobaea* and on eight *Senecio* species

Source	Pupal weight			Development time		
	df	MS	F	df	MS	F
Populations	10	815.85	1.53	10	11.77	1.294
Plants within populations	43	544.82	1.96**	43	9.17	1.233
Error	162	278.67		162	7.43	
Species	5	4,801.22	6.50**	5	340.60	25.73***
Plants within species	20	576.14	2.34**	20	14.33	2.86
Error	75	269.21		75	12.70	

= $P < 0.01$, *= $P < 0.001$

Among the different species, larval survival differed significantly (Table 3 : Kruskal-Wallis: $\chi^2=26.21$, $df=7$, $P < 0.001$). Survival was not only high on *S. jacobaea* (92%) but also on *S. rupestris* (88%), *S. aquaticus* (72%), and *S. alpinus* (72%). No traces of feeding were found on *S. inaequidens* and *S. viscosus* and all larvae died on these species. *S. adonidifolius* was left out of the statistical analysis because of the different experimental set up for this species. However, three out of the four larvae did survive on *S. adonifolius* (average pupal weight: 134.3 mg, SE 17.0; average development time: 34 days, SE 1.2). A one-way nested ANOVA showed that both development time and pupal weight of *T. jacobaeae* were different between the *Senecio* species (Table 4). The development time was shortest on *S. jacobaea*, *S. alpinus* and *S. aquaticus*. Pupal weight was highest in these species as well. On *S. sylvaticus* development time was longest and pupal weight was lowest (Table 3).

Larval performance was not correlated with PA composition (squared euclidean distance) of plants from the *S. jacobaea* populations (Fig. 3A). Furthermore, neither backward nor forward multiple regression analysis showed a significant correlation of PA composition with larval performance. There was no correlation between

larval performance and the total PA concentration of the *S. jacobaea* plants (Fig. 3B).

Larval performance on the eight *Senecio* species was not correlated with PA concentration (Fig. 3D). Larval performance and the euclidean distance of the PA composition of the plants from the different species were also not correlated (Fig. 3C). Although we found differences in larval performance between the species, we could not show any correlation with PAs.

Oviposition choice

Experiment with leaves

In this oviposition experiment 22 females laid eggs. The 48 females that did not lay eggs were excluded from the analysis. The relatively low number of egg-laying females is probably due to the short period in which the experiment took place (3 days). It can take more than a week before a *T. jacobaeae* female lays her eggs (personal observation). Unfortunately the conditions of the leaves during this experiment did not allow the experiment to last longer than 3 days.

Fig. 3 **A** Correlation between larval performance and PA composition of *S. jacobaea* plants ($r=-0.20$, $n=49$, $P=0.17$). **B** Correlation between larval performance and PA concentration of *S. jacobaea* plants ($r=0.22$, $n=53$, $P=0.11$). **C** Correlation between larval performance and PA composition of plant of different *Senecio* species ($r_s=-0.16$, $n=35$, $P=0.35$). **D** Correlation between larval performance and PA concentration of plants of different *Senecio* species ($r_s=0.22$, $n=40$, $P=0.18$)

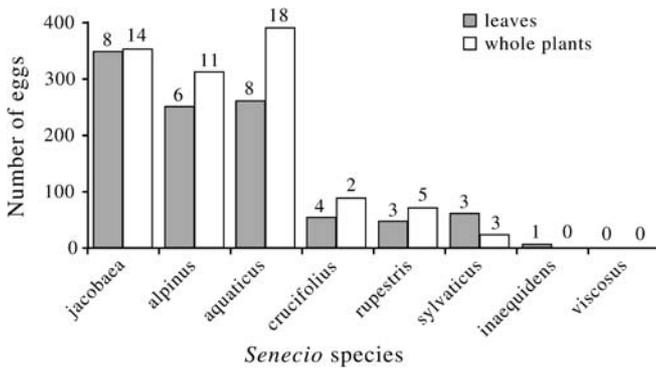
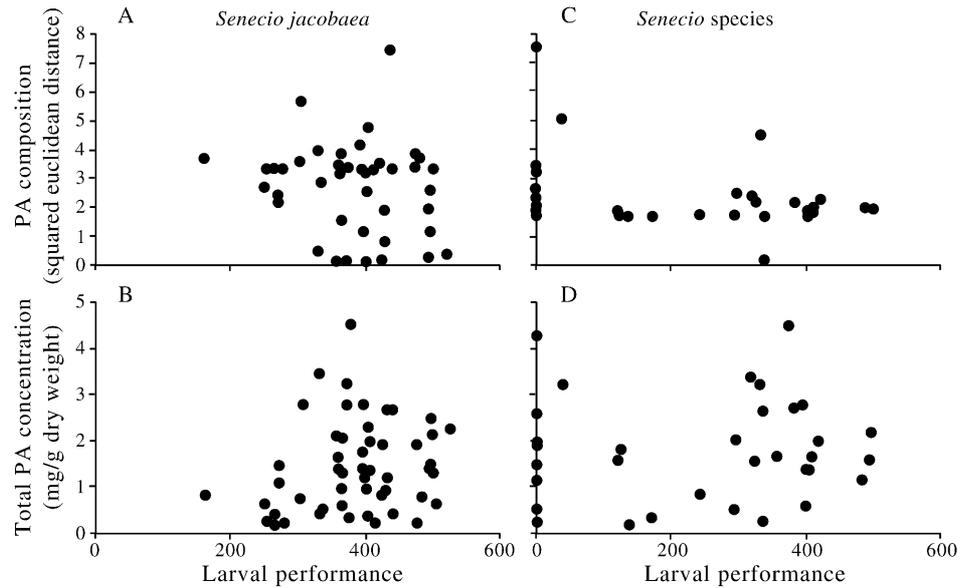


Fig. 4 Oviposition choice of *Tyria jacobaeae* on eight *Senecio* species in choice experiments with leaves and plants. Numbers above the bars indicate number of egg batches

The total number of eggs on the eight *Senecio* species differed significantly among the species (Friedman, $\chi^2=19.94$, $df=7$, $P<0.01$). However, the post hoc multiple comparisons test failed to show which *Senecio* species differed significantly from each other. The most eggs were laid on *S. jacobaea*, *S. alpinus* and *S. aquaticus*. Far less eggs were found on *S. erucifolius*, *S. rupestris* and *S. sylvaticus* and hardly any eggs were laid on *S. viscosus* and *S. inaequidens*, although one small egg batch was found on the latter (Fig. 4). The size of the batches ranged from 3 to 87 eggs. The average size of the egg batches did not differ among the eight *Senecio* species (ANOVA, $F=1.79$, $df=6$, $P=0.31$).

Experiment with whole plants

Most eggs were laid on *S. jacobaea*, *S. aquaticus* and *S. alpinus* (Fig. 4), as was the case in the experiment with leaves. No egg batches were found on *S. viscosus* and *S.*

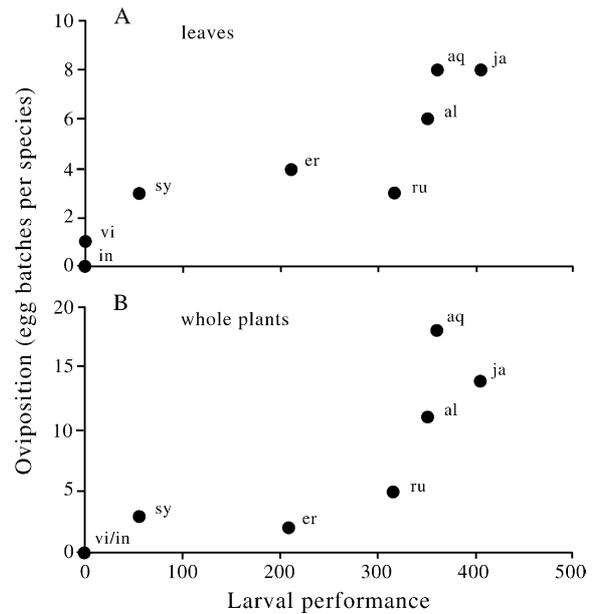


Fig. 5 Relation between larval performance and oviposition choice on eight *Senecio* species. **A** Larval performance on different species and oviposition in the experiment with leaves ($r_s=0.95$, $n=8$, $P<0.001$). **B** Larval performance on different species and oviposition in the whole plant experiment ($r_s=0.95$, $n=8$, $P<0.001$). Letters indicate *Senecio* species

inaequidens. The size of the batches ranged from 2 to 95 eggs. The average number of eggs per batch did not differ significantly among the *Senecio* species (ANOVA, $F=1.16$, $df=5$, $P=0.34$).

The similar preference for leaves as for the whole plants indicates that, between these *Senecio* species, size of the plants is not important for oviposition choice. Oviposition preferences of both the experiments with leaves and with whole plants were highly correlated with larval performance (Fig. 5), but were not correlated with

average PA composition of the species ($r_s=0.28$, $df=8$, $P=0.51$) or with average PA concentration ($r_s=0.13$, $df=8$, $P=0.13$).

Discussion

We did find differences in larval performance and oviposition preference between the *Senecio* species. These differences were, however, not correlated with alkaloid composition. Furthermore, larval performance on the *S. jacobaea* populations was not correlated with alkaloid composition of those populations. All species, except *S. sylvaticus*, mainly contain alkaloids of the senecionine-necine type. *S. sylvaticus* has a completely different set of alkaloids (triangularine type) and it is striking that larval performance and oviposition preference on this species is low. Possibly PAs of a type other than senecionine-necine type do affect the cinnabar moth. Lindigkeit et al. (1997) tested the activity of senecionine-oxygenase (enzyme involved in sequestration of PAs in *T. jacobaea*) on a wide range of PAs. The activity of this enzyme was best on senecionine-necine type PAs and was much lower on PAs of other types, such as triangularine. These PAs can therefore not be completely metabolized by the cinnabar moth and are perhaps more harmful.

The differences in larval performance and oviposition preferences may be due to morphological differences or other chemical differences between the *Senecio* species, rather than alkaloid composition of the species. The poor performance of the larvae on *S. viscosus* was probably due to the glandular hairs on the leaves of this viscid species. In our experiment the larvae did not feed on this species at all, which is in accordance with the findings of Merz (1959). Interestingly, Merz (1959) reported that if leaves were rinsed with methanol, dissolving the glandular secretion, *T. jacobaea* larvae did feed on *S. viscosus*. When the glandular secretion of *S. viscosus* was painted on leaves of acceptable *Senecio* species, these were refused by the larvae. In the oviposition experiments no eggs were found on *S. viscosus* and the moths seemed to be extremely deterred by this *Senecio* species (personal observation).

Larval performance and oviposition was also poor on *S. inaequidens*. *S. inaequidens* is phylogenetically the most distant to *S. jacobaea*, while *S. alpinus* and *S. aquaticus* (two species on which the cinnabar moth does well) are the two most closely related species to *S. jacobaea* (Pelser et al. 2002). Plant characters may differ more strongly between phylogenetically less related species. However, in North America the cinnabar moth is known to feed on *S. triangularis* (Diehl and McEvoy 1990), which is more closely related to *S. inaequidens* than to *S. jacobaea*.

Although we found that the cinnabar moth was able to survive and lay eggs on at least six *Senecio* species, there are only few reports of *T. jacobaea* on host plants species other than *S. jacobaea*. In Great Britain the cinnabar moth is occasionally found on *S. vulgaris*

(Rothschild et al. 1979) and in North America on *S. triangularis* (Diehl and McEvoy 1990). Larvae of *T. jacobaea* were found on *Tussilago farfara* (Sutton and Beaumont 1989) but performance was extremely poor on this species (Tinney et al. 1998). In our experiments, two species (*S. aquaticus* and *S. alpinus*) were equally suitable as host plants as *S. jacobaea*. Probably habitat differences of the host plant species can explain why there are no reports of the cinnabar moth on these two *Senecio* species. The moist conditions in which *S. aquaticus* grows are probably not suitable for (pupal) survival of the cinnabar moth. Vrieling and de Boer (1999) suggested that pupal survival of *T. jacobaea* is better on sandy soils than on more humid clay and peat soils. Dempster (1971) showed that if pupae were kept in contact with water, they did not survive. *S. alpinus* grows in the alpine regions of Europe, while *T. jacobaea* occurs at lower altitudes. *S. rupestris*, on which *T. jacobaea* does only moderately well, also grows in alpine regions and so does *S. adonidifolius* on which larvae of the cinnabar moth can survive as was shown in our experiments. Based on our results, *S. jacobaea* is the most suitable host plant species in the habitat of the cinnabar moth. Tinney et al. (1998) found that larval performance was good on *S. vulgaris* but in oviposition choice experiments *S. jacobaea* was preferred over *S. vulgaris*. They argue that plant sustainability can be important for determining the host plant choice as *S. jacobaea* is a bigger and more sustainable host than the annual *S. vulgaris*. Our data showed that larval performance and oviposition preference were highly correlated, indicating that plant quality also plays an important role in determining the host plant choice of the cinnabar moth. PAs, however, do not seem to play an important role.

Since we used whole plants in our experiments, possible subtle effects of PAs may have been confounded with or obscured by other (not measured) factors that could have been different between the plants. Individual PAs could be tested for their effects on the cinnabar moth. However, our results at least show that PAs are not the main determinant for larval performance and oviposition choice. The use of plants also presents another limitation. The level of variation that can be tested is limited to what can be found in natural populations. The patterns that are found in nature result from possible selection in the past and therefore present a biased sample. This would have been a problem if we would have found that PA composition affected the cinnabar moth. However, we could not show an effect of PAs.

The results presented here indicate that variation within the senecionine-necine type PA does not affect the cinnabar moth and therefore it seems unlikely that this specialist moth is the driving force behind the evolution and maintenance of the various structurally related PAs in its host plant *S. jacobaea*. Alternatively, structurally related PAs could differ in their effects towards (non-adapted) generalist herbivores, as was found for the benzyloisoquinoline alkaloids by Miller and Feeny (1983). Seneciophylline, a PA found in *S. jacobaea*, deterred feeding by three generalist insect herbivores (Hägele and

Rowell-Rahier 2000) but hardly any study has been done on the specificity of the effects of structurally related PAs on generalist herbivores. Van Dam et al. (1995) tested the feeding deterrence of three related PAs of *Cynoglossum officinale* (Boraginaceae) on the generalist herbivore *Spodoptera exigua* (Lepidoptera; Noctuidae) and they found no differences in effect between the three PAs. Other alternative explanations for the diversity of related PAs may be that related compounds may act synergistically on herbivores (Adams and Bernays 1978; Lindroth et al. 1988) or that the diversity may be maintained through selection from several different herbivores and/or pathogens (Simms 1990; Mithen et al. 1995; Juenger and Bergelson 1998).

The diversity of PAs in *Senecio* species could also be the result of a process that is 'selectively neutral' instead of being the result of a coevolutionary process between a plant and its herbivores. Under this model it is expected that structurally related PAs do not differ in their effects on herbivores, whether these effects are none, deterrent, toxic or stimulant. Vrieling and Van Wijk (1994) could not show any costs involved in the production of PAs in *S. jacobaea*. Possibly, new compounds evolve easily and if there is no selection pressure on those new PAs, no benefits (equal effects) and no (extra) costs, these compounds remain and can spread within a population. If the diversity of PAs is selectively neutral, random processes like genetic drift and founder effects could have created the differences in PA composition among the populations of *S. jacobaea*.

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