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Citation

Macel, M., Vrieling, K., & Klinkhamer, P. G. L. (2004). Variation in pyrrolizidine alkaloid patters of *Senecio jacobaea*. *Phytochemistry*, 65(3), 865-873.
doi:10.1016/j.phytochem.2004.02.009

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Variation in pyrrolizidine alkaloid patterns of *Senecio jacobaea*

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Received 30 July 2003; accepted 10 February 2004

Abstract

We studied the variation in pyrrolizidine alkaloid (PA) patterns of lab-grown vegetative plants of 11 European *Senecio jacobaea* populations. Plants were classified as jacobine, erucifoline, mixed or senecionine chemotypes based on presence and absence of the PAs jacobine or erucifoline. Due to the presence of jacobine, total PA concentration in jacobine chemotypes was higher than in erucifoline chemotypes. Both relative and absolute concentrations of individual PAs differed between half-sib and clonal families, which showed that variation in PA patterns had a genetic basis. Within most populations relative abundance of PAs varied considerably between individual plants. Most populations consisted either of the jacobine chemotype or of the erucifoline chemotype, sometimes in combination with mixed or senecionine chemotypes.

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Keywords: *Senecio jacobaea*; Genetic variation; Geographic distribution; Pyrrolizidine alkaloids; Chemotypes; Jacobine; Erucifoline

1. Introduction

Plants from the genus *Senecio* (Asteraceae) are known for the production of a wide variety of pyrrolizidine alkaloids (PAs) (Hartmann and Witte, 1995) that have hepatotoxic and carcinogenic properties (Mattocks, 1968; Cheeke 1988). In *Senecio* species PAs are produced in the roots as senecionine *N*-oxides (Hartmann and Toppel, 1987; Toppel et al., 1987). Senecionine *N*-oxide is transported via the phloem to the above ground plant organs (Hartmann et al., 1989) where it is transformed into several related PAs (Hartmann and Dierich, 1998). The transformation of senecionine into related PAs differs between *Senecio* species and hence species-specific PA patterns are produced (Hartmann and Dierich, 1998). *Senecio jacobaea* can contain more than 10 senecionine related alkaloids (Witte et al., 1992). PA concentration in *S. jacobaea* is partly genetically determined (Vrieling et al., 1993). Witte et al. (1992) described two PA chemotypes for *S. jacobaea*,

the jacobine type and the erucifoline type, based on PA content in inflorescences of plants collected in the field. Jacobine types were characterized by the PAs jacobine and jacobine and lacked erucifoline, while the erucifoline types contained erucifoline and acetylerucifoline but hardly any jacobine (see Fig. 1 for structures). The two chemotypes did not differ in concentrations of other PAs such as senecivernine, senecionine, integerrimine and seneciophylline. Only rarely an intermediate chemotype was found that contained both jacobine and erucifoline. The patterns described by Witte et al. (1992) are interesting in relation to the evolution of diversity of alkaloids. However, generally only one plant per population was sampled and collected in its natural habitat. Therefore, it is not possible to disentangle environmentally induced patterns and genetic effects. Here, we investigated the PA patterns in leaves of vegetative plants from 10 European populations of *S. jacobaea*. To determine whether the different PA patterns have a genetic basis, we studied these patterns in cloned *S. jacobaea* genotypes and half-sib families grown under identical controlled conditions to minimize a possible effect of environment. To determine the variation within and between the different populations, PAs from 5 plants of every population were analyzed.

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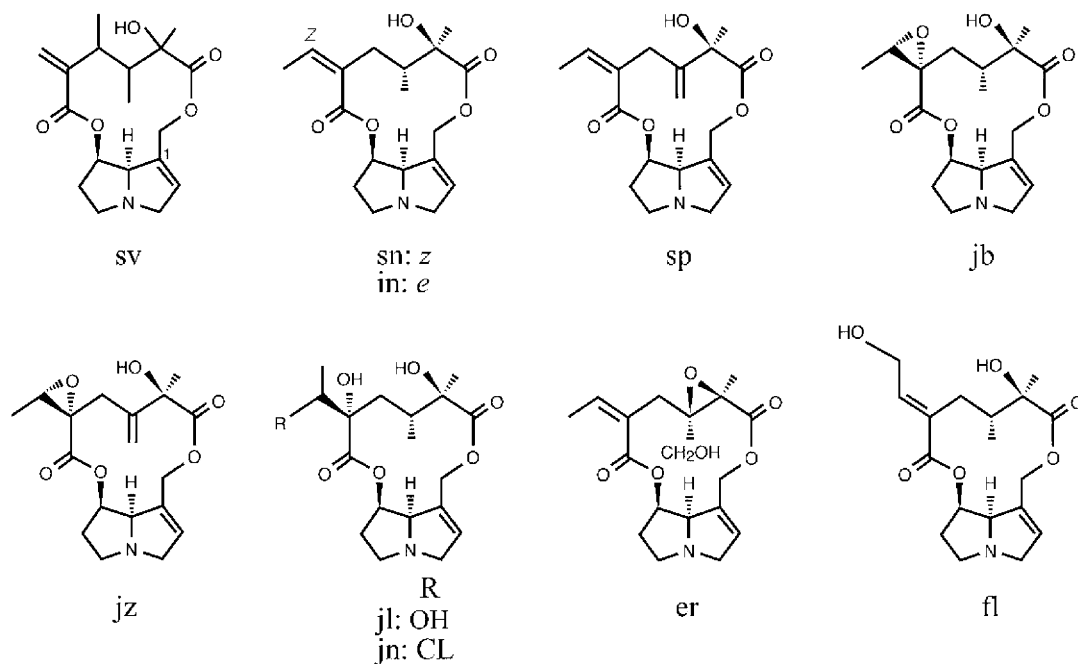


Fig. 1. Structures and codes of PAs found in *S. jacobaea*. sv = senecivernine; sn = senecionine; sp = seneciophylline; in = integerrimine; jb = jacobine; jz = jacozone; jl = jacoline; jn = jaconine; er = erucifoline; fl = eruciflorine.

2. Results and discussion

2.1. Variation in PA composition within clonal families

To show that variation in PA composition among plants is genetically determined, we studied the variation in PA profiles within 10 clonal families of *S. jacobaea* (16–34 plants per family). Each clonal family was from a different population (Table 1). In some plants 6 senecionine related PAs were found while other plants only contained two PAs. The PA composition (relative abundance) of each clonal family is shown in Fig. 2. Clonal families differed in relative percentages of senecionine, seneciophylline, integerrimine, jacobine and erucifoline (KW, $df=9$, for all PAs: $P<0.005$). The clonal families also differed in absolute concentrations of senecionine, seneciophylline, integerrimine, jacobine and

erucifoline (KW, $df=9$, for all PAs: $P<0.005$). Variation in PA composition within clonal families was small compared to variation among these families indicating a strong genetic component. Inheritance of PA patterns could further be investigated by crossing genotypes with a different PA composition.

2.2. Variation in PA composition between half-sib families

We analyzed the PA composition of offspring from 25 plants in two areas (in total 50 half-sib families) of the population in Meijendel (The Netherlands). All 413 plants analyzed, except for one plant, contained mainly jacobine, the percentage of jacobine ranged from 41 to 100% of total PA. One plant contained mainly jacoline (45%). The percentage of erucifoline ranged from 0 to

Table 1

Origin of *S. jacobaea* plants used in this study. Source indicates number of plants of which seeds were used in our study

Code	Population	Analysis	Source
AMS	Amsterdam, <i>The Netherlands</i>	Clone/population	1 plant
BUR	Burghaamstede, <i>The Netherlands</i>	Clone/population	Unknown
DRL	Driel, <i>The Netherlands</i>	Population	3 plants
SLK	Slenaken, <i>The Netherlands</i>	Clone/population	1 plant
MEI	Meijendel, <i>The Netherlands</i>	Clone/population	3 plants
MAV	Mavellier, <i>Switzerland</i>	Clone/population	3 plants
SUN	Sundsvall, <i>Sweden</i>	Clone/population	1 plant
FIL	Filly, <i>Belgium</i>	Clone/population	Unknown
CHE	Chereng, <i>France</i>	Clone/population	1 plant
EBA	Ejby Adal, <i>Denmark</i>	Clone/population	Unknown
SCH	Schiermonnikoog, <i>The Netherlands</i>	Clone	1 plant

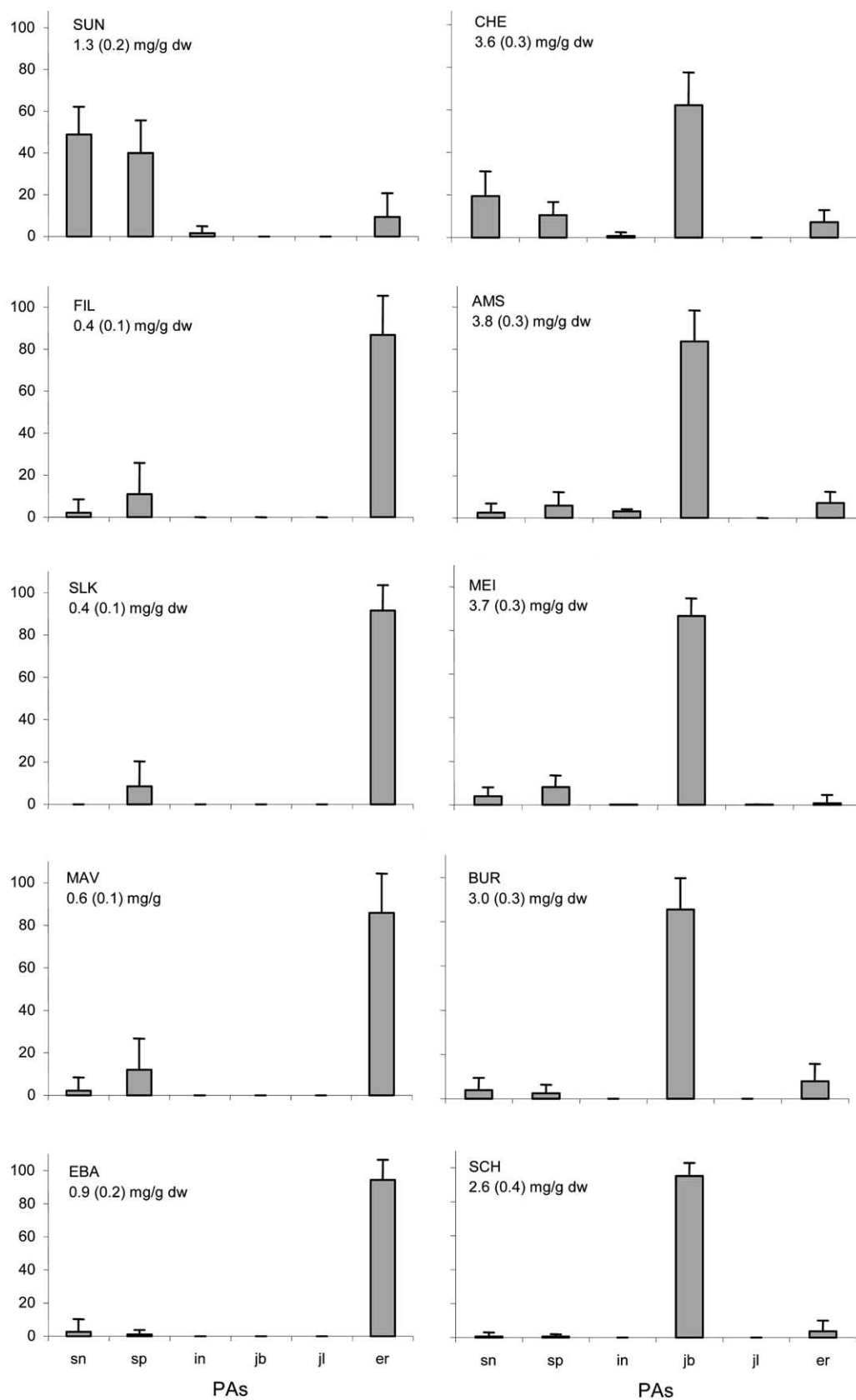


Fig. 2. PA composition of *S. jacobaea* clonal families in average relative abundance of PAs ($\pm$ S.D.). Most clonal families that contained jacobine also contained small amounts of erucifoline. Origin is indicated in the right corner. Average PA concentration per clonal family ($\pm$ SE) is given. $n = 16$ –34.

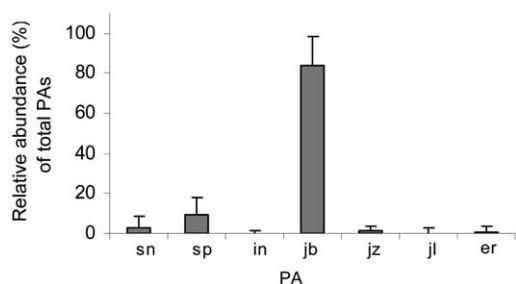


Fig. 3. PA composition of *S. jacobaea* from Meijendel in relative abundance of PAs ($\pm$ S.D.). $n=413$.

19% of total PA. Fig. 3 shows the mean relative abundance (%) of individual PAs over all 413 plants. The percentage of senecionine, seneciophylline, integerrimine, jacobine and jacozone differed among the families (KW, $df=49$, for all PAs $P<0.005$). Percentages erucifoline and jacoline did not differ among the families (er: KW $\chi^2=61.51$, $df=49$, ns; jl: KW $\chi^2=45.93$, $df=49$, ns). In absolute concentrations senecionine, seneciophylline, integerrimine, jacobine and jacozone also differed between the families (KW, $df=49$, for all PAs $P<0.002$). Jacoline and erucifoline concentrations did not differ between the families (KW, ns). Families also did differ in their total PA concentration ranging from 1.57 mg/g ($se\pm 0.30$) to 5.51 mg/g ($se\pm 0.97$) (ANOVA $F=2.64$, $df=49$, $P<0.001$). PA concentration did not differ between plants from the two areas in Meijendel (ANOVA, $F=1.79$, $df=1$, $P=0.18$). These results show that although all plants contained mainly jacobine, considerable (genetic) variation in both PA composition and concentration existed within the Meijendel population.

2.3. Variation within and between populations

From 10 European *S. jacobaea* populations (Table 1) 4–5 plants were analyzed for their PA composition. Ten senecionine related PAs were found in some plants while other plants contained only two or three of these PAs. Fig. 4 shows the average PA composition of the populations. Populations differed significantly in the relative abundance of seneciophylline (KW $\chi^2=24.53$, $df=9$, $P<0.005$) and jacobine (KW $\chi^2=28.08$, $df=9$, $P<0.001$). Relative abundance of all other PAs did not differ between populations (KW, $df=9$, all $P>0.05$). In absolute concentrations only jacobine differed among the populations (KW $\chi^2=24.98$, $df=9$, $P<0.005$), concentrations of the other PAs did not differ between the populations (KW, $df=9$, all $P>0.05$). Although a relatively small number of plants was used of each population, variation in PA composition within populations was considerable and this variation confirms the more extensive data from the Meijendel population above. As an example, Table 2 shows the variation in PA composition of individual plants in three populations. The

plants from the same population that we analyzed were sometimes half-sibs (Table 1) and therefore the actual variation in PA composition in a population might be even greater than is shown by our data.

2.4. Chemotypes

All plants contained the PAs senecionine or seneciophylline. In contrast to the chemotypes described by Witte et al. (1992), plants that contained jacobine had often also small amounts of erucifoline (Fig. 2). The jacobine types described by Witte et al. (1992) completely lacked erucifoline. Here, we will consider (1) plants with mainly jacobine and no or little erucifoline as jacobine chemotypes, (2) plants that contained both jacobine and erucifoline in similar amounts as mixed chemotypes and (3) plants without jacobine but with erucifoline as erucifoline chemotypes. In addition, some plants had only trace amounts of erucifoline and contained no jacobine. We classified these plants as senecionine chemotypes. Fig. 5 shows the PA composition of these chemotypes.

Most populations consisted of either erucifoline chemotypes or jacobine chemotypes, sometimes in combination with mixed or senecionine chemotypes (Table 2, Fig. 4). In five of the ten populations we sampled (DRL, SUN, FIL, CHE, BUR) mixed chemotypes were found (Fig. 4). Only in one population, CHE, plants of pure jacobine, mixed and pure erucifoline chemotype were found (Table 2). It is possible, however, that more 'mixed' populations exist because we may not have a

Table 2

PA composition of plants from three *S. jacobaea* populations as an example for the variation within and between populations

Pop.	Plant	Alkaloid (% relative abundance)								PAs (mg/g dw)
		sv	sn	sp	in	jb	jz	jl	jn	er
AMS	A	–	–	15	–	84	tr	tr	tr	2.2
	B	–	tr	15	–	71	–	tr	–	2.8
	C	tr	3	11	4	64	–	–	15	1.2
	D	–	3	–	–	56	15	9	6	0.7
	E	tr	72	tr	28	–	–	–	–	2.7
SLK	A	8	17	18	7	3	–	10	–	1.4
	B	37	tr	23	8	2	tr	–	–	0.2
	C	tr	8	6	–	–	–	–	–	0.8
	D	6	tr	14	8	–	–	–	–	0.8
	E	62	tr	33	–	–	–	–	–	0.2
CHE	A	tr	13	13	tr	68	–	–	–	0.4
	B	tr	4	10	tr	61	–	tr	tr	2.3
	C	7	7	22	–	50	4	5	7	3.2
	D	2	3	55	3	16	4	6	tr	2.0
	E	tr	19	57	tr	–	tr	tr	–	1.7

The AMS population consists of jacobine and senecionine chemotypes, SLK population consists of erucifoline chemotypes only and the CHE population consists of jacobine, erucifoline and mixed chemotypes based on the relative abundance of individual PAs. tr = trace amounts $<1\%$ of total PA

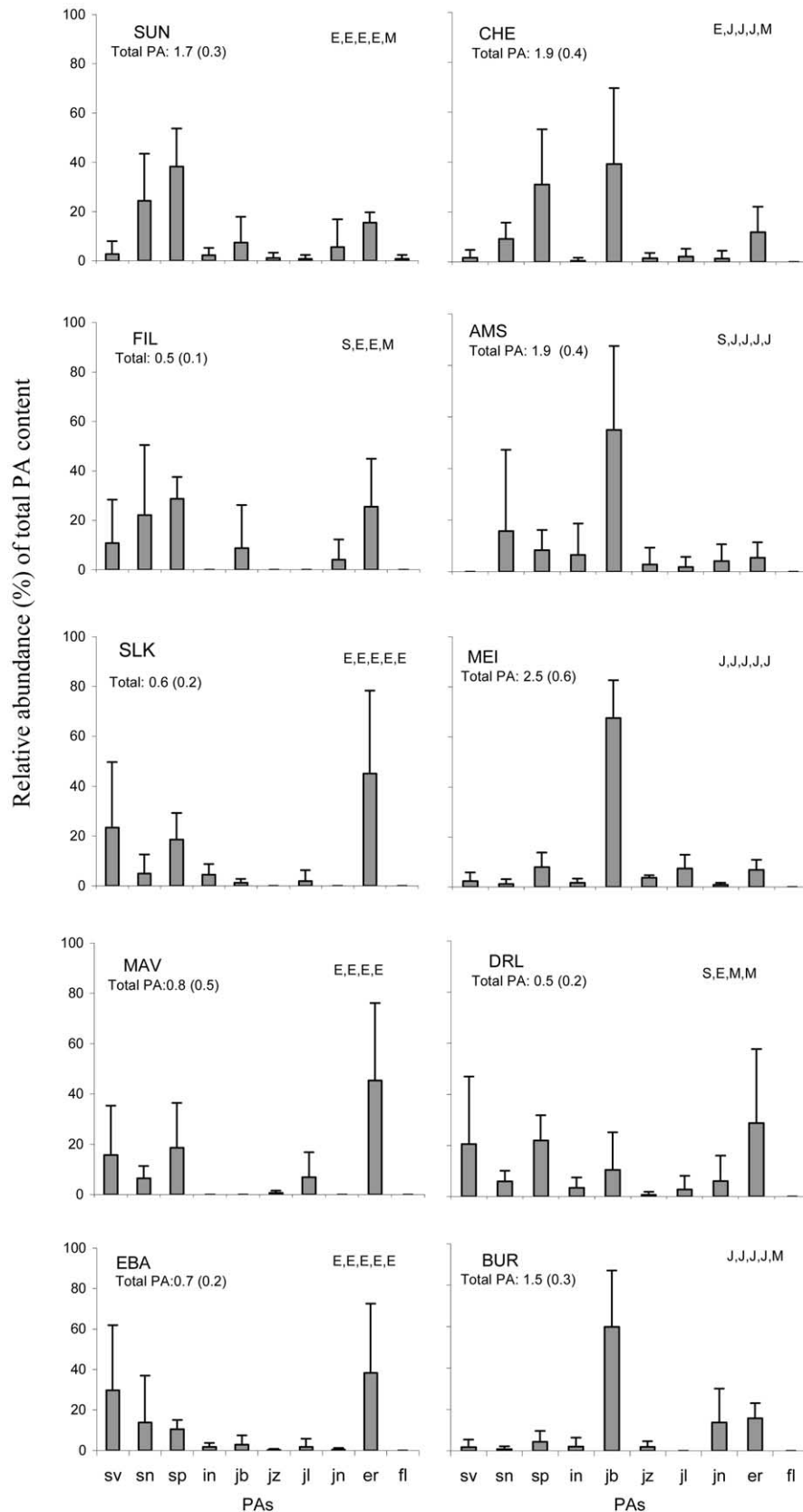


Fig. 4. PA compositions of *S. jacobaea* from European populations given as average relative abundance of PAs ($\pm$ S.D.). Population is indicated in the right corner. Average PA concentration per populations in mg/g dry weight ($\pm$ SE) is given. Letters indicate chemotypes of individual plants: S = senecionine type, E = erucifoline type, J = jacobine type, M = mixed type $n = 4-5$.

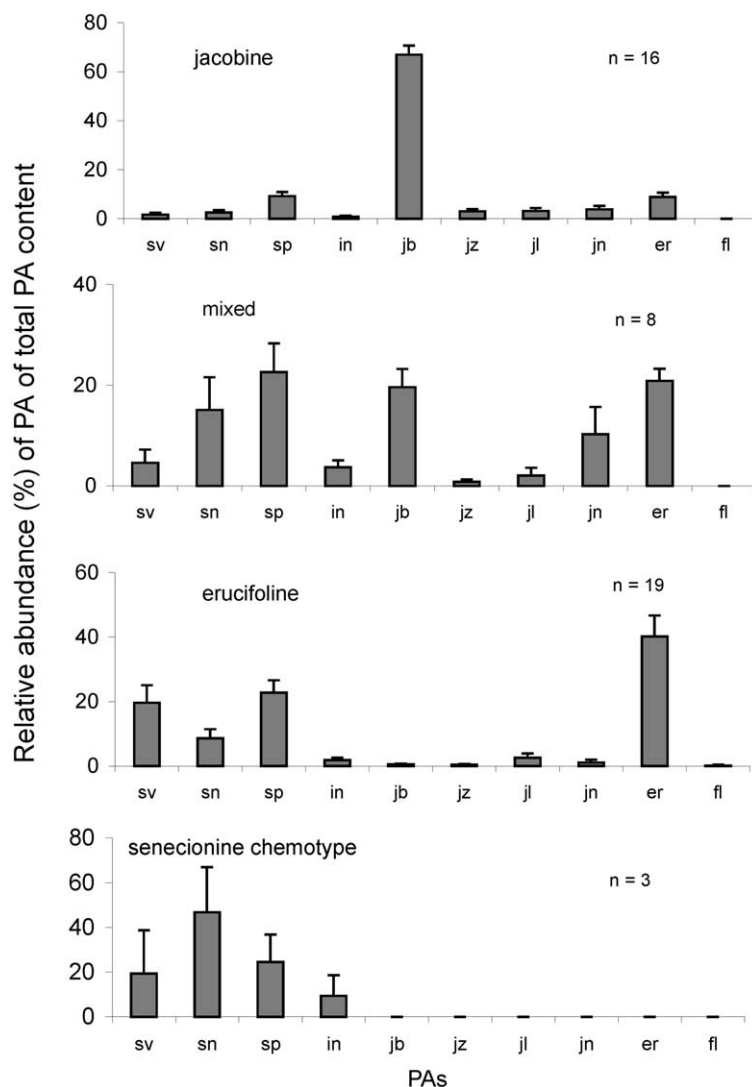


Fig. 5. PA composition of the chemotypes in relative abundance of PAs ($\pm$ SE).

complete overview of all the chemotypes present in a population due to the limited number of genotypes of most populations. Witte et al. (1992) suggested that the distribution of the erucifoline chemotype was limited to the Eastern and Southern Europe. However, in our study, plants of Northern European populations (Sweden, Denmark) were erucifoline chemotypes (Fig. 6). Furthermore, plants of some populations from The Netherlands also were erucifoline chemotypes. The PA patterns found under uniform conditions may perhaps not accurately reflect differences in PA patterns between populations in the field because plants may show different norms of reaction for e.g. nutrient concentration, water availability, day length. Our data do show that not only South-Eastern populations are genetically equipped to produce erucifoline chemotypes.

The total PA concentration of the jacobine chemotypes in both the clones and the populations was higher than the PA concentration of the erucifoline chemo-

types (Fig. 7). Among the plants from the populations, the mixed chemotype and senecionine chemotype had intermediate PA concentrations (Fig. 7). In the clonal families, senecionine chemotypes also had a lower total PA concentration compared to the jacobine chemotypes (Fig. 7). The higher total PA concentration of the jacobine chemotype is due to the “addition” of jacobine (Fig. 7). If we compared the total concentration minus jacobine we found no significant difference between the chemotypes for the populations (ANOVA, $F=1.45$, $df=4$, $P=0.281$). For the clones the total PA concentration without jacobine was significantly higher in the erucifoline and senecionine chemotypes (KW $\chi^2=23.93$, $df=2$, $P<0.001$).

2.5. Natural selection?

Our data for the clonal families show that differences in PA composition between populations of *S. jacobaea*

have a genetic basis. The variation in PA composition between individual plants within the populations was considerable. In the population of Meijendel, half-sib families differed in PA composition and concentration. Molecular genetic analyses (AFLP) of European *S. jacobaea* populations, with similar geographic and bio-

chemical range as studied in this paper, showed that there was considerable variation among different populations. This molecular variation was not linked to the distribution of alkaloid patterns, although one marker was directly linked with presence of jacobine (K. van den Hof, J. Joshi and K. Vrieling, unpublished data).



Fig. 6. Geographic distribution for the chemotypes of generative plants and vegetative plants of *S. jacobaea* in Europe.

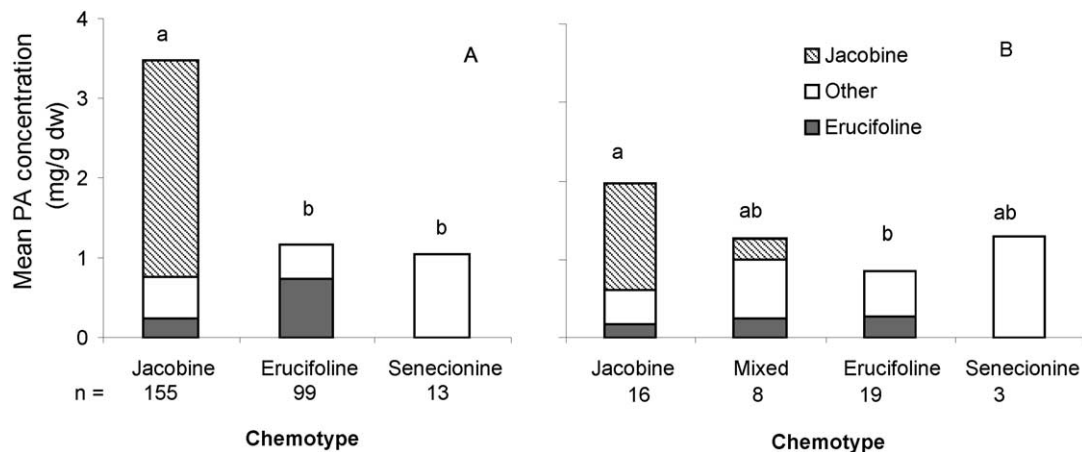


Fig. 7. Average total PA concentrations of the chemotypes from plants of (A) clones or (B) populations. Letters indicate significant differences in total PA concentration, Populations: ANOVA, Bonferroni post-hoc tests, Clonal families: Kruskal–Wallis, post-hoc Mann–Whitney tests with Bonferroni correction.

This seems to indicate that the geographical distribution of the chemotypes in Europe is not caused by common ancestry but by similar selection pressures in a certain region. The genetic variation in PA composition provides a basis upon which natural selection, by e.g. herbivores or pathogens, may act. It is unlikely that the specialist herbivore *Tyria jacobaeae* plays a role in the evolution of the different chemotypes because larval performance and oviposition preference of this specialist moth was not affected by chemotype of *S. jacobaea* (Vrieling and de Boer, 1999; Macel et al., 2002; Macel and Vrieling, 2003). However, other herbivores may have been sensitive to difference in PA composition. It has been shown that the relative effect of a PA can differ between insect species (Macel, 2003) and therefore differences in herbivore community in *S. jacobaea* populations could have led to the evolution of the different chemotypes.

3. Experimental

3.1. Growth conditions

All plants were grown in 50/50 dune sand/peat mixture in 11 cm diameter pots in a growth chamber: photoperiod 8 h light: 16 h dark, 20 °C day/15 °C night, relative humidity 70%. After two months in the pots the plants were given ample nutrients.

3.2. Clonal families, half-sib families and populations

Clonal families: We used 16–34 plants per clonal family. A clonal family consisted of one randomly selected genotype of a population. This genotype was propagated via tissue culture. Plants were grown for two months in pots before analyzing the PA patterns.

Half-sib families: We collected seeds from *S. jacobaea* plants in two areas of the Meijendel population. In each area we sampled 25 plants. We analyzed 4–10 seedlings per plant for their PA profile, in total 413 plants. Plants were grown from seed for two months before analysis.

Populations: Plants were grown from seed for 5 months and then harvested for PA analysis.

3.3. PA analysis

The fifth youngest leaf of each plant was harvested to determine 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 (WCOT, 15 m×0.25 mm; DB-1 and DB-17, J&W Scientific) with following conditions: injector: 250 °C, temperature program 150–300 °C, 6 °C per minute, carrier gas He, pressure DB-1 100 kPa and

DB-17 80 kPa, split mode 1–20, detectors: FID and NPD. Structure of the PAs was verified by GC–MS (Witte et al. 1992). Total PA concentration was determined spectrophotometrically, by a method modified after Mattocks (1967). Heliotrine (Latoxan) was used as ref. compound. The concentration of individual PAs was calculated by total PA concentration×fraction of individual PA measured by GC analysis.

3.4. Statistical analysis

Statistics were performed in SPSS 8.0 (SPSS Inc, 1998). Total PA concentrations of the 413 plants from the half-sib families in Meijendel were log-transformed, after log-transformation differences in PA concentration among families were tested with a one-way ANOVA. Differences between individual PAs were tested with a Kruskal–Wallis test. Differences in PA composition and concentrations among the clonal families and populations were also tested with a Kruskal–Wallis test.

Acknowledgements

We are most grateful to Dr. Jens Hagen, Dr. L. Witte and Prof. Dr. T. Hartmann for their help with the GC and GC–MS analysis. We thank Helene de Vos and Karin van Veen- van Wijk for their technical assistance and Martin Brittijn for the artwork. Urs Schaffner, Stefan Andersson, Els Schlatmann, Henk van Dijk, Nico de Boer and the Botanical Garden in Copenhagen generously provided us with seeds from the different populations. We thank Nicole van Dam, Ed van der Meijden and two anonymous referees for their valuable comments on earlier drafts of the manuscript.

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