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Variation of pyrrolizidine alkaloids in *Senecio vulgaris* plants from native and invasive ranges

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Abstract

Pyrrolizidine alkaloids (PAs), a typical kind of secondary metabolites in plants, have important roles on defense against herbivores and pathogens; however, specialist herbivores adapted to PAs can use them as cues for oviposition and feeding. Thus, in the native ranges, PA diversity and concentration in plants were selected by the balance between pressure from generalist and specialist herbivores. In introduced ranges, where the specialist herbivores are absent, the introduced plants could increase concentration and diversity of PAs. This predication is deduced from the Shift Defense Hypothesis (SDH). In this research, we investigated whether there were any differences between native and invasive *Senecio vulgaris* plants (from Europe and China, respectively) with regards to the PA composition and concentration. We grew the native and invasive *S. vulgaris* plants in an identical condition and harvested them when they started to bloom. Their roots and shoots were separately harvested and dried. PA composition and concentration from powder of the shoots and roots were detected by using liquid chromatography – tanderm mass spectrometry (LC-MS/MS). We identified 14 PAs which belongs to the structural group senecionine – like PAs. Most of them occurred in both the native and invasive *S. vulgaris* plants, except the usaramine *N* – oxide that was only found in the native ones. From the 14 PAs identified, only riddelliine *N* – oxide had significantly higher present frequency in the invasive plants than in the native plants. The invasive *S. vulgaris* plants had significantly lower concentration of 3 individual PAs (seneciphylline *N* – oxide, spartioidine and spartioidine *N* – oxide) than the native ones. These results demonstrated that PA diversity and concentration of some individual PAs tended to reduce in the invasive range of *S. vulgaris*. This is contrary to the predictions of the SDH that the invasive plants would produce more qualitative defense than the native ones, and it is probably an evidence that a little trade – off between defense and growth happened to the *S. vulgaris* in China.

Key words: Biological invasion, Shift Defense Hypothesis (SDH), qualitative defense, Liquid chromatography – tanderm mass spectrometry (LC-MS/MS), Secondary metabolite, Diversity

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1. Introduction

Pyrrolizidine alkaloids (PAs), a typical kind of plant secondary metabolites, have been intensively studied and many PAs have been detected in different species. Most of them are esters of a dibasic necic acid and a pyrrolizidine diol "necine base" (Hartmann 1999). PAs have been found in plants from families such as the Compositae, Boraginaceae, and Leguminosae. They are diverse and can be grouped into five major classes: the senecionine, triangularine, monocrotaline, lycopsamine and phalaenopsine class. The most diverse PAs class is senecionine class which contains more than 100 structures (Rizk 1990; Hartmann & Witte 1995). In *Senecio* species, such as *S. vulgaris*, PAs are primarily produced as *N* – oxide in the roots. The *N* – oxides form are predominantly present than their corresponding form tertiary amines (Hartmann & Zimmer 1986; Hartmann & Toppel 1987). However, the tertiary amine is reported to be more toxic than the *N* – oxides (Macel et al., 2005).

PAs are known as chemical defense compounds served as deterrent or toxic to herbivores and pathogens (Boppré 1986; Schneider 1987; Macel 2003; Molyneux et al., 2011). They play a role in plant resistance to most vertebrates and generalist herbivores (Schneider 1987; Macel 2003; Cheng et al., 2011b) and pathogens (Hol & Van Veen 2002; Singh et al., 2002). PAs functioned in the resistance against generalist herbivores (Leiss et al., 2009b; Macel & Klinkhamer 2010) such as generalist snail (*Helix aspersa*) (Cano et al., 2009), leafminer (*Liriomyzia moth*), western flower thrips (*Franklinella occidentalis*) (Cheng 2012), *Brachycaudus cardii* (Vrieling et al., 1990; Macel 2003; Leiss et al., 2009a). In the study about PAs effect on vertebrates, PAs were considered as carcinogenic, mutagenic, genotoxic, fetotoxic and teratogenic factors (Fu et al., 2004; Wiedenfeld 2011). They are toxic to humans and ruminants (Wiedenfeld & Edgar 2011), livestock (Habermehl et al., 1988; Rizk 1990) such as cattle, horses, sheep, etc. due to causing damages to liver, lungs, and blood vessels, etc. However, specialist herbivores are reported to adapt to PAs. Plants with higher PA concentration are always more attractive to the specialist herbivores (Macel & Klinkhamer 2010; Lee et al., 2011). They can utilize PAs from host plants for their own benefits such as for a cue of food and oviposition (Loon et al., 1992; Macel & Vrieling 2003; Bernays et al., 2004; Cheng 2012). Specialist herbivores, such as *Tyria jacobaeae* (Naumann et al., 2002), even sequestered and metabolizes PAs from host plants for their own defense, sexual pheromones (Trigo 2011). High tertiary amines PA of jacobine – like PAs concentration and some otosenine – like PAs could simulate *T. jacobaeae* to produce more eggs and egg batches on plants (Cheng 2012). Based on the role of PAs on different kinds of

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herbivores, we concluded that plants with high PA concentrations will be more damages if the specialist herbivores are present; and they will be few damages if the specialist herbivores are absent. Therefore, the variation of PA composition and concentration in the invasive plants in new ranges is necessary for them to defend against new guild of herbivores.

Indeed, introduced plants will leave behind their specialist herbivores when they introduced to new ranges as predicted by the Enemy Release Hypothesis (ERH) (Keane & Crawley 2002). Consequently, the introduced plants could reduce resources to produce costly quantitative defenses against the specialist herbivores. A net gain of the resources will partly allocate to produce more cheap qualitative defenses against generalist herbivores without side effect of attracting the specialist herbivores. This allocation of resources results in the higher qualitative defenses in the invasive plants as predicted by the Shift Defense Hypothesis (SDH) (Müller-Scharer et al., 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011). Thus, in this study we hypothesized that the invasive *Senecio vulgaris* plants (from China) will produce more diverse and higher PA concentration than the native ones (from Europe).

The variation of PA profiles has been found between different species and between native versus invasive plants of the same species. It has been intensively studied in different species from *Senecio* genus (Pelser et al., 2005), such as *S. vulgaris* and *S. vernalis* (Hartmann & Zimmer 1986); and in *Jacobaea* genus, such as *J. vulgaris* and *J. erucifolia*. Several studies found that plants from invasive ranges had higher concentration and more diverse of qualitative defense than those from native area (reviewed by Doorduin & Johanna 2012; Lin 2015). For examples, the invasive genotypes of *S. inaequidens* and *S. pterophorus* produced higher total PA concentration than their native genotypes (Cano et al. 2009). *Jacobaea vulgaris* plants from invasive populations contained higher total PAs and tertiary amines PAs concentrations than those from their native range (Joshi & Vrieling 2005; Lin 2015).

In this study, we investigated qualitative and quantitative PAs variation between native and invasive *S. vulgaris* populations from Europe and China, respectively. We expected that there was an evolution happened to the invasive *S. vulgaris* plants in respect of increasing qualitative defense level. The native and invasive plants of *S. vulgaris* from different populations were grown in an identical condition in a greenhouse. PAs were extracted and measured from dry powders of the samples using liquid chromatography – tanderm mass spectrometry (LC-MS/MS). We found and compared the PAs between ranges. We addressed the following questions:

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(1) Are there any differences between the native and invasive *S. vulgaris* plants with regards to the composition and concentration of PAs? If yes,

(2) Do invasive *S. vulgaris* plants produce more diverse and higher amount of PAs than the native ones?

(3) Are there any differences between *S. vulgaris* plants from different populations with regards to the composition and concentration of PAs?

2 Materials and Methods

2.1. Study species

Senecio vulgaris (Common groundsel, Asteraceae) most probably originated from southern Europe (Kadereit 1984). In the 18th century it spread to America, Sahara north Africa, Asia, Australia and New Zealand (Robinson et al., 2003). This species is reported to contain pyrrolizidine alkaloids (PAs) (Hartmann & Zimmer 1986; Hartmann & Toppel 1987; Cano et al. 2009; Yang et al., 2011), which are second metabolite compounds in plants. Pyrrolizidine alkaloids are regarded as major defense compounds of *S. vulgaris* and *Senecio* genus in common (Macel 2011). The *N* – oxides are abundant occurrence in plants than the tertiary bases (Hartmann & Zimmer 1986; Hartmann et al., 1989). In tissue of PA producing species the tertiary alkaloid is rapidly *N* – oxidized. In *Senecio* genus, such as *S. vulgaris*, roots are the exclusive site of biosynthesis of PAs primarily as senecionine *N* – oxide (Toppel & Hartmann 1986; Hartmann & Toppel 1987) before they are diverted to different individual PAs in shoot by specific enzymes (See Figure S1 for the formation of other PAs from senecionine) (Hartmann & Dierich 1998). Subsequently, they are transported to shoots and selectively stored in the target tissues such as inflorescences or epidermal cell layers of stems. They are mainly accumulated in inflorescences as *N* – oxides, and a small proportion of the alkaloid *N* – oxides are accumulated in leaves (Hartmann & Zimmer 1986; Hartmann et al. 1989). Some authors found senecionine (Hartmann & Zimmer 1986), or seneciphylline to be the dominating PA (Lüthy et al., 1983), while some other authors found that both senecionine and seneciphylline are considered as main PAs in *S. vulgaris* (Borstel et al., 1989; Brown & Molyneux 1996; Frischknecht et al., 2001).

2.2. Plant resources

Seeds of *S. vulgaris* were collected from populations in Europe and China (Table 1). In this study, we used 6 native and 6 invasive *S. vulgaris* populations from Europe and China, respectively. Within each population, we selected 5 to 7 mother plants that they contained a large number of

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good seeds and from each mother plant, 4 to 6 seeds were selected. From each population, 36 seeds have been selected. Thus, for this experiment 432 seeds were used.

2.3. Plants growth

Two kinds of substrates were prepared that one was for seed germination and the other one was for transplanting seedlings. The first one was made manually from coconut soil and sand (1:1 by volume). Water was added to the substrate until the substrate was well wetted. This substrate was filled into 12 – cell boxes (size for one cell: 3.7 × 3.7 × 5 cm) for seed germination. The second substrate was similar to the first one except that we added slow release fertilizer or solid fertilizer (N: P: K= 14:13:13, Osmocote, The Scotts Company, USA), with the ratio of 20g fertilizer to 3kg substrate.

One seed was sown in each cell. After sowing, the boxes were covered with a transparent top and placed in a climate room (20^{o}C). The sowed seeds were watered by a small sprayer. When seedlings appeared (3 – 6 days after sowing), we brought them to a greenhouse at Hubei Academy of Forestry to be supplied much sunlight. At the time the seedlings had 2 – 4 true leaves, they were transferred to bigger pots (size: 8 × 8 × 9 cm) and filled with the second substrate prepared as described above. To help seedlings grow well before they could absorb the nutrients from the solid fertilizer, they were supplied with a solution of liquid fertilizer (4 drops/week) during the first three weeks and were watered by a small sprayer. In the greenhouse, seedlings in pots were arranged by populations. When the plants had reached a certain size, five blocks were formed based on the size of seedlings. Each block had 12 individual plants of which each individual came from a different population. The individuals in each block were similar in sizes and randomly arranged in the blocks.

2.4. Plant harvesting and measurement

When plants were beginning to bloom, their capitula were harvested and kept in a freezer at -78^{o}C. A week later, most of the plants had flowered. We harvested all plants and measured their vegetative and reproductive traits (height, fresh and dry weight of shoots and roots, total number of leaves, and number of flowers and buds). The shoots and roots were separated by scissors at their root crowns. The shoots were cleaned under a tap water, and the roots were rinsed with water. They were dried by tissue papers before their fresh weights were separately measured by using a scale (Adventure^{TM} OHAUS). These samples were separately kept in plastic bags in liquid nitrogen before they were kept in a freezer at -78^{o}C. After that the samples were dried with a freeze – dry machine. Their dry weights were measured by another scale (METTLER

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TOLEDO) due to its high sensitivity to low weight. The dry shoots and roots of five blocks were ground into fine powders, and then approximately 10 mg of the powder of shoots and roots were separately prepared in 2 ml – ependofs. The prepared materials were stored in -20°C until PAs extraction.

2.5. PAs extraction and analysis

Approximately 10 mg of the fine powdered plant material was extracted with 1 ml 2% formic acid solution. Heliotrine was added as internal standard to the extraction solvent at a concentration of 1 μg ml^{-1}. The plant extract solution was shaken for 1 hour. Solid plant material was removed by centrifugation at 2600 rpm for 10 min and filtered through a 0.2 μm nylon membrane (Acrodisc 13 mm syringe filter). An aliquot of the filtered solution (25 μl) was diluted with water (975 μl) and 5 μl was injected in the Liquid chromatography – tanderm mass spectrometry (LC-MS/MS) system. All these steps were the same as described by Cheng et al (2011) (Cheng et al., 2011a).

2.6. Data analysis

2.6.1. Analysis of PAs qualitative variation

We found 22 PAs, including 8 PAs were still not identified clearly. Hence, we paid attentions to the 14 PAs identified. All PAs identified in this study were senecionine – type PA and seneciphylline – type PA, which belong to senecionine – like PA (Cheng et al. 2011a) (Table 2, Figure S2). The present frequency of each individual PA in each range or population was calculated as follows: (total samples from each range or population that the individual PA was identified) / (total samples studied in each range or population) × 100 (%). The differences in the present frequencies of individual PAs between ranges were tested using independence Chi – square test.

2.6.2. Analysis of PAs quantitative variation

We defined each individual PA and each group of PAs as a separate dependent variable. Only 8 individual PAs which occurred in all studied samples were analyzed for PA quantitative variation. We tested for normal distribution and homogeneity of variance among populations and among ranges using Shapiro – Wilk normality test and Bartlett's test of homogeneity of variances, respectively. The two tests showed that only 4 variables (concentrations of *N* – oxides PA, total PA, total senecionine – type PA and senecionine *N* – oxide) met the assumptions of ANOVA test. We ignored the level of mother plants because some mother plants had only one replication. We applied one – way ANOVA to test the difference in the concentrations of those

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groups of PAs and the individual PAs between all populations. Interestingly, there were not significantly different in these variables between all populations. We therefore applied one – way ANOVA to test the difference in concentrations of these PAs between ranges.

The other variables that did not meet assumptions of ANOVA test were tertiary amines PA concentration, total seneciphylline – type PA concentration, and concentrations of the following individual PAs: integerrimine, integerrimine *N*-oxide, senecionine, seneciphylline, seneciphylline *N*-oxide, spartioidine and spartioidine *N*-oxide. Fortunately, they met the assumptions of Kruskal – Wallis (KW) test. We therefore tested for differences in concentrations of these individual PAs between populations and between ranges using Kruskal – Wallis tests (KW tests).

All analyses were conducted in R version 3.0.2 (R Core Team 2014).

3. Results

3.1. PAs qualitative variation

PAs qualitative variation between ranges

All 14 PAs identified were grouped into two subgroups (senecionine – type PA and seneciphylline – type PA), which belong to senecionine – like PA (Cheng et al. 2011a). All of them are both tertiary amines PAs and *N* – oxides PAs. Interestingly, we detected senecivernine and usaramine *N* – oxide, but their corresponding forms, senecivernine *N* – oxide and usaramine, were absent in all studied samples (Table 2).

Usaramine *N* – oxide which was found in the native *S. vulgaris* plants (3.33% samples), but it was absent in the invasive plants. Except usaramine *N* – oxide, all of the other PAs detected in the native plants were also occurred in the invasive plants. Retrorsine, retrorsine *N* – oxide, riddelliine and riddelliine *N* – oxide showed higher present frequencies in the native plants than in the invasive ones; except the present frequency of senecivernine was lower in the native plants. However, only present frequency of riddelliine *N* – oxide was significantly different between ranges (independence Chi – square tests, df = 1, p = 0.004). This individual PA presented in plants from the native range with higher frequency (90%) than in plants from the invasive ranges (46.67%).

The 12 individual PAs: senecionine, senecionine *N* – oxide, integerrimine, integerrimine *N* – oxide, retrorsine, retrorsine *N* – oxide, senecivernine, riddelliine, seneciphylline, seneciphylline *N* – oxide, spartioidine, spartioidine *N* – oxide, were not significantly different in present

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frequencies between ranges (independence Chi – square tests for all 12 PAs, df = 1, $p > 0.05$; Table 2).

PA qualitative variation between populations

From the 14 individual detected PAs, the present frequencies of 6 individual PAs (riddelliine, riddelliine *N* – oxide, senecivernine, usaramine *N* – oxide, retrorsine and retrorsine *N* – oxide) range from 0 – 100% in all populations. Especially, riddelliine was only detected in population Eu6 and Ch4 with 40% and 20% present frequencies, respectively; and usaramine *N* – oxide was only detected in population Eu6 with 20% present frequency. The other 8 individual PAs were detected in all populations with 100% present frequencies (Table 3).

3.2. PAs quantitative variation

PAs quantitative variation between ranges

We analyzed quantitative difference in the concentration of 8 individual PAs, which occurred in all studied samples (Table 2), and total concentration of 5 groups of PAs: total PAs, tertiary amines PAs, *N* – oxides PAs, senecionine – type PA and seneciphylline – type PA.

PA quantitative variation between populations

We found that most of all the mean of the PA concentrations tended to be higher in plants from the native range than those from the invasive range, except the concentration of senecionine tended to be higher in plants from the invasive range. There were no significant differences in the total concentrations of all PAs, *N* – oxides PAs, tertiary amines PAs, senecionine – type PA, and concentrations of 5 individual PAs (senecionine *N*-oxide, integerrimine, integerrimine *N* – oxide, senecionine, seneciphylline) between ranges (ANOVA or KW tests; in all cases: df = 1, 58; $p > 0.05$; Table 4), but significant differences were found for the concentration of seneciphylline – type PAs and 3 individual PAs (seneciphylline *N* – oxide, spartioidine, spartioidine *N* – oxide) between ranges (ANOVA or KW tests, in all cases: df = 1, 58; $p < 0.05$; Table 4).

The concentrations of integerrimine *N* – oxide, seneciphylline *N* – oxide, spartioidine, spartioidine *N* – oxide and total concentration of seneciphylline – type PA were significantly different between populations (KW test, in all cases: df = 11, 48; $p < 0.05$, Table 4). The concentrations of other individual PAs and groups of PA were not significantly different between populations (ANOVA or KW test, in all cases: df = 1, 48; $p > 0.05$; Table 4).

4. Discussion

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Compare to the previous works, no novel PAs was detected in the native or invasive *S. vulgaris* plants. Although the PAs of *S. vulgaris* have been extensively studied (Hartmann & Zimmer 1986; Toppel & Hartmann 1986; Pieters & Vlietinck 1988; Cano et al. 2009; Liu et al., 2010; Xie et al., 2010), to our knowledge this study was the first one which compared the PA concentration and composition between the native and invasive *S. vulgaris* plants.

Some previous studies found senecionine PA (Hartmann & Zimmer 1986), or seneciphylline PA to be the dominant (Lüthy et al. 1983), while some others found both the two PAs to be considered as main PAs in *S. vulgaris* (Borstel et al. 1989; Brown & Molyneux 1996; Frischknecht et al. 2001). In this study, we found senecionine PA, not seneciphylline, to be the main PA for plants from both the native and invasive ranges because the concentration of senecionine PA detected in the native and invasive plants were higher than the concentration of seneciphylline PA 4.37 and 7.09 fold, respectively. The same as findings by previous studies (Hartmann & Zimmer 1986; Hartmann & Toppel 1987; Borstel et al. 1989), this study also found PAs occurred in two forms: tertiary amines and *N* – oxides. The *N* – oxides PA concentration detected in the native and invasive *S. vulgaris* plants were higher than the tertiary amines PA concentration 9.26 and 8.24 fold, respectively. Thus, in this study *N* – oxides PAs were dominant in all the native and invasive *S. vulgaris* plants.

The limitation of this study is that a number of individual PAs were not detected in this study while they were reported in other studies such as vulgrarine which was detected as a new PA in *S. vulgaris* from northeast China (Xie et al. 2010), neoplatyphylline, platyphylline, neosenkirkine (Yang et al. 2011), etc. We do not know whether the vulgarine, neoplatyphylline, platyphylline, and neosenkirkine were absent in all studied samples or they were in the 8 unknown PAs. However, the most important is that we detected and compared the concentrations of total PA, tertiary amines PA and *N* – oxides PA which could be considered as the overall PA from the native and invasive samples were detected and compared. Thus, the results found in this study are believable.

The present frequencies of 6 individual PAs (retrorsine, retrorsine *N* – oxide, riddelliine, riddelliine *N* – oxide, senecivernine and usaramine *N* – oxide) range from 0 – 100% in the studied populations (Table 3). And the concentrations of integerrimine *N* – oxide, seneciphylline *N* – oxide, spartioidine, spartioidine *N* – oxide were significantly different between populations. The results indicate that the PA composition and concentration were different between *S. vulgaris* populations. The findings consist with previous studies when comparing the

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composition of secondary metabolites between different populations of plants. For instance, the diversity of essential oil was found in different populations of *Thymus vulgaris* (Thompson et al., 2003) or the concentration and diversity of PA was found in *J. vulgaris* plants from different populations (Lin 2015), etc. Those previous studies demonstrate that the concentration and diversity of secondary metabolites between populations is very popular.

The study found that present frequencies of PAs in the invasive *S. vulgaris* plants tended to be lower than those in the native ones. Especially, usaramine *N* – oxide was only detected in the native *S. vulgaris* plants; and the present frequency of riddelliine *N*-oxide was significantly higher in the native *S. vulgaris* plants (Table 2). The results indicate that the PA composition might be different between the native and invasive plants. The invasive *S. vulgaris* plants tended to produce less diversity of PAs than the native ones. Our results supported the findings by Joshi & Vrieling (2005) and Lin (2015) that the native *J. vulgaris* plants expressed more diversity of PA than the native ones (Joshi & Vrieling 2005; Lin 2015). Castells et al (2014) also found that diversity of PA in *S. pterophorus* (from South Africa) reduced after invasion (Castells et al., 2014). However, the results do not support our prediction and the prediction of the SDH that the invasive plants produce more diverse of PAs than the native ones. In fact, some previous studies supported that plants from invasive populations contain more composition of qualitative compounds than the native ones, such as the case of invasive *Tanacetum vulgare* plants (Wolf et al., 2011).

We also found a trend that the invasive *S. vulgaris* plants might produce lower concentrations of individual PAs than the native plants. These results do not support again our prediction and the predictions of the SDH (Müller-Scharer et al. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011) that the concentrations of PAs are higher in invasive plants. Indeed, results from many previous experiments support the predictions. They compared PA variation between native and invasive plants and reported that the PA levels significantly increased in the invaded area (Doorduin & Vrieling 2011; Wolf et al. 2011). For example, invasive populations of *S. inaequidens*, *S. pterophorus* (Cano et al. 2009), *S. pterophorus* (Castells et al. 2014) and *S. jacobaea* (Joshi & Vrieling 2005; Stastny et al., 2005; Lin 2015) showed significantly higher total PA concentration than their native populations.

In fact, some invasive species evolved towards decreased defense levels, they may develop other compensative mechanisms that contribute to the invasion success. For instance, invasive genotypes of *Sapium sebiferum* evolved to reduced their defense and resistant ability, but the

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compensative mechanisms that contribute to their invasion success were more tolerant and outperformed than the native genotypes under higher levels attack (Siemann & Rogers 2003; Rogers & Siemann 2004; Zou et al., 2008). This study found evidences support that the qualitative defense slightly reduced in the invasive *S. vulgaris* plants; while our previous study demonstrated that the invasive *S. vulgaris* plants grew and reproduced better than those from the native range (unpublished data). Therefore, we could deduce that the better performance of invasive *S. vulgaris* plants could be considered as a compensatory mechanism contributed to their invasion success. And it is also suggested that a little trade – off between resource allocation to growth/ reproduction and defense could be happened in invasive plants. This agrees with the study by Messina (Messina et al., 2002) who found that fast – growing populations was less defense than the slow – growing populations.

5. Conclusions

In conclusion, the native and invasive *S. vulgaris* plants were grown in the identical conditions, thus the differences in concentration of some individual PAs and PA composition between ranges and between populations could be explained by the genetic variation between ranges as well as between populations. The study found a pattern that the invasive *S. vulgaris* plants evolved towards less diverse and lower concentrations of some individuals PAs (qualitative chemical defenses) compared to the native ones. This finding is contrast to our predictions and the predictions of the SDH that the invasive plants would produce more diverse and concentration of qualitative defense than the native ones (Müller-Scharer et al. 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011). And it is probably an evidence that a little trade – off between defense and growth happened to the invasive *S. vulgaris* plants in China.

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Table 1. Sites of origin of native and invasive populations of *Senecio vulgaris*

Range	Country	Source population	Population	Coordinates	Collected year
Native	Spain	Barcelona	Eu1	41º40'28.96"N, 02º43'54.34"E	2012
	Poland	Poland	Eu2	51º23'58.91"N, 21º57'34.88"E	2012
	Scotland	St. Andrews	Eu3	56º19'49.57" N, 02º47'1.32" W	2012
	Switzerland	Marly	Eu4	46º47'8.64"N, 07º9'6.11"E	2012
	Portugal	Óbidos	Eu5	39°21'29"E, 9°9'28".W	2013
	Germany	Potsdam	Eu6	52°24'0", 13°4'0.0	2012
Invasive	China	Shennongjia	Ch1	109° 59' 42", 31° 29' 9"	2012
		Dalian	Ch2	38º52.193"N, 121º33.284"E	2012
		Lashihai-Lijiang	Ch3	26° 54.195", 100° 8. 87"	2012
		Shennongjia	Ch4	31° 27' 35", 110° 24' 03"	2012
		Xianghelu-Lijiang	Ch5	26° 52. 125", 100° 14. 245"	2012
		Dali	Ch6	26° 42' 19", 100° 09' 05"	2012

Table 2. Individual pyrrolizidine alkaloids (PAs) and their present frequencies detected in native and invasive *Senecio vulgaris* plants.

Sub structural group	PA	Code	Present frequency of individual PA (%)	
			In native plants	In invasive plants
senecionine type	senecionine	sn	100	100
	senecionine *N* – oxide	snox	100	100
	integerrimine	ir	100	100
	integerrimine *N* – oxide	irox	100	100
	retrorsine	rt	100	96.67
	retrorsine *N* – oxide	rtox	100	96.67
	senecivernine	sv	30	33.33
	usaramine *N* – oxide	usox	3.33	0
seneciphylline type	riddelliine	rd	6.67	3.33
	riddelliine *N* – oxide	rdox	90	46.67
	seneciphylline	sp	100	100
	seneciphylline *N* – oxide	spox	100	100
	spartioidine	st	100	100
	spartioidine *N*-oxide	stox	100	100

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Table 3. Present frequencies (%) of individual pyrrolizidine alkaloids (PAs) detected in all *Senecio vulgaris* poulations (Pop)

PAs / Pop	ir	irox	sn	snox	sp	spox	st	stox	rt	rtox	rd	rdox	sv	usox
Eu1	100	100	100	100	100	100	100	100	100	100	0	80	20	0
Eu2	100	100	100	100	100	100	100	100	100	100	0	80	0	0
Eu3	100	100	100	100	100	100	100	100	100	100	0	80	80	0
Eu4	100	100	100	100	100	100	100	100	100	100	0	100	0	0
Eu5	100	100	100	100	100	100	100	100	100	100	0	100	80	0
Eu6	100	100	100	100	100	100	100	100	100	100	40	100	0	20
Ch1	100	100	100	100	100	100	100	100	100	100	0	80	100	0
Ch2	100	100	100	100	100	100	100	100	80	100	0	40	0	0
Ch3	100	100	100	100	100	100	100	100	100	100	0	60	20	0
Ch4	100	100	100	100	100	100	100	100	100	100	20	80	80	0
Ch5	100	100	100	100	100	100	100	100	100	80	0	20	0	0
Ch6	100	100	100	100	100	100	100	100	100	100	0	0	0	0

Explanation for variables: **Eu1 – 6**: populations from Europe, **Ch1 – 6**: populations from China (See Table 1 for more details). **ir**: integerrimine, **irox**: integerrimine *N* – oxide, **sn**: senecionine, **snox**: senecionine *N* – oxide, **sp**: seneciphylline, **spox**: seneciphylline *N* – oxide, **st**: spartioidine, **stox**: spartioidine *N* – oxide, **rd**: riddelliine, **rdox**: riddelliine *N* – oxide, **rt**: retrorsine, **rtox**: retrorsine *N* – oxide, **sv**: senecivernine, **usox**: usaramine *N* – oxide.

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Table 4. Results of one – way ANONA or Kruskal – Wallis (KW) tests and mean values ± SE for concentrations of pyrrolizidine alkaloids (PAs, μg/g dry weight) measured from the native and invasive *Senecio vulgaris* plants.

Sub structural group	PA	Code	Mean ± SE of PAs concentration		Populations (df = 11, 48)	Ranges (df = 1, 58)
			In native plants	In invasive plants	F value or KW chi – squared	F value or KW chi – squared
Senecionine – type PA	senecionine	sn[k]	155.13±19.96	159.28±21.01	8.002	002
	senecionine *N* – oxide	snox[a]	1378.28±151.63	1257.78±144.81	1.585	0.33
	integerrimine	ir[k]	27.09±3.52	27.07±3.78	11.641	0.008
	integerrimine *N* – oxide	irox[k]	316.92±45.11	286.45±39.52	20.963*	0.184
	retrorsine	rt[n]	8.24±3.26	2.29±0.47		
	retrorsine *N* – oxide	rtox[n]	82.25±25.89	19.36±2.79		
	senecivernine	sv[n]	2.73±0.92	1.33±0.46		
	usaramine *N* – oxide	usox[n]	0.53±0.53	0.00±0.00		
Seneciphylline – type PA	riddelliine	rd[n]	0.31±0.29	0.03±0.03		
	riddelliine *N* – oxide	rdox[n]	4.48±1.98	0.69±0.23		
	seneciphylline	sp[k]	33.83±5.44	21.98±2.93	10.117	1.973
	seneciphylline *N* – oxide	spox[k]	317.11±52.91	177.76±26.88	19.994*	4.853*
	spartioidine	st[k]	5.54±0.87	3.47±0.55	20.363*	3.984*
	spartioidine *N* - oxide	stox[k]	57.96±10.03	33.84±6.92	25.703**	4.723*
Sum concentration of	total PAs	total[a]	2390.41±260.09	1991.33±229.45	1.695	1.324
	tertiary amines PA	fb[k]	232.87±29.08	215.45±27.37	7.299	0.148
	N – oxides PA	ox[a]	2157.54±238.58	1775.88±208.20	1.778	1.453
	Senecionine – type PA	tsn[a]	1971.18±211.18	1753.56±201.36	1.677	0.556
	Seneciphylline – type PA	tsp[k]	419.23±66.16	237.77±35.93	20.377*	5.807*

Explanation: [a] the variables were analyzed using one – way ANOVA test, [k] the variables were analyzed using Kruskal – Wallis test, and [n] the variables were not analyzed by statistic tests. **ox**: *N* – oxide PAs, **total**: total PA, **tsn**: senecionine – type PA, **snox**: senecionine *N* – oxide, **tsp**: seneciphylline – type PA, **fb**: tertiary amine PAs, **ir**: integerrimine, **irox**: integerrimine *N* – oxide, **sn**: senecionine, **sp**: seneciphylline, **spox**: seneciphylline *N* – oxide, **st**: spartioidine, **stox**: spartioidine *N* – oxide, **rd**: riddelliine, **rdox**: riddelliine *N* – oxide, **rt**: retrorsine, **rtox**: retrorsine *N* – oxide, **sv**: senecivernine, **usox**: usaramine *N* – oxide.

Level of significance: *p < 0.05, **p < 0.01, ***p < 0.001.

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Supplementary Materials

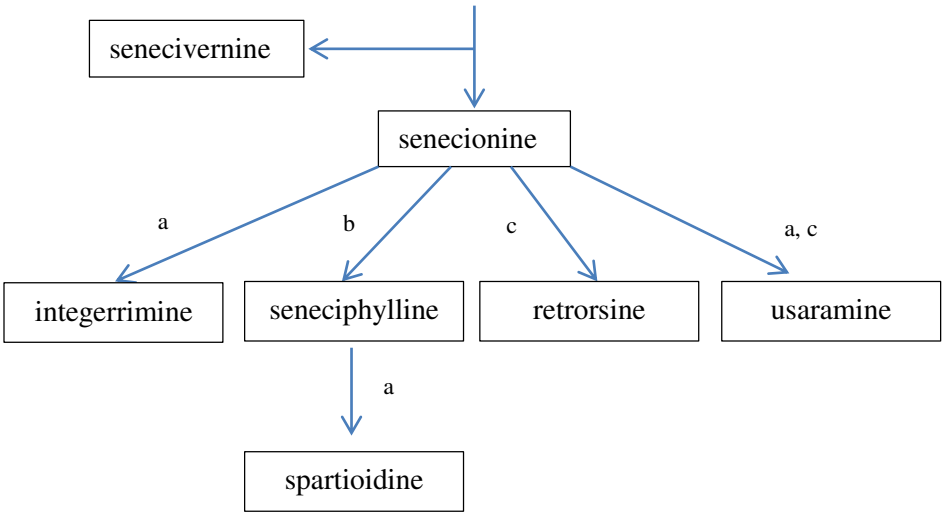

Figure S1. The formation of pyrrolizidine alkaloids from Senecionine. With the exception of senecivernine, senecionine is the common precursor of all other PAs. Further structural diversifiation requires three simple one – step – reactions marked by letters a – c: a = Z/E – isomerization at C20; b = 13, 19 – dehydrogenation; c = site – specific hydroxylations. Adapted from Pelser et al (2005) (Pelser et al. 2005).

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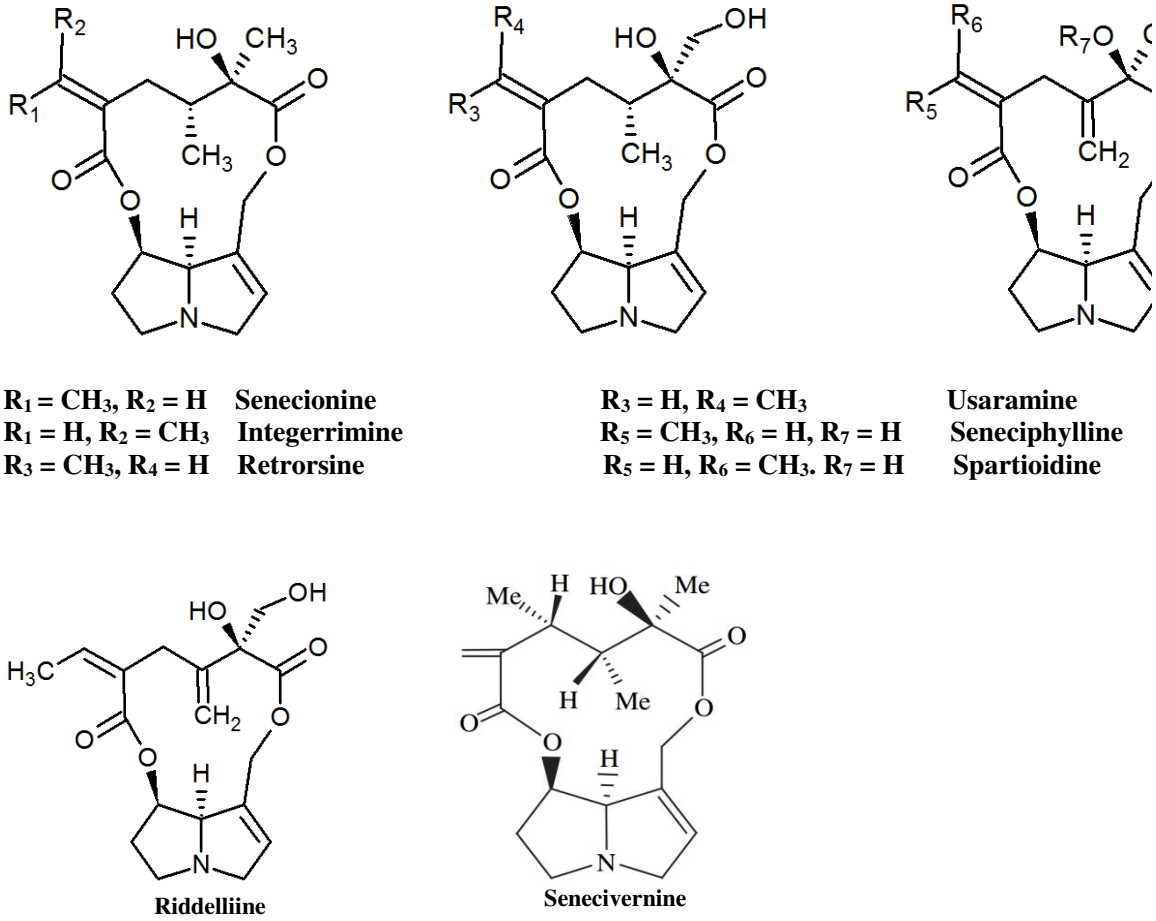

R_1 = CH_3, R_2 = H **Senecionine**
R_1 = H, R_2 = CH_3 **Integerrimine**
R_3 = CH_3, R_4 = H **Retrorsine**
R_3 = H, R_4 = CH_3 **Usaramine**
R_5 = CH_3, R_6 = H, R_7 = H **Seneciphylline**
R_5 = H, R_6 = CH_3. R_7 = H **Spartioidine**

Riddelliine

Senecivernine

Figure S2. Chemical structures of the pyrrolizidine alkaloids (PAs) detected from native and invasive *Senecio vulgaris* L. in this study. Adapted from Dominguez et al (2008) and Cheng (2011) (Dominguez et al., 2008; Cheng 2012).

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