Evolutionary Ecology of Senecio

Evolutionary ecology

The primary focus of evolutionary ecology is to identify and understand the evolution of key traits, by which plants are adapted to their environment, and to understand how biological variation is generated and maintained.

We, therefore, need to know how biotic and abiotic environmental factors influence:

1] The transition from genotype to phenotype;

2] The genetic and developmental constraints that play a role in generating the phenotype; and

3] How (combinations of) trait values translate to differences in success of phenotypes.

Evolutionary Ecology of Senecio

As a tool we need:

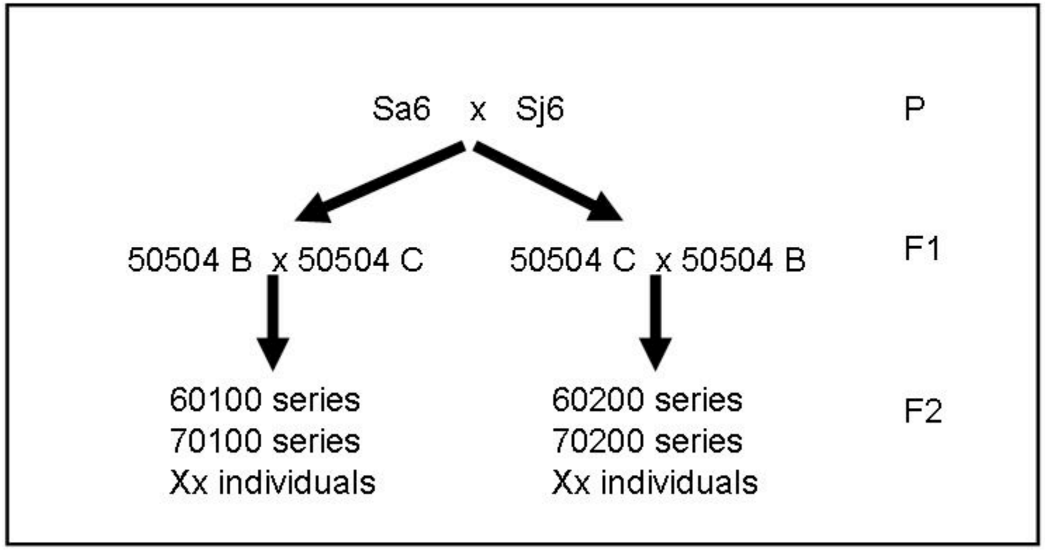
1. Study systems with well-known ecology
2. Sufficient genetic variation in relevant trades
3. The possibility to do ecological experiments
4. The possibility to use well-defined genotypes

Evolutionary Ecology of Senecio

The cross:

- A rayless *Jacobaea vulgaris* subs *dunensis* (individual from the dune area near The Hague (The Netherlands)) was crossed with a rayed *Jacobaea aquatica* subs *aquaticus* individual from the nature reserve Het Zwanenwater (The Netherlands).
- The cross resulted in numerous seeds which were germinated. Two rayed individuals were chosen and crossed reciprocally which each other resulting in two sets of offspring.
- The parental, F1 and 138 F2 individuals are maintained in tissue culture.
- For experiments parental species, F1 and F2 can be cloned to perform experiments with the same genotypes.
- For high resolution mapping the number of F2 plants can be extended endlessly, as both F1's are in tissue culture.

The approach



Molecular data

SNP Discovery

Transcriptome sequenced with Illumina platform

De novo assembly and aligning against *S. vulgaris* EST database

16000 SNPs for *J. vulgaris*

11000 SNPs for *J. aquatica*

30000 - 35000 SNPs between species

We will construct a genetic map and locate all measured ecological and metabolomic traits and e.g. indentify markers for herbivore resistance and metabolites that coincide

Traits that have been measured

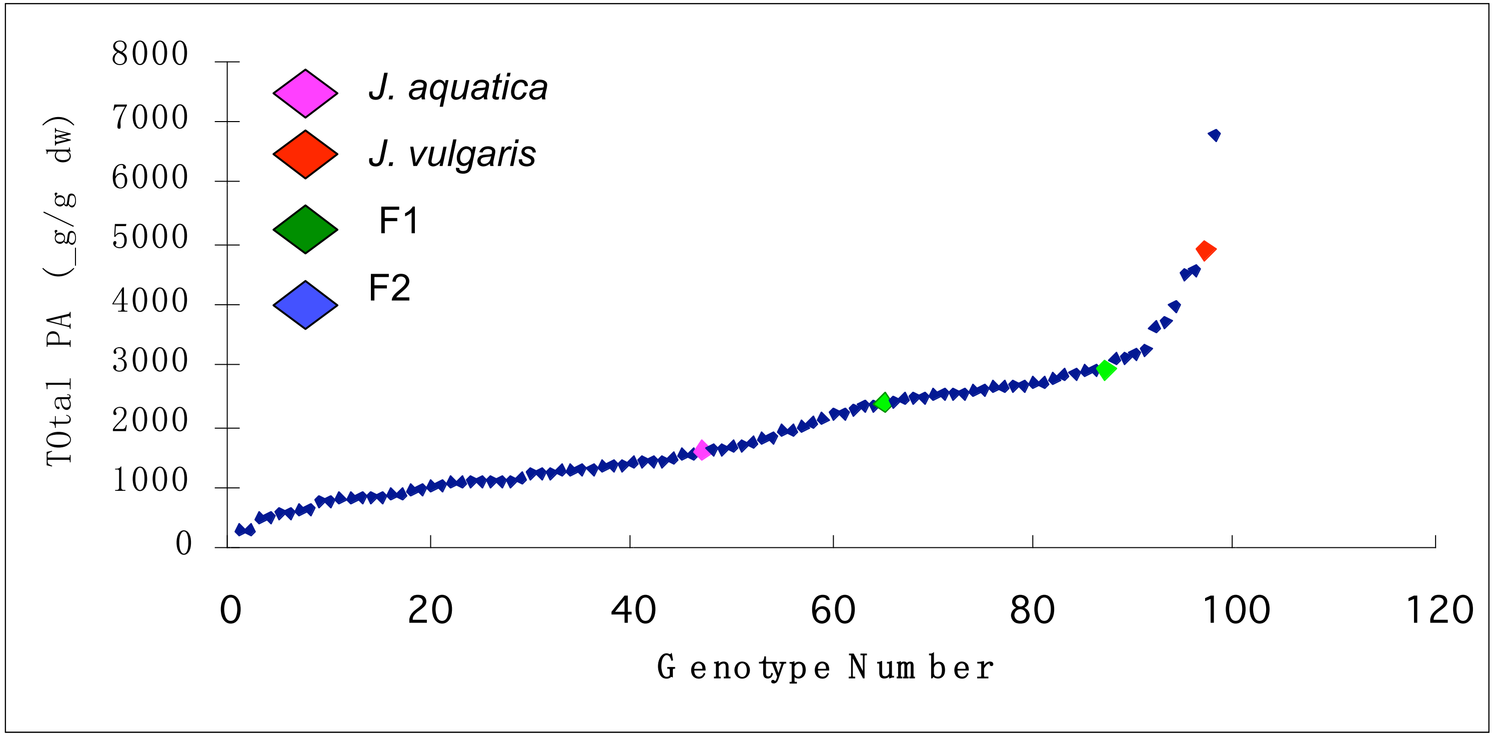
- Growth in the lab
- Growth and survival in the parental habitats
- Life-history in a common garden
- Flower type
- Flooding resistance (40 genotypes)
- Thrips resistance
- Alkaloid patterns ((LC-MS/MS)
- Metabolomic profiles (NMR and LC-MS)

General Results

- For all traits we found a strong genetic component (genotype effect)
- For (nearly all traits) we found transgressive segregation

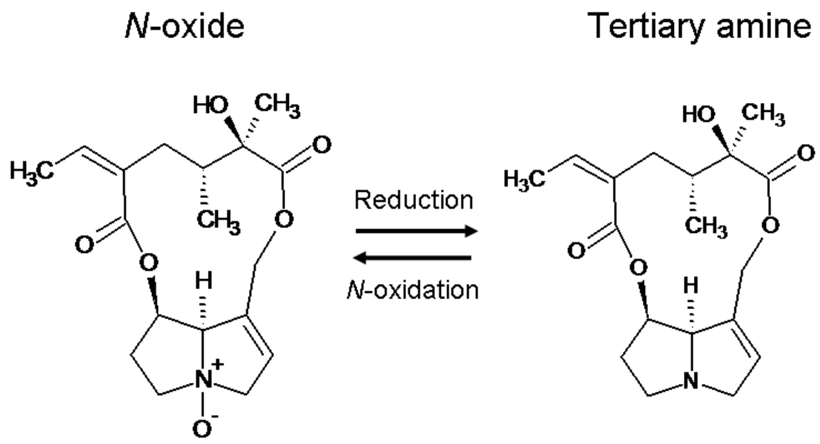
PA measurements for the shoots

- In total 37 different PAs: 14 N-oxides and 23 tertiary amines.
- The concentrations of the individual PAs were genotype dependent (KW, df=97, n=569, for all 37 PAs: $P<0.05$).
- Transgressive segregation occurred for the concentration of most PAs.
- No new alkaloids were found, however new combinations were found



Alkaloids

- Alkaloids occur in free base (tertiary amines) and N-oxide form
- The ratio is genotype dependent and differs for individual PAs
- Within the major PAs especially jacobine type PAs occur in shoots mainly as free base whereas the other types occur nearly fully as N-oxide
- In roots all types occur nearly fully as N-oxide
- Some minor PAs (otosenine type) are only present as free base
- Among genotypes correlations between the ratios freebase/ N-oxide are higher within groups of structurally related PAs



PAs in the shoots : 4 structural types

Senecionine, Senecionine N-oxide
Intergerrimine, Intergerrimine N-oxide
Retrorsine, Retrorsine N-oxide
Usaramine, Usaramine N-oxide
Riddelline, Riddelline N-oxide
Seneciphylline, Seneciphylline N-oxide
Spartiodine, Spartiodine N-oxide
Acetylseneciphylline,
Acetylseneciphylline N-oxide

Jacobine, Jacobine N-oxide
Jacoline, Jacoline N-oxide
Jaconine, Jaconine N-oxide
Jacozine, Jacozine N-oxide
Dehydrojaconine

Erucifoline, Erucifoline N-oxide
Acetylerucifoline
Acetylerucifoline N-oxide

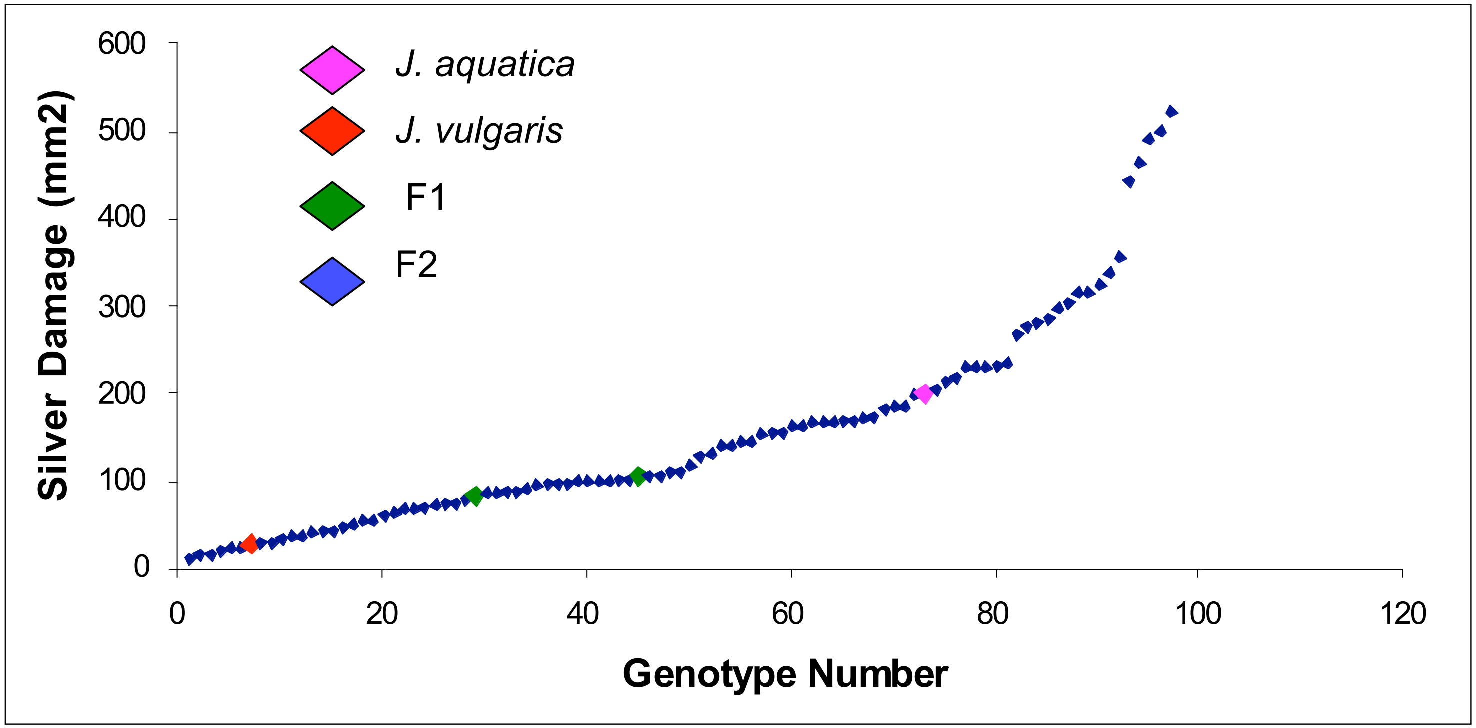
Otosenine
Senecivernine
Senkirkine
Onetine
Desacetyldoronine
Florosenine
Floridanine
Doronine

Thrips bioassay

Anova showed that the silvery damage was genotype dependent.

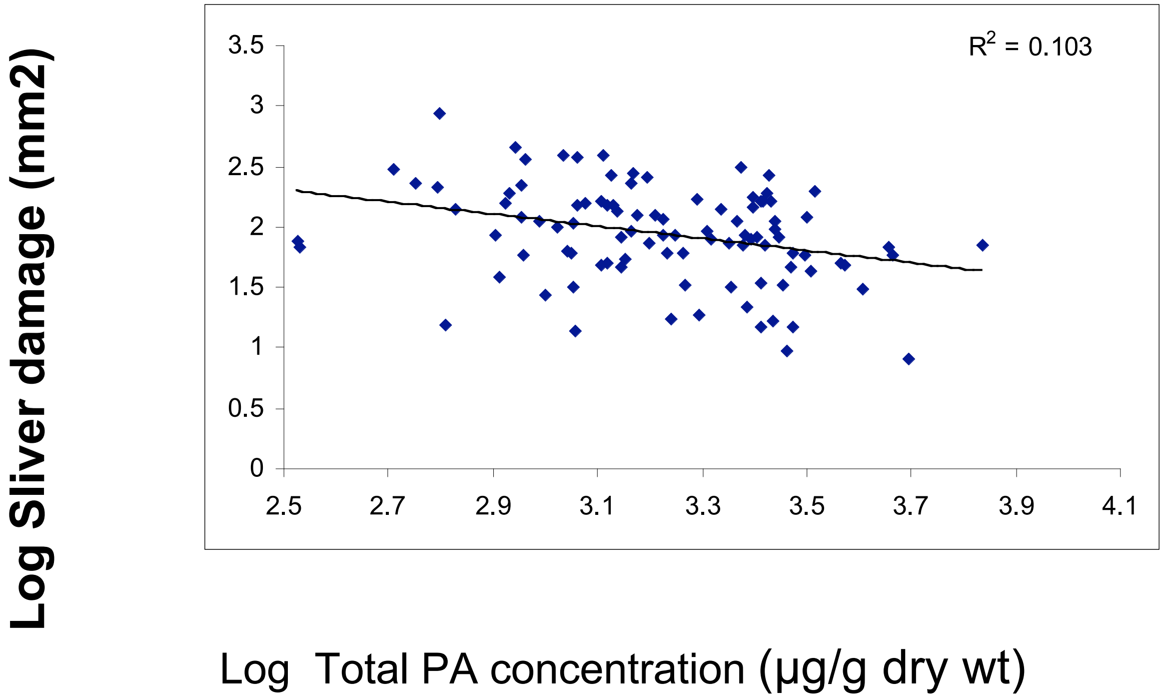
($F_{97,586}$= 5.822, $p < 0.001$)

(silver damage was log transformed and dry mass of the shoots was used as a covariate)



Correlation between PAs and thrips damage

PA concentrations and silver damage are means per genotype



Correlations between the major PAs and thrips damage

PA	p-value	Bonferroni p-value
Total PA	0.001**	0.013*
Jacobine	0.001**	0.012*
Jacobine N-oxide	0.001**	0.009**
Jacoline	0.000**	0.008**
Jacoline N-oxide	0.000**	0.007**
Jaconine	0.001**	0.016*
Jaconine N-oxide	0.001**	0.009**
Jacozine	0.035*	0.315
Acetylerucifoline	0.016*	0.189
Erucifoline	0.200	0.798
Acetylseneciphylline N-oxide	0.509	1.528
Erucifoline N-oxide	0.584	1.168

Pearson correlation between log (genotype mean silver damage per genotype) and log (genotype mean PA concentrations), df=98.

Correlations between the major PAs and thrips damage

Log PA Concentration	p-value	Bonferroni p-value
Senecionine	0.042*	0.252
Senecionine N-oxide	0.042 *	0.291
Intergerrimine	0.005**	0.064
Intergerrimine N-oxide	0.026*	0.257
Seneciphylline	0.018 *	0.203
Seneciphylline N-oxide	0.087*	0.437
Acetylseneciphylline	0.916	0.916
Acetylseneciphylline N-oxide	0.509	1.528

Pearson correlation between log (genotype mean silver damage per genotype) and log (genotype mean PA concentrations), df=98.

Preliminary results for genetic markers based on 180 AFLP markers

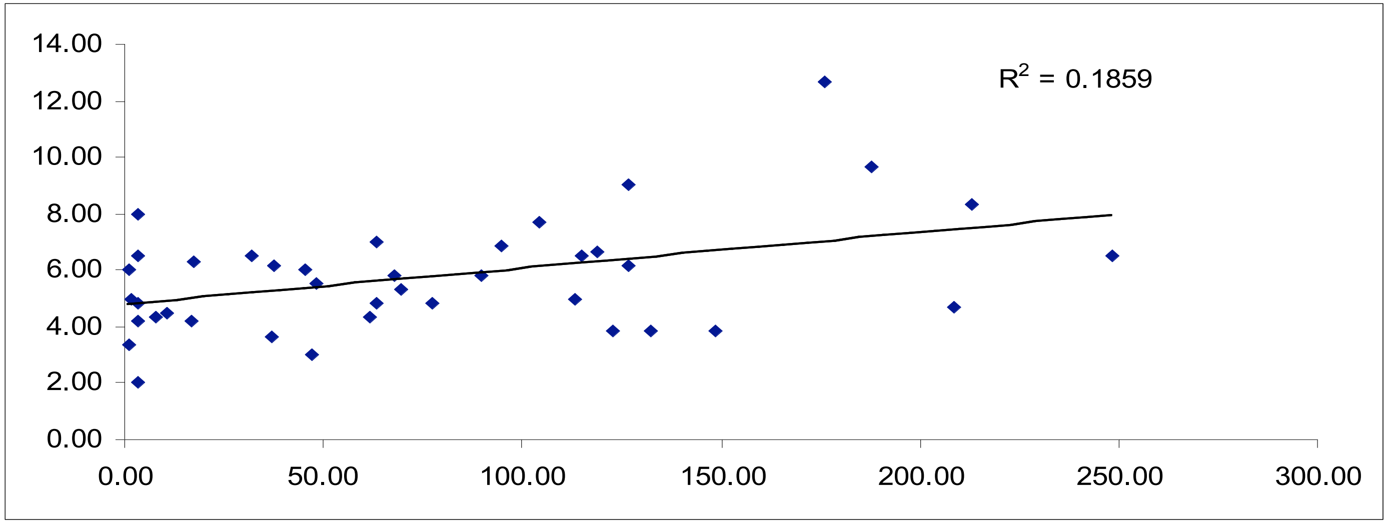
1. For both thrips damage (10) and jacobine (8) we detected a number of genetic markers.
2. 3 of them were associated with both.
3. In all cases, for the shared markers, the alleles for high jacobine concentrations are alleles associated with low thrips damage.

Why do not all plants have high PA (jacobine concentrations)

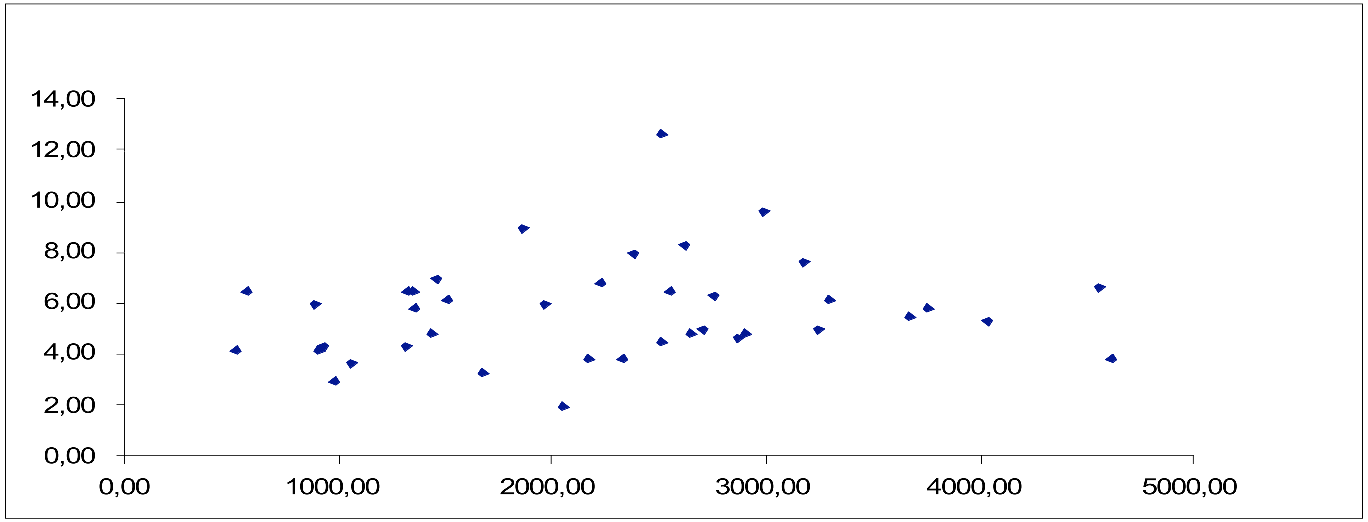
- PAs (Jacobine) are effective against an important generalist herbivore
- PAs are cheap to produce (most attempts to show any cost failed)

Relationship between oviposition by the specialist Tyria jacobaeae and alkaloid concentration

Mean no. of egg batches



Jacobine



Total PA

Genotype mean concentration (µg/g dry wt)

Conclusion

- Structurally related Pas have different effects on both specialist and generalist herbivores
- Oviposition was positively related to jacobine type alkaloids, but only with the free base form and not with N-oxides. Oviposition was not related to other type alkaloids
- Oviposition was not related to total PA concentration
- Thrips resistance was positively related to both forms of jacobine type alkaloids
- Genotypes that are well protected against the generalist thrips are attractive to the specialist Tyria (specialist/ generalist dilemma, van der Meijden 1996)

Outlook

- Further development of genetic map (SNPs)
- Mapping of QTLs for all traits
- Extension of trait database (flower morphology, drought resistance, flooding resistance, herbivore resistance, pathogen resistance, growth and survival in different habitats)
- Testing of evolutionary constraints
- Gene discovery with the aid of next generation sequencing (Proposals with the Beijing Genomic Institute and Trent University Canada)
- Making genotypes and data available for other researchers