

The effect of hybridization on secondary metabolites and herbivore resistance: implications for the evolution of chemical diversity in plants

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Abstract The diversity of secondary metabolites (SMs) has been poorly understood from both a mechanistic and a functional perspective. Hybridization is suggested to contribute to the evolution of diversity of SMs. In this paper we discuss the effects of hybridization on SMs and herbivore resistance by evaluating the literature and with special reference to our own research results from the hybrids between *Jacobaea vulgaris* (syn. *Senecio jacobaea*) and *Jacobaea aquatica* (syn. *Senecio aquaticus*). We also review the possible genetic mechanism which causes the variation of SMs and herbivore resistance in hybrids. Most SMs in hybrids are present in the parents as well. But hybrids may miss some parental SMs or have novel SMs. The concentration of

parental SMs in hybrids generally is constrained by that in parental plants, but transgressive expression was present in some hybrids. Hybrids may be as susceptible (resistant) as the parents or more susceptible than the parents, but rarely more resistant than the parents. However, different hybrid classes (F1, F2, backcrossing and mixed genotypes) show different patterns in relation to herbivore resistance. The variation in SMs and herbivore resistance occurring in hybrids could be explained by complicated genetic mechanisms rather than a simple one-gene model. Most previous work in this field only reported mean trait values for hybrid classes and few studies focused on genotype differences within hybrid classes. Our study in *Jacobaea* hybrids showed transgressive segregation in most SMs and herbivore resistance. To summarize, our article shows that hybridization may increase the variation of SMs and affect herbivore resistance, which may partially explain the evolution of chemical diversity in plants.

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Abbreviations

AFLP Amplified fragment length polymorphism
BC Backcrossing
dw Dry weigh of plant material
PA Pyrrolizidine alkaloid
QTL Quantitative Trait Locus
SM Secondary metabolite

Introduction

The role of hybridization with respect to the origin of biodiversity has been debated for over a century. Recent research indicates that hybridization plays a role in the generation of novel traits, transgressive segregation, introgression of traits between species and speciation itself (e.g. Stebbins 1959; Arnold 1992; Rieseberg and Carney 1998). Furthermore hybridization may contribute to the success of invasive species and cause the displacement and extinction of local species (Huxel 1999; Prentis et al. 2007). To elucidate the diversity in SMs and plant resistance from an ecological and evolutionary perspective, hybrids are regarded as a good study system (Fritz et al. 1999; Orians 2000). In this paper we will focus on the consequences of hybridization for the generation of qualitative and quantitative variation in secondary chemistry and the possible effects on hybrids fitness in relation to herbivore resistance. We asked the following questions: (1) Do hybrids have a greater qualitative or quantitative variation of SMs and herbivore resistance relative to the parental taxa? (2) Does this pattern differ between different classes of hybrids? (3) Is the variation in SMs and herbivore resistance determined by simple genetic mechanisms? From an evolutionary point of view it is important to know if among a hybrid population some individuals show more extreme trait values than either of the parental species. Unfortunately, most studies reported mean trait values for hybrid classes and only very few studies focused on genotype differences within hybrid classes. Finally, we therefore, will summarize the development of our research on *Jacobaea* hybrids as a case study with special attention on within hybrid genotype variation.

Hybridization occurs frequently in *Senecio* and *Jacobaea* species. The interaction of herbivorous insects and plants of these species in relation to the pyrrolizidine alkaloids (PAs) have been intensively studied (van der Meijden et al. 1989, 1992; Vrieling et al. 1991; Hartmann 1999; Macel et al. 2002, 2005; Leiss et al. 2009a).

The effect of hybridization on SMs

Qualitative variation of SMs in hybrids

Hybrids may: (1) express all of the SMs of the parental taxa; (2) fail to express certain parental SMs;

(3) express novel SMs that are absent in each parent or (4) express a parental SM in other tissues than the parents. The last two are regarded as novelty. Rieseberg and Ellstrand (1993) showed that, among 743 SMs identified in parents and their F1 hybrids of 21 genera from 24 studies, 68% were present both in the hybrids and their parents, 27% only occurred in parental plants, and 5% of the SMs were only found in the hybrids. The frequency of novelty was ca. 8% in later generation hybrids and even higher (ca.18%) in hybrid-derived species. The survey by Orians (2000) based on 31 studies including 80 hybrid crosses showed that at least one specific SM was missing in the hybrids in ca. 60% of the crosses and at least one novel SM was found in ca. 40% of the crosses. We extended the reviews of Rieseberg and Ellstrand (1993) and Orians (2000) by including 7 studies published from 2000 to 2010. From these studies we scored the presence and absence of SMs in different hybrid classes. The combined results show that 24.2% of the 1,112 SMs studied were not present in the hybrids, 5.5% were novel SMs only present in the hybrids and 70.3% were present in both the parents and hybrids (Table 1). Adding more studies did not change the results obtained by the previous review suggesting that the data are solid (Table 1, chi-square = 2.8, $df = 2$, $P = 0.24$).

Quantitative variation of SMs in hybrids

Orians (2000) divided the quantitative variation of the concentration of SMs in F1 hybrids relative to the parents in five categories: (1) higher than in either parent (O-overexpression); (2) intermediate between those of the two parental taxa (Im-intermediate expression); (3) lower than in either parent (U-underexpression); (4) similar to that in one of the two parental taxa (D-dominance expression); or (5) not different from either parent when the parents show similar concentrations (ND-no difference). From an evolutionary point of view, we think the fourth category (Dominance) should be divided to two subcategories: DI-dominance to the lower parent and Dh-dominance to the higher parent. A meta-analysis of 5 studies showed that among the 96 parental SMs present in the F1 hybrids, most are either expressed at concentrations similar to one of the parents or at intermediate concentrations (33 and 29%, respectively), some are over expressed (19%), while others occur at lower concentrations than in

Table 1 Qualitative and quantitative variation of the secondary metabolites (SMs) in hybrid plants

Taxa	SM	Hybrid class	Quantitative variation			Quantitative variation						Reference
			Missing SMs	Complementary SMs	Novel SMs	ND	Im	DI	Dh	U	O	
<i>Allium cepa</i> × <i>A. kermesinum</i> ^a	Cysteine sulfoxides and volatile sulfur compounds	F1	0	26	0	7	8	4	1	2	4	Storsberg et al. (2004)
<i>Corymbia citriodora</i> subsp. <i>variegata</i> × <i>C. torelliana</i> ^b	Hexane extracts of leaves	F1	17	29	5	0	7	1	2	0	4	Nahrung et al. (2009)
<i>Citrus meyeri</i> × <i>C. limon</i> cv. Cavone	Essential oils	F1	5	55	1	38	5	7	4	1	1	Verzera et al. (2005)
<i>C. medica</i> × <i>C. limon</i> cv. Cavone		F1	1	49	0	39	3	4	3	0	0	
<i>Eucalyptus globulus</i> ^c	Sideroxylonal and macrocarpal	F1(SN)	0	6	0	0	0	1	0	1	4	O'Reilly-Wapstra et al. (2005)
		F1(NS)	0	6	0	1	0	1	3	0	1	
<i>Eucalyptus grandis</i> × <i>E. camaldulensis</i> ^d	Essential oils	F1	14	10	3	0	7	1	0	1	1	Tolosa et al. (2008)
<i>E. grandis</i> × <i>E. tereticornis</i> ^d		F1	15	10	3	0	5	0	1	2	2	
<i>Quercus gambelii</i> × <i>Q. grisea</i> ^e	Ellagitannins	F1	0	10	0	0	5	0	0	5	0	Yarnes et al. (2008)
		BC(gr)	0	10	0	2	4	2	0	1	1	
		BC(ga)	0	10	0	3	4	1	1	1	0	
<i>Salix caprea</i> × <i>S. repens</i>	Phenolic glucosides	F1	0	7	0	0	2	4	1	0	0	Hallgren et al. (2003)
		F2	0	7	0	0	5	0	2	0	0	
		BC(c)	0	7	0	0	0	6	1	0	0	
		BC(r)	0	7	0	1	5	0	1	0	0	
Results previous reviews:(qualitative data from Rieseberg and Ellstrand 1993 and quantitative data from Orians 2000)			217	533	49	5	28	32 ^f	13	18		Rieseberg and Ellstrand (1993), Orians (2000)
Total			269	782	61	96	88	84 ^f	27	36		
Percentage			24.2%	70.3%	5.5%	29.0%	26.6%	25.4% ^f	8.2%	10.9%		

F1, *F2*, *BC* (pure species parents indicated by the first or first two letters), *ND* (no difference from both of the parents), *DI* (intermediate between the parents), *DI* (similar to the lower parent), *Dh* (similar to the higher parent), *U* (underexpression), *O* (overexpression)

^a The average concentration of individual SMs of 6 F1 hybrids

^b SMs appearing in >50% of hybrid individuals scored as present and those appearing <50% scored as missing

^c Intraspecies hybrids of two geographically distinct populations (races) of *E. globulus*; north-eastern Tasmania (N) and south-eastern Tasmania (S). The female parent is stated as the first letter in the cross, and the male parent is stated as the second letter in the cross

^d Mean concentration of every SMs reported

^e Natural hybrids, only samples collected in July

^f Combination of DI and Dh

either parent (14%, Orians 2000). We extended the review of Orians (2000) by including 7 studies published from 2000 to 2010. Most of these studies reported the mean concentration of the SMs for different hybrid classes with a statistical test which showed whether the hybrid classes and the parents were significantly different from one another. The combined results show that the parental SMs present in the hybrids were mostly expressed at concentrations similar to both of the parents, one of the parents, or at intermediate concentrations (30.8, 20.8 and 28.2%, respectively). The other SMs were over expressed (11.5%) or under expressed (8.7%, Table 1). Addition of the new studies showed that the number of over and under expressed SMs declined. These are the most interesting cases as values beyond the range present in the parents are reached, which can be subject to natural selection. However, as we will discuss later it would be more valuable to include individual variation among genotypes as well.

The effect of hybridization on herbivore resistance

Given the fact that the patterns of expression of SMs show large variation among different hybrid crosses it is expected that the patterns of resistance are highly diverse too. Fritz et al. (1994, 1999) produced a simple scheme analogous to the scheme of Orians (2000) for SMs, the only exception being that the dominant category was split into a category similar to the resistant parent and a category similar to the susceptible parent. They postulated that, resistance in hybrids may be: (1) higher than in either parent (the hybrid resistance hypothesis, R); (2) intermediate between both parents (the hybrid additive hypothesis, A); (3) lower than in either parent (the hybrid susceptibility hypothesis, S); (4) similar to the parent with the higher resistance (the hybrid resistance-dominant hypothesis, Dr); (5) similar to the parent with the lower resistance (the hybrid susceptibility-dominant hypothesis, Ds), or (6) not different from both parents (the hybrid no difference hypothesis, ND).

Fritz et al. (1999) surveyed 118 tests from 22 studies that compared responses of herbivore resistance to hybrids and parental plants and they found that the four most commonly reported responses of the hybrids to insect herbivores are: no difference,

additive, susceptibility-dominant and hybrid susceptibility while there was little support for the hybrid resistance hypothesis (Table 2). We added 12 studies published between 1999 and 2010, which reported herbivore resistance on 27 hybrid taxa in 168 tests. Herbivore resistance was evaluated by various methods such as plant damage, herbivore performance (density, growth rate, developmental time, weight, etc.) or herbivore preference. These new studies involved 7 genera, among which the most intensively studied ones being *Salix* (4 studies), *Eucalyptus* (3) and *Quercus* (2). The majority of studies were concerned with trees or shrubs and only two genera with herbs (*Ipomopsis* and *Myriophyllum*) were included. Among the 27 hybrids taxa involved in these studies, 11 were F1, 5 were F2, 8 were backcrosses and 3 were mixed genotypes. Most mixed hybrids were unspecified crosses from natural hybrids zones. We combined our own survey with that from the previous work (Fritz et al. 1999). The combined result shows support for the no difference hypothesis (22.0%) and the additive hypothesis (21.7%). The hybrid susceptibility hypothesis (22.4%) was more frequent supported than the hybrid resistance hypothesis (5.9%). Support for the resistance-dominant hypothesis (12.9%) is close to that of the susceptibility-dominant hypothesis (15.0%, Table 2). If expression of SMs would be related to herbivore resistance, one would have expected the pattern of quantitative variation of SMs may be similar to that of herbivorous resistance in hybrid plants. But the results of our survey do not support this assumption as the pattern of SM variation in hybrids is dissimilar to that of herbivore resistance (Fig. 1, Chi-square: 33.7, $df = 1$, $P < 0.001$, Ds and Dr pooled for D). For instance, hybrids are more often susceptible to herbivores than can be expected on basis of the frequency of under expression of SMs. Also the different hybrid classes (F1, F2, BC and MX) show different distribution patterns over the 6 hypotheses (Fig. 2, Chi-square: 49.9, $df = 4$, $P < 0.001$). Strikingly in the F2 class the resistant category is absent and the susceptible category is increased. The increase in the hybrid susceptibility might be related to hybrid breakdown in later generations (Czesak et al. 2004). Another explanation can be that the hybrids contain SMs in combinations that attract the total suit of herbivores present on one or both of the parental species.

Table 2 Survey of studies on plant resistance in hybrid plants

		Hypotheses supported						
		ND	A	Ds	Dr	S	R	
<i>Corymbia citriodora</i> × <i>C. torelliana</i>	F1	0	1	1	0	0	2	Nahrung et al. (2009)
<i>Eucalyptus globulus</i> ^a	F1	0	2	0	0	0	0	O'Reilly-Wapstra et al. (2005)
<i>Eucalyptus amygdalina</i> × <i>E. risdonii</i>	F1	0	0	0	0	4	0	Dungey and Potts (2003)
<i>Ipomopsis aggregata</i> × <i>I. tenuituba</i>	F1	1	0	0	1	1	0	Campbell et al. (2002)
<i>Myriophyllum spicatum</i> × <i>M. sibiricum</i>	F1	7	4	0	1	0	0	Roley and Newman (2006)
<i>Populus fremontii</i> × <i>P. angustifolia</i>	F1	0	0	0	0	2	0	McIntyre and Whitham (2003)
<i>Quercus gambelii</i> × <i>Q. grisea</i>	F1	0	2	0	3	0	0	Yarnes et al. (2008)
<i>Salix eriocephala</i> × <i>S. sericea</i>	F1	0	4	0	0	0	0	Czesak et al. (2004)
<i>Salix sericea</i> × <i>S. eriocephala</i>	F1	0	1	0	0	0	0	Fritz et al. (2001)
<i>Salix caprea</i> × <i>S. repens</i>	F1	2	0	0	0	0	0	Hallgren et al. (2003)
<i>Salix eriocephala</i> × <i>S. sericea</i>	F1	1	3	2	2	5	1	Hochwender and Fritz (2004)
<i>Eucalyptus amygdalina</i> × <i>E. risdonii</i>	F2	0	0	0	0	4	0	Dungey and Potts (2003)
<i>Ipomopsis aggregata</i> × <i>I. tenuituba</i>	F2	1	0	1	0	1	0	Campbell et al. (2002)
<i>Salix eriocephala</i> × <i>S. sericea</i>	F2	0	1	0	1	2	0	Czesak et al. (2004)
<i>Salix caprea</i> × <i>S. repens</i>	F2	0	0	0	0	2	0	Hallgren et al. (2003)
<i>Salix eriocephala</i> × <i>S. sericea</i>	F2	0	2	3	4	5	0	Hochwender and Fritz (2004)
<i>Salix caprea</i> × <i>S. repens</i>	BC(c)	1	0	0	0	1	0	Hallgren et al. (2003)
	BC(r)	1	0	0	0	1	0	
<i>Salix eriocephala</i> × <i>S. sericea</i>	BC(e)	0	3	0	0	1	0	Czesak et al. (2004)
	BC(s)	0	1	0	2	1	0	
<i>Salix eriocephala</i> × <i>S. sericea</i>	BC(e)	0	4	3	1	2	4	Hochwender and Fritz (2004)
	BC(s)	1	2	4	2	4	1	
<i>Quercus gambelii</i> × <i>Q. grisea</i>	BC(ga)	0	1	1	3	0	0	Yarnes et al. (2008)
	BC(gr)	0	1	0	3	0	1	
<i>Eucalyptus amygdalina</i> × <i>E. risdonii</i>	MX	9	2	6	6	7	0	Dungey et al. (2000)
<i>Ipomopsis aggregata</i> × <i>I. tenuituba</i>	MX	0	3	3	0	0	0	Campbell et al. (2002)
<i>Quercus crassifolia</i> × <i>Q. crassipes</i>	MX	0	2	0	0	0	0	Tovar-Sanchez and Oyama (2006)
Result from previous review		39	23	19	8	21	8	Fritz et al. (1999)
Total		63	62	43	37	64	17	
Percentage		22.0%	21.7%	15.0%	12.9%	22.4%	5.9%	

Hybrids were grouped by supporting 6 resistance hypotheses: *ND* no difference between parent, *A* additive resistance, *D* dominant resistance, *S* susceptible, *R* resistant. For detailed information see text. Hybrid classes: F1, F2, BC. In the BC crosses the parental species to which the F1 is backcrossed is indicated by the first or first two letters. *MX* mixed hybrid populations

^a Intraspecies hybrids of two geographically distinct populations (races) of *E. globulus*

Genetic basis for variation of SMs and herbivore resistance in hybrids

Genetic basis for qualitative variation of SMs

The mode of qualitative inheritance of SMs is mostly considered to be Mendelian with the presence of SMs being dominant. The chemical expression in later

generation hybrids and backcrosses often segregates according to Mendelian ratios. If both parents produce a chemical, hybrids almost always produced it and when only one parent produces a chemical, the hybrids usually produce it as well (Rieseberg and Ellstrand 1993; Orians 2000). This explains why the hybrids usually show complementary patterns with respect to the qualitative chemical variation. The

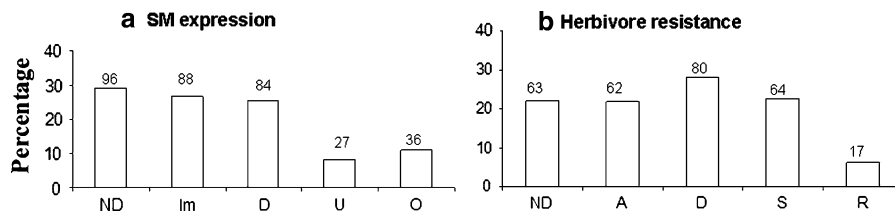


Fig. 1 Division of tests of secondary metabolite (SM) expression (a) and herbivore resistance in hybrid plants (b) over the different categories in percentages of the total. Numbers on the top of the bars indicate the number of SMs or herbivore resistance tests. For SM expression: *ND* no difference, *Im*

intermediate expression, *D* dominant expression, *U* under expression, *O* over expression. For herbivore resistance: *ND* no difference, *A* additive resistance, *D* dominant resistance, *S* susceptible, *R* resistant. Data resources are shown in Tables 1 and 2. For detailed information see the text

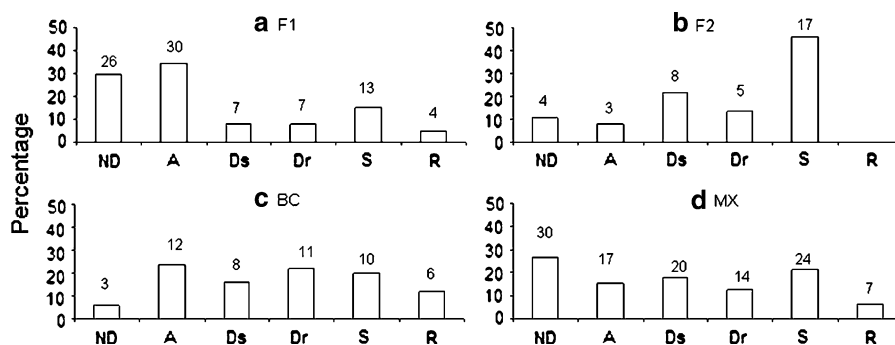


Fig. 2 Division of tests of herbivore resistance in hybrid plants over the different categories in percentages of the total. a F1 hybrid populations, b F2 hybrid populations, c BC backcross populations, d MX mixed hybrid populations.

Numbers on the top of bars indicate the number of tests. *ND* no difference, *A* additive resistance, *D* dominant resistance, *S* susceptible, *R* resistant. Data resources are shown in Table 2. For detailed information see the text

explanations to the deviation from a strictly complementary pattern of chemical expression always involve the biosynthetic pathway. The loss of SMs in a hybrid individual can be caused by the gain of a gene or alleles coding for an elongation of the biosynthetic pathway. The former end product in the hybrid has then become an intermediate which is immediately transformed into the next compound in the biosynthetic pathway. This not necessarily leads to new compounds as such compounds can be already present in the parent, from which the genes or alleles are obtained. Similarly, biochemical novelty may arise if: (1) the obstruction of a biosynthetic pathway leads to accumulation of intermediate compounds that are normally only present as transients or (2) enzymes present in only one parent can lead to the formation of a compound by modifying a chemical structure that is only present in the other parent. The combination of genes and the enzymes that they code for can lead to the formation of

compounds not present in either of the parental species. All the above effects can also be obtained by changes in the regulatory genes. Finally, disruption of regulatory genes following hybridization can cause a shift in where the SMs is produced (Rieseberg and Ellstrand 1993; Orians 2000; Firn and Jones 2003). On the level of large segregating populations with backcrossing to the parental species we expect that within the hybrid swarm a large variation is present as all combinations of genes and alleles can end up in the individuals of a hybrid swarm and hence leading to a large biochemical diversity (see Kirk et al. 2004).

Genetic basis for quantitative variation of SMs

The quantitative expression of SMs is often controlled by more than one gene with dominant, over-dominant, recessive, additive, or epistatic allelic effects within a locus or between loci. As the F1 hybrids nearly always show an intermediate pattern

of the chemical expression, additive inheritance is regarded as the most common model (e.g. Hallgren et al. 2003) which is supported by Table 1. The categories of ND and Im make up for 56%. Variation in quantitative expression can come about by the effect of different alleles on loci coding for the enzymes in the biosynthetic pathway or by different alleles at regulatory genes.

Genetic basis for variation of herbivore resistance

Mendelian models which assume resistance is due to a single gene are usually applied when the hybrids showed segregation of herbivore resistance response. Quantitative models assume that resistance is polygenic and hybrids have continuous variation in resistance (Fritz et al. 1994, 1999). The results (in Table 2) clearly show that a simple model of one gene with Mendelian inheritance in several cases can not explain the results. The hybrid susceptibility and resistance hypothesis and the over and under expression of SMs can not occur under such models. However they are present in about 38% of the cases for hybrid resistance and in about 19% of the cases for SMs. These results indicate that multiple genes must be involved in these traits to explain this transgressive segregation. Also one would expect that in F2 and BC hybrids would never support the hybrid susceptibility and resistance hypothesis under a one-gene model. Yet whole F2 and BC populations do support in a few cases the hybrid susceptibility and resistance hypothesis.

Variation of SM expression and herbivore resistance within hybrid class

It is useful to get a general picture of hybridization effects by comparing averages of the different hybrid classes such as F1, F2 and BC to the parents. But this method has the drawback that extreme phenotypes in hybrids such as transgressive segregation and the variation among individual genotypes may be lost (Kirk et al. 2004). Rieseberg et al. (1999, 2003) showed that most quantitative traits had at least one antagonistic QTL and transgressive segregation is common as a result from the recombination of the concerned QTLs in segregating hybrid populations. Genotypic differences can be significant within the

same hybrid class both in SM expression and herbivore resistance. For instance, the profile of the cysteine sulfoxides as well as the volatile secondary metabolites in the F1 hybrids between two *Allium* species was not uniform (Storsberg et al. 2004). The concentrations of tannin and phenolic glycosides in different F1 hybrid families from two *Salix* species were different (Orians et al. 2000). McIntyre and Whitham (2003) reported F1 hybrids between two cottonwood species which differed significantly in resistance to the gall mite, *Aceria parapopuli*. They measured gall mite population growth rates among F1 hybrid genotypes for 4 years. The gall mite populations remained at extremely low densities on some host genotypes, but other host genotypes supported phenomenal growth. It is exactly this individual variation on which selection operates. The final composition of natural hybrid swarms will result from the continuous process of hybridization and backcrosses and natural selection operating to increase resistance. If within a hybridization event only a few individuals are more resistant than either of the two parents these genotypes can be selected for and on a longer timescale can dominate the hybrid population and determine its success even though on average early generation hybrids were far less resistant than the parents (Barton 2001).

Case study of *Jacobaea* hybrids

Senecio sl. is a large genus (between 1,000 and 3,000 species), including *Jacobaea* species, with a worldwide distribution. The genus is notorious for the occurrence of pyrrolizidine alkaloids. *Senecio* species vary remarkably in morphological and ecological traits. Interspecies hybridization is widespread in this genus (e.g. Vincent 1996). For example, hybridization between *Senecio squalidus* and *Senecio vulgaris* has led to the origin of three new fertile hybrid taxa and *S. squalidus* itself also is a hybrid species from a cross between *Senecio aethnensis* and *Senecio chrysanthemifolius* (Abbott and Lowe 2004; James and Abbott 2005; Abbott et al. 2009). There are many other well-documented cases of hybridization between *Senecio* species (e.g. Beck et al. 1992; Hodalova 2002; Lopez et al. 2008).

Jacobaea vulgaris (Tansy ragwort or Common ragwort) is native in Europe and west Asia but

invasive in North America, Australia and New Zealand. *Jacobaea aquatica* (Marsh ragwort) is a closely related to but not a sister species of *J. vulgaris* (Pelser et al. 2003). *J. vulgaris* mainly grows in disturbed dry, sandy soils with low levels of organic matter while *J. aquatica* is a species from wet habitats with high levels of organic matter. Natural hybrids between *J. vulgaris* and *J. aquatica* are reported and grow in locations which appear to be intermediate to parental sites with regards to soil organic content and humidity (Kirk et al. 2005b). Putative hybrids from the Zwanenwater (The Netherlands) were initially identified in 1979 based on highly variable and usually intermediate flower and leaf lobe morphology compared to *J. vulgaris* and *J. aquatica*. Kirk et al. (2004) confirmed these were hybrids between *J. vulgaris* and *J. aquatica* by using the AFLP markers and PA composition and showed that the hybrid population is strongly backcrossed with *J. vulgaris* (Kirk et al. 2004, 2005a).

PA expression in *Jacobaea* hybrids

J. vulgaris and *J. aquatica* have different PA compositions. Plants of *J. vulgaris* that hybridized with *J. aquatica* in the Zwanenwater are rich in jacobine type PAs such as jacobine, jaconine, jacobine and jacozone, while *J. aquatica* often lack these PAs or contain them in very small quantities. Compared to *J. vulgaris* levels of senecionine and erucifoline are much higher in *J. aquatica* (Macel et al. 2002; Pelser et al. 2005). Kirk et al. (2010) examined the PA composition in later generation natural hybrids, artificially generated F1 hybrids and pure *J. vulgaris* and *J. aquatica*. She reported that florosenine, a putatively novel PA, was present in both of the natural and artificial hybrids. In addition, hybrids showed larger variation in total PA concentration and more extreme PA diversity than the parental plants over various environmental conditions. For instance, in the shoots of F1 hybrids, the total concentration varied from 0.02 to 3.02 mg/g dw and the number of PAs ranged from 1 to 10 in the wet nutrient soil, but under the same condition, the range in the parental shoots was 0.03–1.62 mg/g dw and 3–6 different PAs. These results suggest transgressive segregation in both directions.

Based on the work mentioned above, an artificial F2 hybrid system between *J. vulgaris* subs. *dunensis* and *J. aquatica* subs. *aquatica* has been established. The seeds were collected for *J. vulgaris* at Meijndel, a coastal dune area in north of The Hague (The Netherlands) and for *J. aquatica* at the Zwanenwater Nature Reserve (The Netherlands). The two parental species were crossed and two F1 individuals were chosen and crossed resulting in more than 100 F2 individuals. Parents, F1 and F2 are maintained in tissue culture and can be propagated endlessly. Plants of the 2 parental genotypes, the 2 F1 and 102 F2 hybrid genotypes were grown in a climate room for 6 weeks. Parental and F1 genotypes had 12 replicates. Each F2 genotype was represented by at least 3 clones and most had 6 clones. Shoots and roots were harvested and freeze dried for PA investigation by LC-MS/MS (see methods in Joosten et al. 2009). In total, 37 PAs, including tertiary amines and the corresponding *N*-oxides were detected. The PA expression in F1 hybrids was often intermediate between the two parents. In contrast, transgressive segregation in two directions was found for the concentration of total PA and most individual PAs in the shoots of F2 genotypes. The mean total PA concentration for the parental genotypes was 1,627 (\pm SE 337) μ g/g dw in *J. aquatica* and 4,942 (\pm SE 467) μ g/g dw in *J. vulgaris*. Total PA concentration for all tested F1 and F2 genotypes ranged from 334 to 6,834 μ g/g dw. Both the F1 hybrids had intermediate levels of total PAs of 2,392 (\pm SE 470) and 2,979 (\pm SE 438) μ g/g dw. Only one F2 genotype had a higher amount of total PA than *J. vulgaris* while 49 (about 50%) of the F2 lines had lower total PA concentration than *J. aquatica* (Fig. 3a). For the individual PAs overexpression was observed more frequently. About 14% of the F2 hybrids showed a higher expression level of senecionine free base than in either of the parents (Fig. 3b). According to the genotype mean concentration, none of the F2 hybrid genotypes over-expressed jacobine free base in either of the parents while 20% of the F2 hybrid genotypes under-expressed jacobine (Fig. 3c). Presence and absence of PAs in F2 hybrids was more variable than in either of the parental genotypes (Cheng et al., unpublished data). This confirmed the results of Kirk et al. (2010) who showed that new combinations of PAs are found in hybrids.

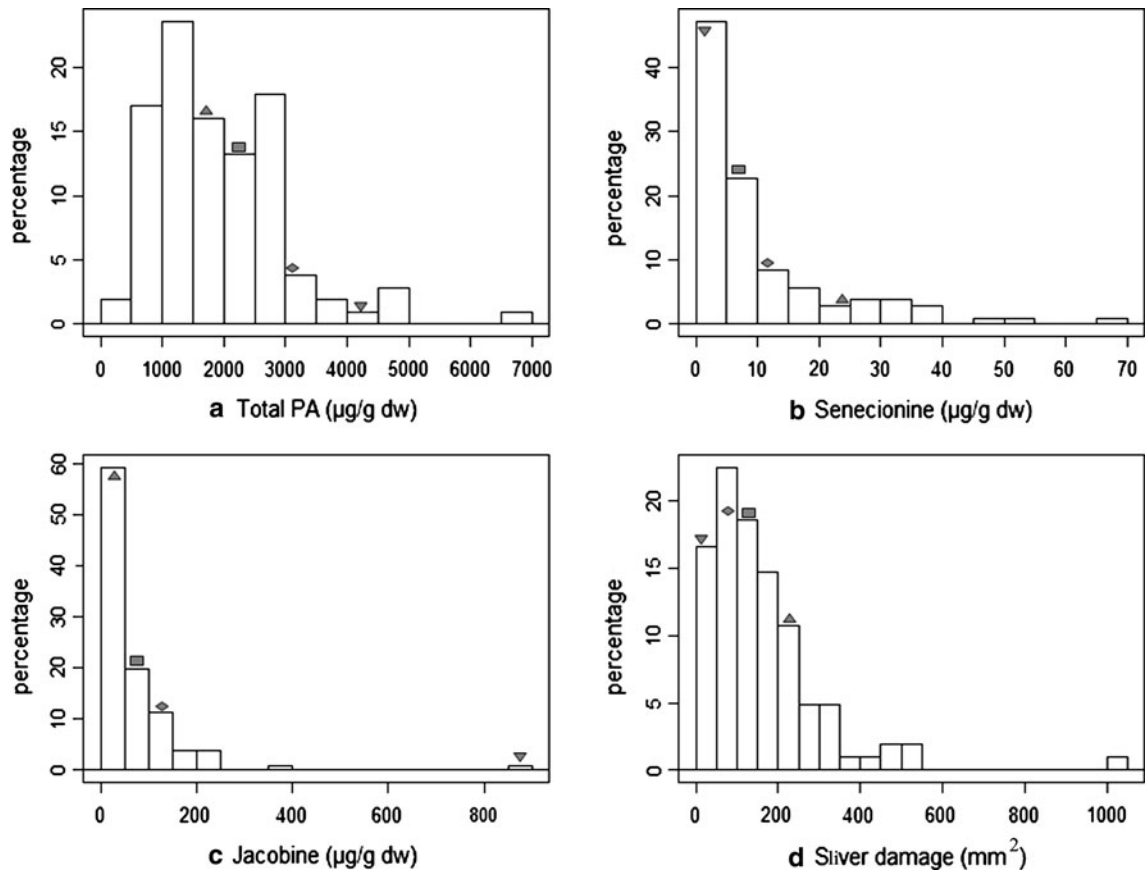


Fig. 3 Distribution of genotype means of **a** total PA concentration, **b** senecionine concentration, **c** jacobine concentration and **d** thrips damage in the shoots of *J. aquaticus*, *J. vulgaris*, F1 and F2 hybrids. Total PA includes free bases and *N*-oxides. For senecionine and jacobine only the free base concentration

is given. Symbols above the bars indicate the position of parental and F1 genotype. Filled triangle *J. aquatica* parent, filled inverted triangle *J. vulgaris* parent, filled square F1-A, filled diamond F1-B. Data from Cheng et al. (unpublished)

Herbivore resistance in *Jacobaea* hybrids

Leiss et al. (2009a) identified four thrips (*Frankliniella occidentalis*)—resistant and four thrips—susceptible F2 genotypes in the hybrids between *J. vulgaris* and *J. aquatica*. They analyzed the PA composition and found that higher amounts of jacobine *N*-oxide and jaconine *N*-oxide were found in thrips-resistant hybrids, especially in younger leaves. A more extensive study including 94 F2 hybrids and 3–6 replicates for each genotype showed transgressive segregation of *F. occidentalis* resistance in both directions although it was far more pronounced in the direction of higher susceptibility (Fig. 3d). Eight PAs: jacobine, jaconine, jacoline, jacozone and their corresponding *N*-oxides were positively correlated with thrips resistance (Cheng

et al., unpublished data). This strongly suggested that the segregation pattern of thrips resistance within F2 hybrids is caused by underlying segregation patterns of jacobine type alkaloids.

Outlook

The study of hybrids and their parental species offers many opportunities to investigate the variation of secondary chemistry and herbivore resistance in plants. The frequently occurring transgressive segregation in F2 hybrids offers potentially large variation in concentration and composition of secondary chemistry, which can be used for ecological experiments to test the function of such compounds in natural settings. If these are combined with in vitro tissue

culture techniques, hybrids can be maintained and replicated infinitely. This allows the repetition and new experiments on the same genotypes. Plants which have short lifecycle such as *Jacobaea* have the advantage to reduce the experimental cycle. The fast developing metabolic detection and analyses methods will facilitate identification of the compounds in interest. (e.g. Kim et al. 2009; Kuzina et al. 2009; Leiss et al. 2009a, b). QTL mapping of such hybrid crosses adds another opportunity to further unravel the genetics behind secondary chemistry and resistance.

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