



Ecological consequences of plant hybridization in willows: inheritance patterns of secondary compounds and herbivore foraging behaviour.

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Abstract

Segregation of genetic variation into species is traditionally viewed as the principal unit of evolution while intraspecific hybridization was regarded as a mistake in nature. Nevertheless, intraspecific hybridization is common between many plant species and recent studies have suggested that hybridization may be beneficial to individuals. hybridization is also of interest as it influence species that are interacting with the hybridising species, for example herbivores that need to decide whether or not to forage on hybrids between host plants and non-host plants.

To understand how herbivores are influenced by hybridization, and how herbivory influences hybrid plants, I have studied the inheritance of plant resistance characters, foraging preference and performance of herbivores (leaf beetles and voles) and the degree of herbivore damage on pure and hybrid willows. The studied willow species, *Salix caprea*, *S. repens* and *S. aurita* differ in secondary metabolite composition.

The results show that both studied groups of secondary metabolites, phenolic glucosides and condensed tannins, are additively inherited in hybrids between *S. repens* and *S. caprea*, while condensed tannins are equal in *S. caprea*, *S. aurita* and hybrids between the two parental species (Paper I and II). There is no common response of the studied herbivore community. Instead, it seems that specialist herbivores either discriminate against hybrids and non-host parental species or do not separate between hybrids and host parental species. In contrast, generalists usually show either intermediate preference for hybrids, or do not discriminate between hybrids and parental species. One generalist species shows a preference that indicates a breakdown in resistance (Paper II, III, and IV). When adding together the effects of all herbivores, it appears that herbivores inflict more damage to hybrids than parental species (Thesis, paper II and VI).

Keywords: Leaf beetles, voles, performance, preference, plant resistance, phenolics, phenolic glucosides, condensed tannins

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Appendix

Papers I - VI

The present thesis is based on the following papers, which will be referred to by their Roman numerals.

- I. Hallgren, P, Ikonen, I, Hjältén, J. and Roininen, H. Inheritance patterns of phenolics in F1, F2 and back-cross hybrids of willows: implications for herbivore responses to hybrid plants. Manuscript submitted to Journal of Chemical Ecology.
- II. Hjältén, J., Hallgren, P. and Qian, H. 2002. The importance of parent host status for hybrid susceptibility to herbivores: A test with two hybrid lines of willows. *Ecoscience* 9(3): 339-346.
- III. Hallgren, P and Hjältén, J. Feeding preference of eight leaf beetle species for *Salix caprea*, *S. repens* and F1 hybrids. Manuscript.
- IV. Hallgren, P and Hjältén, J. Vole preference for *Salix caprea*, *S. repens* and their F1, F2 and backcross hybrids. Manuscript.
- V. Hallgren, P. Effects of willow hybridization and simulated browsing on the growth and survival of the leaf beetle *Phratora vitellinae*. Manuscript.
- VI. Hjältén, J. and Hallgren, P. The resistance of hybrid willows to specialist and generalist herbivores and pathogens: the potential role of secondary chemistry and parent host plant status. in Wagner, M. R., Clancy, K. M., Lieutier, F. and Paine, T. D. (eds.), *Mechanisms and Deployment of Resistance in Trees to Insects*, pp. 153-168, Kluwer Academic Publishers, Dordrecht.

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Introduction

"The segregation of the total genetic variability of nature into discrete packages, so called species, which are separated from each other by reproductive barriers, prevents the production of too great a number of disharmonious incompatible gene combinations. This is the basic biological meaning of species and this is the reason why there are discontinuities between sympatric species. We do know that genotypes are extremely complex epigenetic systems. There are severe limits to the amount of genetic variability that can be accommodated in a single gene pool without producing too many incompatible gene combinations." (Mayr, 1969, p. 316).

Ernst Mayr's also explains that the biological species concept is a "a concrete phenomenon of nature" although he admits that in reality hybridization occurs frequently between seemingly good sympatric species (Mayr, 1996). Recently, it has been suggested that hybridization plays an important role in speciation (Barton & Hewitt, 1985; Hewitt, 1988), maintenance of small populations, (Grant & Grant, 1992) and development of species through introgression of new traits (Anderson, 1949; Keim et al., 1989; Fritz, 1999; Hochwender, Fritz & Orians, 2001).

Some researchers who study hybrids and herbivore resistance question the traditional view of hybridization as an event that decreases fitness. These researchers view hybridization as a process that increases genetic diversity and thus may influence adaptation (Hochwender, Fritz & Orians, 2001).

However, plant hybridization is an important process also for herbivores that utilize the hybridising taxa. Irrespective of how one views hybridization; a process that increases genetic diversity, a process that acts adaptively through introgression of traits, or a random event that should be regarded as a mistake of nature; hybridization introduces variability that herbivores need to handle to be able to make good foraging decisions. Plant hybrids may show combinations of plant traits or even novel traits due to the recombination of genes; this makes the choice about foraging and host plants even more complex for the herbivore (Arnold, 1997; Fritz, 1999). hybridization may cause variability in host recognition characters, herbivore defence characters, and nutrition levels independently and thus may introduce a novel decision situation for herbivores.

One of the major difficulties for herbivorous animals is to satisfy their energetic and nutritional demands, mainly because of the low nutrient content of plants, the complex bounding of energy in plant tissue, and the many poisonous chemicals present in plant tissue (Lawton & McNeil, 1979). The value of plants as food for herbivores varies between plant species but also between plant individuals due to plant responses to biotic and abiotic factors mediating changes in primary metabolites, secondary metabolites and other plant traits (Lawton & McNeil, 1979). Nitrogen through amino acids are important for the growth of animals and plant nitrogen content thus often directs the development rate and foraging of herbivores (Raubenheimer & Simpson, 1999; Wait, Jones & Coleman, 1998; Schoonhoven, Jermy & van Loon, 1998). An

increase in plant available nitrogen may lead to an increase in nitrogen content of plant tissues, increasing availability to herbivores, but may instead also increase plant growth and thus not lead to changes in herbivore nitrogen intake rate (Wait, Jones & Coleman, 1998). It is assumed that herbivores usually have a negative effect on plants causing an evolution of anti herbivore traits (Ehrlich & Raven, 1964; Fraenkel, 1959; Wink, 1999). Apart from primary metabolites, herbivore foraging is also influenced by secondary metabolites, morphological traits, for example leaf hairs and spines, and phenology of the plant (Larsson, 2002). Plant secondary metabolites are very diverse, more than 100000 individual compounds have been isolated and characterized and many of the compounds are specific to species, taxa or families of plants, while others are more generally occurring (Wink, 1999). The role of many secondary plant compounds are currently unknown but many substances are assumed to act as a defense against herbivores and pathogens (Ehrlich & Raven, 1964; Fraenkel, 1959; Wink, 1999; Larsson, 2002). However, it has also been questioned if herbivory is the reason behind the evolution of plant secondary metabolites (Jeremy, 1984; Beart, Lilley & Haslam, 1985). The origin of the evolution of secondary metabolites has instead been suggested to be for example to act as carbon sinks or to protect plants against ultraviolet radiation (Close & McArthur, 2002; Wink, 1999). Still there are numerous studies showing that secondary metabolites act as protection against natural enemies (Kennedy & Barbour, 1992; Wink, 1999), suggesting that at least the expression of some secondary substances has evolved as a response to herbivory. In addition, the apparent quality of a plant as food differs between herbivore species due to the specific adaptation to plant characters (Jaenike, 1990). Adaptation to specific plants and thus reduced diet variability (specialization) is one strategy for herbivores to overcome the problems with using plants as food (Fry, 1990; Jaenike, 1990). Herbivores have developed systems to recognize the plants that they are adapted to and to avoid plants that are poisonous or unsuitable as food (Bernays & Chapman, 1994; Bernays, 1998, 2001). But also herbivores that are not very specialized evaluate the food plants and if given a choice, select food that gives the highest gain (Singer et al., 1992; Stephens & Krebs, 1986).

Recently, several studies have examined the effects of hybridization on plant resistance to herbivores (Strauss, 1994; Fritz, 1999; Orians, 2000). Different studies show different results and all possible patterns have been reported. Sometimes F1 hybrid resistance is intermediate between parental species, equal to one of the parental species, or superior respective inferior to the parental species (Fritz, Nichols-Orians & Brunsfeld, 1994; Fritz, 1999). A pattern where F1 and/or F2 hybrids are more susceptible to herbivores than the parental species corresponds to the traditional view that hybrids have a lower fitness than pure species. This view is based on the logic that speciation is adaptive and that a mix of species leads to a loss of the adapted characters with a decrease in fitness as a result (Whitham, 1989; Mayr, 1996). However, studies and experiments of hybrid resistance to herbivores and pathogens do not confirm this view for all combinations of hybrids and herbivores. This may be due to several reasons. Most of the early studies of hybrid plant susceptibility and resistance to herbivores were performed with natural hy-

brids and thus make it difficult to distinguish between environmental and genetic explanations for differences between pure and hybrid zones (Messina, Richards & McArthur, 1996; Fritz, Roche & Brunsfeld, 1998). One way to limit environmental influences is to conduct experiments with hybrid plants and pure species in the laboratory or in a common garden (Messina, Richards & McArthur, 1996). As a consequence, experimental studies are stressed (Strauss, 1994).

It is significant that both pure species and hybrids may be influenced differently by environmental conditions; for example, taxa that are most susceptible under dry conditions are not necessarily the most susceptible under wet conditions (Graham, McArthur & Freeman, 2001). Because herbivores sometimes cause an induction of plant defences, (Karban & Baldwin, 1997) different herbivores that feed on pure and hybrid plants could cause differences in susceptibility to other herbivores that are not influenced by hybridization, making the results hard to understand. Therefore, it is important to study if parental species and hybrids respond differently to herbivores.

Resistance is the effect of several different processes such as host selection of the individual herbivore, foraging effort by the herbivore, and population dynamics of the herbivore population (Larsson, 2002). In addition, the effect of herbivores on plant fitness is determined by the influence of the whole herbivore community that feeds on a plant together with the plants ability to tolerate damage. It is therefore not possible, to draw any conclusions about fitness effects of the plants from herbivores based only on observations of single herbivore species preference without considering other important conditions.

To understand how plant hybridization influences herbivores and plant-herbivore interactions, we need to consider how plant characters are inherited in hybrids and how herbivores respond to these changes. The following questions will be addressed in this thesis:

1. What is the inheritance pattern of secondary metabolites such as phenolic glucosides and condensed tannins in hybrids between *S. caprea* and *S. repens* (paper II and paper II)?
2. What is the preference pattern of leaf beetles and voles for *S. caprea*, *S. repens*, and hybrids (paper II, paper III, paper IV)?
3. Does growth and survival of *Phratora vitellinae* larvae differ between *S. repens*, *S. caprea*, and F1 hybrids previously damaged and undamaged by mammalian herbivores (paper V)?
4. Does the total amount of herbivore leaf damage differ between *S. caprea*, *S. repens*, and F1, F2, and backcross hybrids (paper I)?
5. Can the interspecific variation in herbivore responses to hybrid plants be explained by differences in their adaptations to secondary metabolites (paper VI and thesis)?

Material and methods

Willow hybrids

Willows hybridize frequently. For example, *Salix caprea* (L., willow, Salicaceae), *S. aurita* (L.), and *S. repens* (L., creeping willow) hybridize in northern parts of coastal Sweden (Lid & Tande Lid, 1979). Willows are intensively used by many different herbivores (Tahvanainen, Julkunen-Tiitto & Kettunen, 1985; Tahvanainen et al., 1985). The most abundant secondary chemicals in willows are phenolics, mainly flavonoids, tannins and simple phenolic glucosides (Julkunen-Tiitto, 1989). Willows may broadly be divided in two main groups characterised by their secondary metabolites. One group consists of species that contains high levels of condensed tannins but only low levels of phenolic glucosides, such as *S. caprea* and *S. aurita*. In contrast, the other group consists of species that contains several phenolic glucosides in high concentrations, such as *S. repens* and *S. myrsinifolia* (Julkunen-Tiitto, 1986, 1989). The phenolics in willows are assumed to play an important role for herbivore and pathogen resistance but may also be important for bud and leaf development and UV absorption (Julkunen-Tiitto, 1989). Numerous studies have shown that phenolic glucosides can influence selection of herbivores on willows (Tahvanainen, Julkunen-Tiitto & Kettunen, 1985; Tahvanainen et al., 1985; Kelly & Curry, 1991; Pasteels & Rowell-Rahier, 1992; Kolehmainen et al., 1995). My main emphasis is thus on these groups of substances and their role as herbivore resistance characters.

To control the origin of the plant material, I used controlled crosses by hand-pollinating naturally growing pure willows. The crossings were performed during 1995, 1997, and 1999. During the crossing in 1997, F₂ and backcross hybrids were produced by crossing F₁ hybrids from 1995 with either F₁ hybrids from 1995 or with pure naturally growing willows. For detailed descriptions of the techniques used for hand-pollinating see Hjältén (1997) and paper II-V.

After the seedling stage, the plants were grown in pots in a greenhouse or in an experimental field. For most of the studies (question 1, 2, and 3), the plants were grown in pots on an experimental field close to the Swedish University of Agricultural Sciences in Umeå (63°44' N, 20°18' E) for 2-3 months. These plants received water when needed either using water sprinklers (plant material planted 1996 and 1997) or using CNL (compensated non-leakage) watering systems (plant material from 1999 used in paper III).

The plants used for the insect performance studies (planted 1999) were only grown in the greenhouse. To study the interaction between mammalian herbivores and leaf beetles, winter dormant shoots were cut to simulate vole damage during the winter. Moose damage was simulated by leaf stripping on elongating spring shoots.

For the vole experiment and field study (question 2), the plants from the crossing made in 1995 and 1997 were replanted during 1999 in two experi-

mental fields, one at the Forest Research Institute of Sweden ("Skogforsk") in Sävar 25 km north of Umeå and one in Pengsjö 30 km west of Umeå.

Insect herbivores

Generalist and specialist leaf beetles (Coleoptera: Chrysomelidae) are among the most important insect herbivores that feed on willows (Koch, 1992; Jolivet & Hawkeswood, 1995). Leaf beetles were also the most abundant herbivores on the experimental plants, but I have also observed lepidoptera larvae, hymenoptera larvae, and galls from galling sawflies. The leaf beetle species that were used in this study can be divided into species that use willows with low concentrations of phenolic glucosides in the leaves, *Lochmaea caprea* (L. all insect nomenclature from Gustavsson, 2002), *Crepidodera fulvicornis* (Fabr.), *Phratora vulgatissima* (L.), *P. polaris* (Suff.), *Galerucella lineola* (Fabr.), *Gonioctena viminalis* (L.) and *Plagioderia versicolora* (Laich.), and species that are adapted to higher concentrations of phenolic glucosides, *P. vitellinae* (L.), *Chrysomela populi* (L.) and *Gonioctena linnaeana* (Schrank).

Analysis of leaf chemistry, question 1

To analyse secondary chemicals in the leaves, we randomly collected between 20 (*S. caprea*) and 150 (*S. repens*) leaves from each plant. The leaves were air-dried at room temperature (18-22C) in cardboard boxes (Julkunen-Tiitto & Sorsa, 2001) and ground in a plant mill to fine powder. A sub-sample (150mg) was homogenised and extracted using methanol. Low-molecular weight phenolics of the extracts were analysed using Hewlett-Packard HPLC apparatus. From each sample, we quantified all detected phenolic glucosides. Individual compounds were identified by comparing their UV-vis spectra and retention times to those of known compounds. We also detected two unknown compounds whose spectra very closely resembled those of the known phenolic glucosides. Polymeric condensed tannins were measured with the Butanol-HCl -assay (Waterman & Mole, 1994).

Herbivore preference, question 2

To better understand the mechanisms behind the susceptibility of the hybrid plants and how individual herbivores respond to and are influenced by hybridization, I studied foraging preference of several leaf beetle species in cafeteria experiments. Leaves from different taxa were removed from the plant and placed in the same area. Leaf beetles were collected from the local populations on different willows species in the neighbourhood of Umeå. In the cafeteria experiments in 1995, whole leaves were used and during the experiments in 1997 a standardised leaf disk was cut from the leaf. During 2000, the leaves were covered by a transparent acrylate board with holes of a standardised size (100 mm²) to avoid cutting the leaves. In the first two experiments, the amount eaten was measured after 16 hours and in the exper-

iment in 2000 measurements were taken after 2, 4, 6, and 8 hours; preference was calculated using Rodger's index (Krebs, 1999).

Willows are also preferred food for several mammalian herbivores such as moose, hares, and voles (Tahvanainen et al., 1985). During two periods, voles damaged my experimental willows. I counted the number of individual plants in each plant category that had foraging damage from voles. To further examine vole preference between hybrids (F1, F2, and backcross hybrids), I performed cafeteria experiments with voles.

The bank voles, *Clethrionomys glareolus*, used for cafeteria experiments were caught using a live-cage trap (Ugglan, ethical permit A 127 - 00 from Umeå research animal ethical committee) and were kept in cages (size 40, 25, 15 cm). The cafeteria experiment was performed with the willow shoots randomly distributed in a U-formation. The willow shoots were cut from winter dormant plants and then kept frozen before the experiment. The experiments were performed under natural temperature conditions ranging from -2 to -15 C. The bark area eaten on each plant category was measured after two hours.

Insect performance experiment, question 3

Performance of insect larvae were studied on different willow taxa using first instar *P. vitellinae* larvae collected (1st of July 2000) on *S. myrsinifolia* (L.). Each larva was enclosed on a randomly chosen shoot, with a sufficient number of leaves to support its growth on each experimental plant growing in a green house (13 blocks, 3 plant taxa, and 3 treatments per block, for a total of 117 larvae). Larvae for each block were taken from the same shoot on the plant, and thus probably originated from the same clutch of eggs. The larvae were left to forage and develop on the plant for 46 days when the first beetles were hatching from their pupae. At this point, I measured survival, the developmental stage the larvae had attained, the area of leaf consumed, and leaf density of the plants. When the experiments were terminated, no larvae had eaten all of the enclosed leaves.

Total herbivory, question 4

The susceptibility of the experimental willows to leaf chewing insect herbivores was determined from leaves that were collected for analyses of phenolic glucosides and condensed tannins during 1999. Before drying, 10 random leaves from each plant were put in a photocopier and the leaf area, the leaf area consumed by leaf chewing herbivores, and the leaf area consumed by leaf beetles were measured from the photocopies using a mm^2 measuring grid. It was possible to separate foraging traces of leaf beetle larvae from the other herbivores because as far as we know they are the only herbivores that do not consume the leaf veins.

Results and discussion

Most studies have only reported patterns of herbivore preference for hybrids and few successful attempts have been made to explain the observed patterns. The only clear conclusion from earlier studies is that different herbivores differ in their response to plant hybrids (Whitham, Morrow & Potts, 1994; Fritz, 1999). This is not surprising since the response of herbivores depends on both the inheritance, additive or dominant, of plant characters that affect herbivore foraging behaviour, and the response of specific herbivores to these changes (Orians, 2000). We should therefore not expect all herbivores to respond equally to plant hybrids.

To explain and predict the patterns, we need to consider generalist and specialist herbivores separately. Specialisation is an evolutionary process that makes species evolve adaptations to certain plant resistance characters. The host range of that herbivore is usually also narrowed to plant species that share common characters such as the resistance character that the herbivore is adapted to (Jaenike, 1990). The most specialised herbivores should be expected to discriminate more against plants that show dissimilarities with the specialists host plant while less specialised herbivores may include plants that are rather different from their normal hosts. Specialists may use specific plant characters, which are usually regarded as resistance characters such as phenolics, as a feeding stimuli (Bernays, 1998, 2001).

Specialists more commonly react according to a threshold level of a certain plant character while generalists more commonly respond in proportion to characters that influence the herbivores gain of the food (Jaenike, 1990). This seems simple; however, because several different plant characters such as primary and secondary metabolites need to be considered as well as potential synergistic effects between resistance characters, it is difficult to predict.

Secondary chemistry, question 1

Both phenolic glucosides and condensed tannins are known to significantly affect herbivores. Condensed tannins is a group of phenolic polymers generally known to reduce protein uptake, whereas phenolic glucosides are phenolic monomers, some of which are known to have toxic effects on animals (Lindroth & Weisbrod, 1991). In most cases, phenolic glucosides act as repellents for generalist herbivores (Kelly & Curry, 1991; Tahvanainen, Julkunen-Tiitto & Kettunen, 1985). However, for some specialist insect herbivores, they can act as positive cues that trigger oviposition and feeding (Kolehmainen et al., 1995; Soetens et al., 1998; Roininen et al., 1999). Willows with low levels of phenolic glucosides are usually considered palatable to both generalist and specialist herbivores while willows with high concentrations of phenolic glucosides are mainly used by herbivores that are adapted to these substances (Tahvanainen, Julkunen-Tiitto & Kettunen, 1985; Kelly & Curry, 1991; Kolehmainen et al., 1995; Roininen et al., 1999).

Table 1: This table shows the results from experiments with F1, F2, backcrosses (BCc, back-crossed to *S. caprea* and BCr to *S. repens* and pure species (S. c = *S. caprea*, S. r = *S. repens*). The column labeled test shows the type of study (pref. = preference or chem. = chemical analyses). The chemicals are abbreviated: CT = condensed tannins; PHG = phenolic glucosides. The pattern is shown with stacked lines for each experiment; a difference of two lines between stacks in the same experiment shows a significant difference between categories and a difference of one line indicates a non-significant trend.

| Test | Animal or substance | S. c. | BCc | F1 | F2 | BCr | S. r. | Paper |
|--------------|---------------------|-------|-----|----|----|-----|-------|-------|
| chem. | CT | ≡≡≡ | ≡≡≡ | ≡≡ | ≡≡ | ≡ | ≡ | I |
| chem. | PHG | — | — | ≡≡ | ≡≡ | ≡≡≡ | ≡≡≡ | I |
| pref. | Vole | — | — | ≡ | ≡≡ | ≡≡ | ≡≡ | IV |
| plant damage | leaf chewers | ≡ | ≡≡ | ≡ | ≡≡ | ≡≡ | ≡ | I |

Leaf chemistry results were very consistent (Table 1 and 2). Both condensed tannins and phenolic glucosides show a pattern resembling an additive inheritance where hybrids are intermediate between the two extreme parental species. *S. caprea* and hybrids backcrossed to *S. caprea* have the highest concentrations of condensed tannins (125 mg/g dry weight). The concentration decreases through the F1 (75 mg/g) and F2 (55 mg/g), to the lowest concentration in *S. repens* and backcrosses to *S. repens* (30 mg/g). The inheritance pattern for phenolic glucosides are close to the opposite; *S. repens* have the highest concentration (125 mg/g dry weight), then the hybrid backcrossed to *S. repens* (80 mg/g), the F1 and F2 hybrids (50 mg/g), the hybrid backcrossed to *S. caprea* (5 mg/g), and *S. caprea* which are very close to zero. Additive inheritance of chemical resistance traits seems to be the most common pattern (Orians, 2000; Orians & Fritz, 1995; Orians et al., 2000). However, other patterns (including expression of novel compounds) have been reported (reviewed by Orians, 2000).

In addition, there was a negative correlation between condensed tannins and phenolic glucosides for all plant categories that have several individuals with both substances (all categories except *S. caprea* and back-crosses against *S. caprea*). These different chemical groups represent what is supposed to be two different herbivore defence systems for different willow species. Thus, these substances are not expressed independent but rather dependent of the expression of the other substance. This could be either due to a resource trade-off or that they are controlled from the same set of genes.

Herbivore preference, question 2

It has proven difficult to predict the response of specific herbivores to hybrid plants; however, based on the results of the additive inheritance pattern of phenolic glucosides and condensed tannins one may make predictions for specialists and generalists leaf beetle preferences. One group of studied species, *Ph. vitellinae* (Tahvanainen, Julkunen-Tiitto & Kettunen, 1985; Kolehmainen et al., 1995), *C. populi* (Koch, 1992; Ikonen, 2002), and *G. linnaeana* (Koch, 1992) are adapted to high concentrations of phenolic glucosides in their food plants and are usually assumed to be rather specialised (Tahvanainen, Julkunen-Tiitto & Kettunen, 1985). They are reported to use a few willow species with different composition of phenolic glucosides, and are not regarded as very specific, although more specific than other leaf beetles on willows. Thus, I predicted them to prefer *S. repens* over the F1 hybrid and the F1 hybrid over *S. caprea*. The other species used include *L. caprea*, *G. lineola* (Tahvanainen, Julkunen-Tiitto & Kettunen, 1985; Kolehmainen et al., 1995), *Pl. versicolora* (Tahvanainen, Julkunen-Tiitto & Kettunen, 1985; Soetens, Rowell-Rahier & Pasteels, 1991), *Ph. vulgatisima* (Kelly & Curry, 1991), and *G. viminalis* (Koch, 1992) which are more generalistic and avoid high concentrations of phenolic glucosides, especially salicylates (Tahvanainen, Julkunen-Tiitto & Kettunen, 1985; Kolehmainen et al., 1995). We should, therefore, expect them to prefer *S. caprea* over the F1 hybrid and the F1 hybrid over *S. repens*.

Two of the specialist leaf beetles, *Ph. vitellinae* and *Ch. populi*, prefer *S. repens*, which chemically is most similar to their main host plants, *S. myrsinifolia* and *P. tremula* (Table 2). Because these beetles do feed on willows with high but also variable phenolic glucoside concentration and composition, I predicted that they should show intermediate preference for the F1 hybrid. Instead they discriminate against the F1 hybrid and prefer the phenolic glucoside rich parental species *S. repens*, and seemingly exhibited a threshold response to the changes in phenolic glucosides in F1 hybrids, indicating that they are more specialised than what I predicted. *G. linnaeana*, the other tested leaf beetle that specialises on phenolic glucosides, did not show any significant preference for any plant category (Table 2).

Two generalists, *Ph. vulgatisima* and *Pl. versicolora*, preferred *S. caprea* and show intermediate preference for the F1 hybrid. In addition, *L. caprea* resembles this pattern in two of my experiments but shows no preference in a third experiment (Table 2). This is what was predicted for these generalistic leaf beetles which suggests that they exhibit a dose dependent response to changes in secondary chemistry. In the preference test with *S. aurita*, that is chemically similar to *S. caprea*, *L. caprea* did not show any preference (Table 2). The reason for this is probably that both *S. caprea* and *S. aurita* are hosts for *L. caprea*. The chemical analyses indicate that F1 hybrids are quite similar to both parental species.

One generalist species, *G. viminalis*, preferred F1 hybrids compared to parental species (Table 2). This is also indicated in the experiment with *Cr. fulvicornis* (Table 2). A higher than expected preference for the F1 hybrid

Table 2: Summary of experiments with F1 hybrids and pure species *S. caprea*, *S. repens* and *S. aurita*(S. a). The animals are abbreviated, L. c. = *L. caprea*, Cr. f. = *Cr. fulvicornis*, Ph. vi. = *Ph. vitellinae*, Ch. p. = *C. populi*, G. v. = *G. viminalis*, G. l = *G. linnaeana*, Pl. v. = *Pl. versicolora*, Ph. vu. = *Ph. vulgatisima*, the chemicals CT = condensed tannins, PHG = phenolic glucosides and the diet breadth in the column diet as S for specialist and G for generalist. 0 means that the plant category was not used by the herbivore. For further explanation and abbreviations see Table 1.

| Plant Species | | Test | Diet breadth | Animal or substance | Pattern | | | Paper |
|---------------|-------|-------|--------------|---------------------|---------|----|----|-------|
| P1 | P2 | | | | P1 | F1 | P2 | |
| S. c. | S. r. | chem. | - | CT | ≡ | ≡ | = | II |
| S. c. | S. r. | pref. | S | Ph. vi. | — | — | ≡ | III |
| S. c. | S. r. | perf. | S | Ph. vi. | = | = | ≡ | V |
| S. c. | S. r. | pref. | S | G. l. | o | = | — | III |
| S. c. | S. r. | pref. | S | Ch. p. | o | o | = | III |
| S. c. | S. r. | pref. | G | Cr. f. | ≡ | ≡ | = | II |
| S. c. | S. r. | pref. | G | L. c. | ≡ | ≡ | = | II |
| S. c. | S. r. | pref. | G | L. c. | ≡ | ≡ | = | III |
| S. c. | S. r. | pref. | G | Pl. v. | ≡ | — | o | III |
| S. c. | S. r. | pref. | G | Ph. vu. | ≡ | = | o | III |
| S. c. | S. r. | pref. | G | G. v. | — | ≡ | = | III |
| S. c. | S. a. | chem. | - | CT | ≡ | ≡ | ≡ | II |
| S. c. | S. a. | pref. | G | L. c. | ≡ | ≡ | ≡ | II |
| S. c. | S. a. | pref. | G | Cr. f. | ≡ | = | = | II |

could be due to the defence compounds of the parental species, phenolic glucosides and condensed tannins, which are both at least partly potent against the herbivores. However, the intermediate levels in the hybrid are too low to provide significant protection. However, on hybrids between *S. aurita* and *S. caprea*, *Cr. fulvicornis* preferred *S. caprea* while the F1 hybrid and *S. aurita* were equally preferred (Table 2). This indicates that other plant characters are important to *Cr. fulvicornis* preference.

Bank voles, *C. glareolus*, which are generalists, showed preference for *S. repens* and a decreasing trend of use for pure *S. repens* to pure *S. caprea* (Table 1). This indicates a dose dependent response to the changes in secondary

metabolites and shows that although voles are to be regarded as generalistic herbivores and compared to most herbivorous insects very generalistic, they are still highly selective when offered a choice.

Larvae performance, question 3

To better understand the importance and consequences of herbivore preference, we also need to consider performance of the herbivore and its offspring. Generally, we should expect a good correlation between herbivore preference and performance, but preference is not uncommonly more restricted than necessary for performance (Fox & Lalonde, 1993). One reason for this may be that a perfectly acceptable plant is similar to a unacceptable plant (Fox & Lalonde, 1993; Bernays, 2001; Larsson & Ekbohm, 1995).

The studies of *Ph. vitellinae* larvae performance reveal similar results for the foraging preference of adult *Ph. vitellinae* beetles; i.e. development rate are significantly better on *S. repens* than on *S. caprea* and the F1 hybrid and there is no difference between the F1 hybrid and *S. caprea* (Table 2). Simulated browsing caused a decrease in development of *Ph. vitellinae* larvae on damaged *S. caprea* and F1 hybrid plants compared to control plants, but this trend was not found on *S. repens*. One reason for the differences between plant categories could be differences in plant architecture (Honkanen & Haukioja, 1998; Aarssen, 1995). *S. caprea* is treeformed and thus has a rather high apical dominance; *S. repens* has a creeping growth form and consequently low apical dominance; and the hybrid is intermediate in growth form between the parental species. However, for other leaf beetle species, weaker correlations have been found between preference and performance on hybrid plants. Orians et al. (1997) found differences in preference and performance of three leaf beetles species on *S. eriocephala*, *S. sericea* and hybrids.

Total herbivory, question 4

The effect of herbivores on hybrid fitness may not be predicted simply by preference patterns of a few herbivore species. Instead, the combined effects of all herbivores should be taken into account. Hybrids may receive more herbivore damage because they are generally more preferred by herbivores and because they are used by herbivores from both parental species (Whitham, Morrow & Potts, 1994; Fritz, Nichols-Orians & Brunsfeld, 1994). This means that a higher number of herbivore species use hybrids compared with the parental species.

Observations of total damage from leaf chewing herbivores do not resemble the patterns in leaf chemistry. Instead, F1 hybrids and pure species are equally damaged while F2 and backcross hybrids are more susceptible to herbivores. This could be the result of a disruption of co-adapted resistance mechanisms in F2 and backcross hybrids. Similar results have been reported by studies on mouse (*Mus muscus*) resistance against the pinworm (*Aspiscularis tetraptera*) where F1 hybrids are resistant, but F2 and back-cross hybrids are less resistant

(Mouliia, 1999). These results indicate lower fitness of the advanced hybrids then could lead to a barrier against introgression of characters between the parental species.

Synthesis, question 5

Table 3: This table shows the results from previous experiments in willow hybrid systems reviewed in paper VI (*S. phylicifolia* = S. p.). Herbivores are marked into groups, Lb. = leaf beetles, Lgs. = leaf galling sawflies subgenus *Pontania* and *Phyllocolpa*, Lf. = leaf folders, Lgm. = leaf galling mite genus *Iteomyia*, Bgm. = Bud galling mite genus *Dasineura*, and with latin names (some times abbreviations) in parenthesis. The original studies are from Hjältén (1997) marked A, Hjältén (1998) marked B, Hjältén, Ericson & Roininen (2000) marked C and Hjältén unpubl. marked D. For further explanation and abbreviations see Table 1 and 2.

| Plant Species | Test | Diet Breadth | Animal | Pattern | | | Reference |
|---------------|-------|--------------|---------------------------------|---------|----|---|-----------|
| | | | | P | F1 | P | |
| S. c. S. p. | pref. | G | Lb. (L. c) | ≡ | ≡ | ≡ | A |
| S. c. S. p. | pref. | G | Lb. (L. c) | = | ≡ | ≡ | A |
| S. c. S. p. | dens. | S | Lgs.(<i>P. pedunculi</i>) | = | ≡ | ≡ | C |
| S. c. S. p. | dens. | S | Lgs.(<i>P. bridgmanii</i>) | ≡ | ≡ | ≡ | C |
| S. c. S. p. | dens. | S | Lf. (<i>Phyllocolpa</i> . sp.) | = | ≡ | ≡ | C |
| S. c. S. r. | dens. | S | Lgs. (<i>P. pedunculi</i>) | = | ≡ | ≡ | C |
| S. c. S. r. | dens. | S | Lgs. (<i>P. pedunculi</i>) | = | = | ≡ | D |
| S. c. S. r. | dens. | S | Lgs. (<i>P. bridgmanii</i>) | = | ≡ | ≡ | B |
| S. c. S. r. | dens. | S | Lgm. (<i>I. capreae</i>) | = | ≡ | ≡ | B |
| S. c. S. r. | dens. | G | Bgm. (<i>D. rosaria</i>) | ≡ | ≡ | ≡ | B |
| S. c. S. r. | dens. | G | Lb. (Cr. f.) | ≡ | ≡ | ≡ | B |
| S. c. S. r. | dens. | - | Lb larvae | ≡ | ≡ | ≡ | B |

In accordance with my predictions, 2 of the 5 studied specialist species for the *S. repens* - *S. caprea* hybrid system showed preference for one of the parental species and discriminated against the F1 hybrid (*Ph. vitellinae* and *Ch. populi*, Table 2 and 4). Two of the specialists (*P. bridgmanii* and *I. capreae*, Table 3) did not discriminate between the F1 hybrid and the pure host (*S. caprea* in both cases) and one species showed variable results between different studies (*P. pedunculi*, Table 3 and 4). It seems that these species have not developed enough specificity to discriminate the hybrid as different from

the pure host species. This occurs either because there is no reduced fitness connected to choosing the F1 hybrid, because there has not been enough time to develop discrimination or because the cost for mistakenly rejecting the host plant is greater than the cost of accepting the non-host F1 hybrid (Bernays, 1998, 2001; Fox & Lalonde, 1993; Larsson & Ekbom, 1995; Moorehead, Taper & Case, 1993).

Table 4: This table shows the number of herbivore species that have shown the different response patterns (Table 1, 2, 3). The species are divided into specialists (Spec.) and generalists (Gen.) and the responses are divided between the different hybrid systems that have been studied. For further explanation and abbreviations see Table 1 and 2.

| Pattern | S. r * S. c | | S. p * S. c | |
|------------------|-------------|------|-------------|------|
| | Spec. | Gen. | Spec. | Gen. |
| == ≡ ≡ | - | 3 | - | - |
| == ≡ = | 1 | 1 | - | - |
| == = ≡ | 2 | - | - | 1 |
| = ≡ ≡ | 2 | - | 2 | - |
| ≡ ≡ ≡ | - | 1 | 1 | - |
| Variable results | 1 | 1 | - | 1 |

Three of the six studied species that are generalists show a response where F1 hybrids were intermediate to the parental species (Table 4). This may depend on the inheritance pattern of phenolics (paper II and I) and if they are responding in proportion to the concentration of either phenolic glucosides or condensed tannins. As these species generally respond negatively to phenolic glucosides, the former seems more likely. However, it is also possible that their response depends on a combination of plant traits.

Adding together the results from preference tests for all the studied herbivores, the results give some support to the observations of leaf damage. In nine of the 12 studied cases, hybrids were less resistant than the most resistant parental species and there were only three cases where the F1 hybrids were as resistant as the most resistant parental species (Table 4). In addition, because hybrids are used by herbivores from both parents, herbivores may potentially select against hybrids between *S. repens* and *S. caprea*. Similar results have been found in other herbivore-hybrid plant systems as well. Whitham, Morrow & Potts (1994) studied a hybrid zone between *Eucalyptus amygdalina* and *E. risdonii* and found higher density and diversity of herbivores and parasites on hybrids than on pure species. In addition, this study identified backcrosses as more susceptible than F1 hybrids. However, to know

that herbivores actually select against hybrids and thus potentially lead to the evolution of reproductive barriers between these species, we need to consider plant resistance and preference of herbivores as well as how herbivores affect the plant's fitness. hybridization occurs often in many plant families, which suggest that the fitness disadvantages could be out-weighted by advantages.

Conclusions and future research

In hybrids between *S. caprea* and *S. repens* secondary metabolites, such as condensed tannins, and phenolic glucosides follow an additive inheritance pattern. There was no common response of the studied herbivore community to hybrid plants. Instead, it seems that specialist herbivores either discriminate against hybrids and non-host parental species or do not separate between hybrids and host parental species. Generalists usually show intermediate preference for hybrids or no preference between hybrids and parental species. However, one species shows a preference that indicates a breakdown in resistance. When adding together the effects of the herbivore community, it appears that herbivores inflict more damage to hybrids than to parental species.

To further explore effects of hybridization on foraging behaviour in this system, we need more detailed studies on the effect of hybridization on plant traits. These studies should focus on secondary metabolites, primary metabolites, and morphology and how these influence herbivore foraging decisions, performance, and population dynamics. Furthermore, we need to determine the relationship between plant resistance characters and plant fitness. To connect observations from experiments to natural field conditions it is important to determine the influence of environmental factors on hybrids and parental species and hence experiments need to be performed as reciprocal transplant experiments.

It is also important to study natural plant populations to examine the frequency of hybridization and if there are any signs of introgression of traits between the parental populations. This is preferably studied using molecular population genetic analyses.

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References

- Aarssen, L. W. 1995. Hypothesis for the evolution of apical dominance in plants: implications for the interpretation of overcompensation. *Oikos* *74*, 149–156.
- Anderson, E. 1949. *Introgressive hybridization*. New York.
- Arnold, M. 1997. *Natural hybridization and evolution*. New York: Oxford University Press.
- Barton, N. H. & Hewitt, G. W. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* *16*, 113–148.
- Beart, J. L. Lilley, T. H. & Haslam, E. 1985. Plant polyphenols - secondary metabolites and chemical defese. *Phytochemistry* *24*, 33–38.
- Bernays, E. 1998. The Value of Being a Resource Specialist: Behavioral Support for a Neural Hypothesis. *The American Naturalist* *151*, 451–464.
- Bernays, E. 2001. Neural limitations in phytophagous insects: Implications for diet breadth and evolution of host affiliation. *Annual review of entomology* *46*, 703–727.
- Bernays, E. & Chapman, R. 1994. *Host-plant selection by phytophagous insects*. New York: Chapman & Hall.
- Close, D. C. & McArthur, C. 2002. Rethinking the role of many plant phenolics - protection from photo damage not herbivores? *Oikos* *99*, 166–172.
- Ehrlich, P. R. & Raven, . 1964. Butterflies and plants: a study in coevolution. *Evolution* *18*, 586–608.
- Fox, C. & Lalonde, R. 1993. Host confusion and the evolution of insect diet breadths. *Oikos* *67*, 577–581.
- Fraenkel, G. S. 1959. The *raison d'être* of secondary plant substances. *Science* *129*, 1466–1470.
- Fritz, R. Roche, B. & Brunsfeld, S. 1998. Genetic variation in resistance of hybrid willows to herbivores. *Oikos* *83*, 117–128.
- Fritz, R. S. 1999. Resistance of hybrid plants to herbivores: genes, environment, or both? *Ecology* *80*, 382–391.
- Fritz, R. S. Nichols-Orians, C. & Brunsfeld, S. 1994. Interspecific hybridiza-

- tion of plants and resistance to herbivores: hypotheses, genetics, and variable responses in a diverse herbivore community. *Oecologia* 97, 106–117.
- Fry, J. 1990. Trade-offs in fitness on different hosts: evidence from a selection experiment with a phytophagous mite. *The American Naturalist* 136, 569–580.
- Graham, J. McArthur, D. & Freeman, C. 2001. Narrow hybridzone between two subspecies of big sagebrush (*Artemisia tridentata*: Asteraceae): Galls on sagebrush in a reciprocal transplant garden. *Oecologia* 126, 239–246.
- Grant, P. & Grant, R. 1992. Demography and the genetically effective sizes of two populations of darwins finches. *Ecology* 73, 766–784.
- Gustavsson, B. 2002. *Catalogus Coleopterorum Sueciae*, 16/5. <http://www.nrm.se/en/catalogus.html.se>.
- Hewitt, G. M. 1988. Hybrid zones - natural laboratories for evolutionary studies. *Trends in Ecology and Evolution* 3, 158–167.
- Hjältén, J. 1997. Willow hybrids and herbivory: a test of hypotheses of phytophage response to hybrid plants using the generalist leaf-feeder *Lochmea caprea* (chrysomelidae). *Oecologia* 109, 571–574.
- Hjältén, J. 1998. An experimental test of hybrid resistance to insects and pathogenes using *Salix caprea*, *S. repens* and their f1 hybrids. *Oecologia* 117, 127–132.
- Hjältén, J. Ericson, L. & Roininen, H. 2000. Resistance of salix caprea, s-phylicifolia, and their f1 hybrids to herbivores and pathogens. *Ecoscience* 7, 51–56.
- Hochwender, C. G. Fritz, R. S. & Orians, C. M. 2001. Using hybrid systems to explore the evolution of tolerance to damage. *Evolutionary Ecology* 14, 509–521.
- Honkanen, T. & Haukioja, E. 1998. Intra-plant regulation of growth and plant-herbivore interactions. *Ecoscience* 5, 470–479.
- Ikonen, A. 2002. Preferences of six leaf beetle species among qualitatively different leaf age classes of three salicaceous host species. *Chemoecology* 28, 23–28.
- Jaenike, J. 1990. Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics* 21, 243–273.
- Jeremy, T. 1984. Evolution of insect/host plant relationships. *American Naturalist* 124, 609–630.
- Jolivet, P. & Hawkeswood, T. 1995. Backhuys publishers.
- Julkunen-Tiitto, R. 1986. A chemotaxonomic survey of phenolics in leaves of northern salicaceae species. *Phytochemistry* 25, 663–667.
- Julkunen-Tiitto, R. 1989. *Distribution of certain phenolics in Salix species (Salicaceae)*. Ph. D. thesis, University of Joensuu, Joensuu.
- Julkunen-Tiitto, R. & Sorsa, S. 2001. Testing the effects of drying methods on willow flavonoids, tannins, and salicylates. *Journal of chemical ecology* 27, 779–789.
- Karban, R. & Baldwin, I. 1997. *Induced responses to herbivory*. London: The University of Chicago Press.
- Keim, P. Paige, K. N. Whitham, T. G. & Lark, K. G. 1989. Genetic analyses of an interspecific swarm of *Populus*: Occurrence of unidirectional introgression. *Genetics* 123, 557–565.
- Kelly, M. & Curry, J. 1991. The influence of phenolic-compounds on the suit-

- ability of 3 *Salix* species as hosts for the willow beetle *Phratora vulgatissima*. *Entomologia experimentalis et applicata* 61, 25–32.
- Kennedy, G. & Barbour, J. 1992. Chapter Resistance variation in natural and managed systems, pp. 13–41. The University of Chicago Press.
- Koch, K. 1992. *Die Käfer Mitteleuropas, Ökologi*, Volume 3. Goecke & Evers.
- Kolehmainen, J. Julkunen-Tiitto, R. Roininen, H. & Tahvanainen, J. 1995. Phenolic glucosides as feeding cues for willow-feeding leaf beetles. *Entomologia experimentalis et applicata* 74, 235–243.
- Krebs, C. 1999. *Ecological methodology*. Menlo Park: Benjamin Cummings.
- Larsson, S. 2002. *Mechanisms and deployment of resistance in trees to insects*, Chapter Mechanisms and interactions - an overview. Dordrecht: Kluwer Academic Publishers.
- Larsson, S. & Ekblom, B. 1995. Oviposition mistakes in herbivorous insects: confusion or a step towards a new host plant? *Oikos* 72, 155–160.
- Lawton, J. & McNeil, S. 1979. Between the devil and the deep blue sea: on the problem of being a herbivore. pp. 223–244. Blackwell scientific publications.
- Lid, J. & Tande Lid, D. 1979. *Flora of Norway and Sweden (in Norwegian)*. Det Norske Samlaget.
- Lindroth, R. L. & Weisbrod, A. V. 1991. Genetic variation in response to aspen phenolic glycosides. *Biochemical systematics and Ecology* 19, 97–103.
- Mayr, E. 1969. *Principles of Systematic Zoology*. New York: McGraw-Hill.
- Mayr, E. 1996. What is a species, and what is not? *Philosophy of Science* 63, 262–277.
- Messina, F. Richards, J. & McArthur, E. 1996. Variable responses of insects to hybrid versus parental sagebrush in common gardens. *Oecologia* 107, 513–521.
- Moorehead, J. Taper, M. & Case, T. 1993. Utilization of hybrid oak hosts by a monophagous gall wasp: How little host character is sufficient? *Oecologia* 95, 385–392.
- Mouliat, C. 1999. Parasitism of plant and animal hybrids: are facts and fates the same? *Ecology* 80, 392–406.
- Orians, C. 2000. The effects of hybridization in plants on secondary chemistry: implications for the ecology and evolution of plant-herbivore interactions. *American journal of botany* 87, 1749–1756.
- Orians, C. & Fritz, R. 1995. Secondary chemistry of hybrid and parental willows: phenolic glycosides and condensed tannins in *Salix sericea*, *S. eriocephala*, and their hybrids. *Journal of chemical ecology* 21, 1245–1253.
- Orians, C. Griffiths, M. Roche, B. & Fritz, R. 2000. Phenolic glucosides and condensed tannins in *Salix sericea*, *S. eriocephala* and their fl hybrids: not all hybrids are created equal. *Biochemical systematics and ecology* 28, 619–632.
- Orians, C. Huang, C. Wild, A. Dorfman, K. Zee, P. Dao, M. & Fritz, R. 1997. Willow hybridization differentially affects preference and performance of herbivorous beetles. *Entomologia experimentalis et applicata* 83, 285–294.
- Pasteels, J. & Rowell-Rahier, M. 1992. The chemical ecology of herbivory on willows. *Proceedings of the Royal Society of Edinburgh section Biological sciences* 98, 63–73.
- Raubenheimer, D. & Simpson, S. J. 1999. Integrating nutrition: a geometrical

- approach. *Entomologia Experimentalis et Applicata* 91, 67–82.
- Roininen, H. Price, P. Julkunen-Tiitto, R. Tahvanainen, J. & Ikonen, A. 1999. Oviposition stimulant for a gall-inducing sawfly, *Euura lasiolepis*, on willow is a phenolic glucoside. *Journal of chemical ecology* 25, 943–953.
- Schoonhoven, L. Jermy, T. & van Loon, J. 1998. Chapman & Hall.
- Singer, M. Vasco, D. Parmesan, C. Thomas, C. & Ng, D. 1992. Distinguishing between 'preference' and 'motivation' in food choice: an example from insect oviposition. *Anim. Behav.* 44, 463–471.
- Soetens, P. Pasteels, J. Daloze, D. & Kaisin, M. 1998. Host plant influence on the composition of the defensive secretion of *Chrysomela vigintipunctata* larvae (coleoptera: Chrysomelidae). *Biochemical systematics and ecology* 26, 703–712.
- Soetens, P. Rowell-Rahier, M. & Pasteels, J. 1991. Influence of phenolglucosides and trichome density on the distribution of insect herbivores on willows. *Entomologia experimentalis et applicata* 59, 175–187.
- Stephens, D. W. & Krebs, J. R. 1986. *Foraging theory*. Monographs in behavior and ecology. Princeton, New Jersey: Princeton University Press.
- Strauss, S. 1994. Levels of herbivory and parasitism in host hybrid zones. *Trends in ecology and evolution* 9, 209–214.
- Tahvanainen, J. Helle, E. Julkunen-Tiitto, R. & Lavola, A. 1985. Phenolic compounds of willow bark as deterrents against feeding by mountain hare. *Oecologia* 65, 319–323.
- Tahvanainen, J. Julkunen-Tiitto, R. & Kettunen, J. 1985. Phenolic glycosides govern the food selection pattern of willow feeding leaf beetles. *Oecologia* 67, 52–56.
- Wait, A. D. Jones, C. G. & Coleman, J. S. 1998. Effects of nitrogen fertilization on leaf chemistry and beetle feeding are mediated by leaf development. *Oikos* 82, 502–514.
- Waterman, P. G. & Mole, S. 1994. *Analyses of phenolic plant metabolites*. Oxford: Blackwell Scientific.
- Whitham, T. 1989. Plant hybrid zones as sinks for pests. *Science* 244, 1490–1493.
- Whitham, T. Morrow, P. & Potts, B. 1994. Plant hybrid zones as centers of biodiversity: the herbivore community of two endemic tasmanian eucalypts. *Oecologia* 97, 481–490.
- Wink, M. 1999. *Functions of plant secondary metabolites and their exploitation in biotechnology*, Volume 3 of *Annual plant reviews*. Sheffield, England: Sheffield Academic Press.