Phenolic glycosides and condensed tannins in *Salix sericea*, *S. eriocephala* and their F1 hybrids: not all hybrids are created equal

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Received 26 May 1999; accepted 26 July 1999

Abstract

The performance of hybrids depends upon the inheritance and expression of resistance traits. Secondary chemicals are one such resistance trait. In this study, we measured the concentrations of phenolic glycosides and condensed tannins in parental and F1 hybrid willows to examine the sources of chemical variation among hybrids. *S. sericea* produces phenolic glycosides, salicortin and 2'-cinnamoylsalicortin, and low concentrations of condensed tannin in its leaves. In contrast, *S. eriocephala* produces no phenolic glycosides but high concentrations of condensed tannins in its leaves. These traits are inherited quantitatively in hybrids. On average, F1 hybrids are intermediate for condensed tannins, suggesting predominantly additive inheritance or balanced ambidirectional dominance of this defensive chemical from the parental species. In contrast, the concentration of phenolic glycosides is lower than the parental midpoint, indicating directional dominance. However, there is extensive variation among F1 hybrids. The concentration of tannin and phenolic glycosides in F1 hybrid families is either (1) lower than the midpoint, (2) higher than the midpoint, or (3) indistinguishable from the midpoint of the two parental taxa. It appears that the production of the phenolic glycosides, especially 2'-cinnamoylsalicortin, is controlled by one or more recessive alleles. We also observed a two-fold or greater difference in concentration between some hybrid families. We discuss how chemical variation may effect the relative susceptibility of hybrid willows to herbivores. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Salicaceae; Willows; Hybridization; F1 hybrids; Phenolic glycosides; Condensed tannins
1. Introduction

Hybridization is now regarded as a common feature of many plant species, and can lead to speciation and introgression (Rieseberg, 1991, 1995; Rieseberg and Brunsfeld, 1992; Rieseberg and Ellstrand, 1993; Ellstrand et al., 1996; Arnold, 1997). In the last 10 yr, there have been numerous studies suggesting that hybridization, via changes in resistance, also affects the ecology and evolution of plant-herbivore interactions (e.g., Whitham, 1989; Boecklen and Spellenberg, 1990; Whitham et al., 1991, 1994, 1999; Aguilar and Boecklen, 1992; Floate and Whitham, 1993; Paige and Capman, 1993; Fritz et al., 1994, 1996; Strauss, 1994; Messina et al., 1996; Orians et al., 1997; Fritz, 1999; Pilson, 1999). We know that the relative resistance of hybrids and parental taxa to herbivores is highly variable (reviewed by Strauss, 1994; Fritz, 1999), but what determines resistance is not well understood. Although multiple traits are involved, secondary chemicals, such as phenolics, terpenoids, alkaloids and glucosinolates, are often key components of the resistance of plants to herbivores (Rosenthal and Janzen, 1979; Roitberg and Isman, 1992; Bernays and Chapman, 1994; Cardé and Bell, 1995; Chew and Renwick, 1995). Given the general importance of secondary chemistry to plant-herbivore interactions, it is surprising that relatively few researchers have studied the secondary chemistry of hybrids in relation to plant-herbivore interactions (Huesing et al., 1989; Weber et al., 1994; Orians et al., 1997). We believe that knowledge of how secondary chemicals are expressed in hybrids will help researchers understand patterns of hybrid resistance.

The concentration of secondary chemicals in hybrids typically differs qualitatively and quantitatively from their parents (Connor and Purdie, 1976; Harborne and Turner, 1984; Meier et al., 1989; Altman et al., 1990; Levy and Milo, 1991; Rieseberg and Ellstrand, 1993; Orians and Fritz, 1995). Qualitatively, novel chemicals may be found or parental chemicals may be lost. Quantitatively, patterns of expression depend upon the genetic control of production. Parental chemicals may be overexpressed (over-dominance), underexpressed (incomplete dominance), intermediate (no dominance), similar to one of the parental species (dominance), or similar to both parents (co-dominance) (Van Brederode et al. 1974; Falconer, 1989). Quantitative chemical differences can affect the resistance of a plant (Huesing et al., 1989; Orians et al., 1997). What is lacking is an understanding of how secondary chemistry varies among hybrids in ways that could affect patterns of resistance to herbivores.

We have been studying the consequences of hybridization in willow for a number of years. Willows and other Salicaceous plant species produce two main secondary chemicals: phenolic glycosides and condensed tannins, both of which are known to influence the susceptibility of plants to insect and mammalian herbivores (Zucker, 1983; Rowell-Rahier, 1984; Tahvanainen et al., 1985a,b; Basey et al., 1988; Lindroth and Peterson, 1988; Lindroth et al., 1988; Clausen et al., 1989; Schultz, 1989; Denno et al., 1990; Kolehmainen et al., 1994; Orians et al., 1997). Some willow species produce only condensed tannins, others produce mostly phenolic glycosides, and others produce both classes of compounds (Julkunen-Tiitto, 1986, 1989; Orians and Fritz, 1995).
In our system, *S. sericea* produces high concentrations of phenolic glycosides and low concentrations of condensed tannin, while *S. eriocephala* produces high concentrations of condensed tannin but no phenolic glycosides (Orians and Fritz, 1995). The concentrations of both phenolic glycosides and condensed tannins are intermediate, not significantly different than the midpoint, in naturally occurring hybrids of these two species (Orians and Fritz, 1995). However, these hybrids were of unknown pedigree. What is the chemistry of known F1 hybrids? Is there chemical variation among F1 hybrids? How does the chemistry of experimental hybrids differ from natural hybrids? These are all questions that will help us understand how secondary chemistry might determine patterns of hybrid survival. Here we report on the concentrations of condensed tannin and phenolic glycosides in two year old seedlings of *Salix sericea*, *Salix eriocephala* and their F1 hybrids.

2. Materials and methods

2.1. Plants

Both *Salix sericea* Marshall and *Salix eriocephala* Michx. are abundant in the northeastern United States and eastern Canada (Argus, 1986). The species often co-occur and hybridization between the two is common (Argus, 1986; Mosseler and Papadopol, 1989; Fritz et al., 1994). Willows are dioecious and in this system the initial hybridization event is unidirectional (*S. eriocephala* is the paternal parent and *S. sericea* is the maternal parent), due to stigma-pollen incompatibility in female *S. eriocephala* (Mosseler, 1990).

2.2. Generation of seedlings (parental and F1 hybrid)

We performed intraspecific and interspecific crosses using known pure parents, based on RAPD analysis, to generate pure parental and F1 hybrid progenies. From a larger set of crosses (Fritz et al., 1998), we selected seven groups of progenies where the parents of each F1 hybrid cross were used in the matched intraspecific cross (Table 1). Our goal was to avoid using a parental clone twice. However, one *S. sericea* parent (s17) was involved in two crosses.

To ensure purity of each family, the female catkins were covered with mesh bags prior to stigma emergence to prevent visitation by insect pollinators. Bags were removed only while making crosses. The resulting seeds were mass planted in trays of MetroMix 360™ in late May, and, covered with white, shear seed cloth to maintain a high moisture level and to prevent accidental colonization of the trays by naturally dispersing willow seeds. The cloth was removed after about two weeks, when natural seed dispersal had ended. Four-week-old seedlings were transplanted into 3.7 l pots filled with our standard soil mixture (4 parts topsoil, 1 part peat moss, and 1 part vermiculite). Due to mortality, the number of siblings per cross varied (Table 1). The next spring the seedlings were transplanted into 7.4 l pots containing the same soil
Table 1
Crosses performed to generate related parental and F1 hybrid progeny

<table>
<thead>
<tr>
<th>Group</th>
<th>S. sericea</th>
<th>F1 Hybrids</th>
<th>S. eriocephala</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>s17 × s22 (n = 5)</td>
<td>s17 × e18 (n = 4)</td>
<td>e15 × e18 (n = 4)</td>
</tr>
<tr>
<td>II</td>
<td>s28 × s16 (n = 5)</td>
<td>s17 × ne36 (n = 5)</td>
<td>e23 × ne36 (n = 4)</td>
</tr>
<tr>
<td>III</td>
<td>s28 × s27 (n = 7)</td>
<td>s28 × ne35 (n = 2)</td>
<td>e23 × ne35 (n = 4)</td>
</tr>
<tr>
<td>IV</td>
<td>s37 × s55 (n = 3)</td>
<td>s37 × ne33 (n = 9)</td>
<td>e29 × e115 (n = 5)</td>
</tr>
<tr>
<td>V</td>
<td>s83 × s14 (n = 4)</td>
<td>s83 × ne37 (n = 5)</td>
<td>e3 × ne37 (n = 5)</td>
</tr>
<tr>
<td>VI</td>
<td>s1 × s95 (n = 5)</td>
<td>s1 × hhe46 (n = 4)</td>
<td>e64 × hhe46 (n = 4)</td>
</tr>
<tr>
<td>VII</td>
<td>s78 × S60 (n = 5)</td>
<td>s78 × hhe12 (n = 4)</td>
<td>e60 × hhe12 (n = 4)</td>
</tr>
</tbody>
</table>

Note: F1 hybrids within each group have a female in common with the Salix sericea progeny and male in common with the S. eriocephala progeny.

mixture mixed with 13 g of slow-release fertilizer (10 : 10 : 10 NPK). Plants were randomized within blocks.

Leaf samples for chemical analyses were collected on 18 July. Twelve fully expanded leaves were collected from each plant (the first two fully expanded leaves from each of six shoots). Leaves were cut at the petiole, placed on ice, transported to the lab, and vacuum-dried for at least 24 h. Vacuum-drying leaves has been shown to prevent the loss of both condensed tannin and phenolic glycosides (Orians, 1995). Once dry, leaves were ground to a fine powder using a Wiley Mill equipped with a size 30 mesh and then stored in a −20°C freezer.

2.3. Chemical analyses

The phenolic glycosides were assayed using standard techniques (Orians, 1995). Briefly, leaf powder (30 ± 3 mg) was extracted in cold MeOH (10 mg leaf powder/1 ml MeOH) with sonication for 10 min. Cold water was constantly flushed through the sonicator to prevent the heating of samples. We centrifuged and filtered (0.45 μm filter) each sample before placing extracts in crimp-top vials. Extracts were kept in the freezer until analysis (48 h or less). We quantified the concentration of glycosides with an HPLC equipped with an autosampler and a UV detector set at 274 nm (Hewlett-Packard). A reverse-phase NOVA-PAK C18 (4 μm) column (Waters) and a gradient system of distilled water and MeOH was used. 1,3-dimethoxybenzene was used as the internal standard. Standard curves were determined for salicortin and 2'-cinnamoylsalicortin.

The condensed tannins also were extracted using standard techniques (Orians, 1995). Briefly, approximately 300 mg leaf powder was washed with ether and extracted with 70% acetone for 3 h in a 40°C water bath. Acetone was removed under reduced pressure and all extracts were diluted to 8 ml with distilled water. The butanol/HCl method was used to quantify tannin concentrations (Hagerman and Butler, 1989). The concentration of the condensed tannin (mg/g dry leaf) was determined using purified tannins collected from the two willow species and the hybrids.
2.4. Statistical analyses

A one way analysis of variance was used to determine the overall effects of taxon on condensed tannins (using the means of each of the 7 families/taxon). The natural log of condensed tannin concentration was used in the analysis because variance increased with the mean. For the phenolic glycosides only *S. sericea* and hybrids were included in the model, because *S. eriocephala* generally does not contain phenolic glycosides.

Contrasts were performed to determine if the hybrids were significantly different from the midpoint of the two parental taxa (non-transformed data were used for the contrasts), that is, if one averages the parental values do the hybrids differ from that average. This was done using the means comparison contrasts in SuperANOVA (Abacus Concepts, 1989). These comparisons were performed with all taxa included in the model.

Separate analyses were done for each group of families (Table 1). As above, an analysis of variance was used to determine the effects of family (or taxon) on chemistry, and contrasts, as described above, were performed to determine if the concentrations of the chemicals in the hybrid family was significantly different from the average of the two parental families.

Regression analysis was used to determine the relationship between parental and hybrid chemistry, and between the production of phenolic glycosides and condensed tannins.

3. Results

In general, the concentrations of condensed tannins is high in *S. eriocephala*, low in *S. sericea* and intermediate in hybrids, while the concentration of the two phenolic glycosides shows the opposite pattern (Table 2). The concentrations measured in these plants are similar to that measured in field plants (unpublished data), and the patterns are similar to those reported previously (Orians and Fritz, 1995). In general, *S. eriocephala* does not produce phenolic glycosides in its leaves (see also Orians and Fritz, 1995). However, two of the four siblings of one *S. eriocephala* cross (e60 × hhe12) produced phenolic glycosides (data not shown in Table 2), and the four s78 × hhe12 siblings had levels of phenolic glycosides and tannins similar to *S. sericea*. Although these results suggest there is something unusual about hhe12, other crosses involving hhe12 (e64 × hhe12 and s57 × hhe12) were not unusual (unpublished data). Currently, we are unable to explain the anomalous results obtained with the hhe12 crosses.

Because of these anomalous results, we excluded crosses involving hhe12 before testing for taxon effects. Overall, there was a significant effect of taxon on chemistry and all three taxa were significantly different from one another (ANOVA, $F \geq 125.6$, $p \leq 0.0001$) (Table 2). Contrasts revealed significant deviation from the average (or midpoint) of the two parental taxa for salicortin ($p < 0.01$) and 2’-cinnamoylsalicortin ($p < 0.001$), but not for condensed tannins ($p = 0.99$). For both phenolic glycosides, the concentration was lower than the midpoint, indicating a small dominance deviation toward reduced expression.
Table 2
Mean concentration (MG/G dry leaf weight), standard errors (in parentheses), and coefficients of variation (cv) for condensed tannin and the phenolic glycosides in *S. sericea*, F1 hybrids and *S. eriocephala* leaves

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Chemical type</th>
<th>Condensed tannin</th>
<th>Salicortin</th>
<th>2' cinnsctn</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. sericea</em></td>
<td></td>
<td>27.8 (1.8) a</td>
<td>104.8 (4.1) a</td>
<td>18.5 (1.4) a</td>
</tr>
<tr>
<td></td>
<td>cv = 7.6</td>
<td>cv = 10.2</td>
<td>cv = 20.0</td>
<td></td>
</tr>
<tr>
<td>F1 hybrid</td>
<td></td>
<td>71.7 (11.9) b</td>
<td>48.0 (10.7) b</td>
<td>4.5 (2.4) b</td>
</tr>
<tr>
<td></td>
<td>cv = 19.7</td>
<td>cv = 33.5</td>
<td>cv = 44.1</td>
<td></td>
</tr>
<tr>
<td><em>S. eriocephala</em></td>
<td></td>
<td>126.6 (4.2) c</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>cv = 10.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: 2'-cinsctn = 2'-cinnamoylsalicortin. Different letters denote significant differences among taxa (p < 0.001).

In addition, there was more among-family variation for hybrids than for the parental taxa (see Coefficients of Variation in Table 2). This was especially evident for the phenolic glycosides. Some hybrid families produced very low concentrations of salicortin and 2'-cinnamoylsalicortin (26.3 and 0.6 mg/g dry leaf weight, respectively for Group IV) while other families produced relatively high concentrations (60.6 and 2.1 mg/g dry leaf weight, respectively for Group VI). Thus some families produce over twice the concentration of other families. If we include the s78 x hhe12 cross salicortin and 2'-cinnamoylsalicortin concentrations were even higher 106.7 and 18.4 mg/g dry leaf weight, respectively.

For each group of crosses, there was a highly significant difference among families (or taxa) ($F > 19.5$; $p < 0.001$) (Table 3). Based on our previous results, we expected that F1 chemistry would be indistinguishable from the midpoint of the parental taxa. However, contrasts revealed that some F1 hybrids were lower than the midpoint, while others were higher than the midpoint (Fig. 1). Excluding the cross involving hhe12 (Group VII), condensed tannin concentration was significantly different than the parental midpoint for 2 of 6 hybrid families, one higher and one lower. Salicortin concentration was significantly lower than the midpoint for 3 of 6 families. In contrast, 2'-cinnsalicortin concentrations were significantly lower for all 6 hybrid families. For Group VII, condensed tannin was lower and the phenolic glycosides were higher than the parental midpoint (Fig. 1). Generally, the production of phenolic glycosides, especially 2'-cinnamoylsalicortin, showed directional dominance or recessive deviations.

There were significant positive correlations between parental and hybrid chemistry. The concentration of condensed tannins in the hybrids was positively correlated with the concentration in the *S. sericea* parent ($R^2 = 0.79$, $p = 0.02$), but not with the *S. eriocephala* parent ($R^2 = 0.08$, $p = 0.59$). There was a positive correlation for 2'-cinnamoylsalicortin ($R^2 = 0.64$, $p = 0.05$). However, there was no correlation for salicortin ($R^2 = 0.04$, $p = 0.33$).
Table 3
Family mean (standard error) concentration, MG/G dry leaf weight, for condensed tannin and phenolic glycosides in S. sericea (SS), F1 hybrids and S. eriocephala (SE)

<table>
<thead>
<tr>
<th>Group</th>
<th>Family</th>
<th>Tannin</th>
<th>Salicortin</th>
<th>2’cinnslct</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>SS</td>
<td>s17 × s22</td>
<td>22.8(3.3) a</td>
<td>110.8 (7.2) a</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>s17 × e18</td>
<td>66.8 (10.3) b</td>
<td>43.9 (6.7) b</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>e15 × e18</td>
<td>122.4(4.7) c</td>
<td>NONE</td>
</tr>
<tr>
<td>II</td>
<td>SS</td>
<td>s17 × s16</td>
<td>20.8(0.6) a</td>
<td>121.2 (4.4) a</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>s17 × ne36</td>
<td>48.6 (10.1) b</td>
<td>39.3 (8.1) b</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>e25 × ne36</td>
<td>106.4(7.0) c</td>
<td>NONE</td>
</tr>
<tr>
<td>III</td>
<td>SS</td>
<td>s28 × s27</td>
<td>24.7(2.2) a</td>
<td>98.7 (6.2) a</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>s28 × ne35</td>
<td>87.4(7.1) b</td>
<td>31.0 (9.5) b</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>e25 × ne35</td>
<td>113.0(3.0) c</td>
<td>NONE</td>
</tr>
<tr>
<td>IV</td>
<td>SS</td>
<td>s37 × s55</td>
<td>25.3(4.6) a</td>
<td>97.4 (6.3) a</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>s37 × ne33</td>
<td>86.1(8.0) b</td>
<td>26.3 (3.9) b</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>e29 × e115</td>
<td>122.4(5.5) c</td>
<td>NONE</td>
</tr>
<tr>
<td>V</td>
<td>SS</td>
<td>s83 × s14</td>
<td>24.7(3.5) a</td>
<td>88.9 (8.9) a</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>s83 × ne37</td>
<td>70.5 (10.8) b</td>
<td>28.6 (4.5) b</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>e3 × ne37</td>
<td>117.5(3.3) c</td>
<td>NONE</td>
</tr>
<tr>
<td>VI</td>
<td>SS</td>
<td>s1 × s95</td>
<td>22.6(1.6) a</td>
<td>105.4 (7.6) a</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>s1 × hhe46</td>
<td>72.8(4.4) b</td>
<td>60.6 (6.6) b</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>e64 × hhe46</td>
<td>143.9(4.8) c</td>
<td>NONE</td>
</tr>
<tr>
<td>VII</td>
<td>SS</td>
<td>s78 × S60</td>
<td>21.4(0.9) a</td>
<td>111.2(7.9) a</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>s78 × hhe12</td>
<td>15.8(1.1) a</td>
<td>106.7(4.2) a</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>e60 × hhe12</td>
<td>116.1 (13.5) b</td>
<td>24.9 (17.3) b</td>
</tr>
</tbody>
</table>

Note: 2’cinslet = 2’-cinnamoylsalicortin. Different letters denote significant differences among families within each group (p < 0.001).

Among hybrids we found an inverse correlation between the concentration of tannin and salicortin (Fig. 2) and between tannin and 2’-cinnamoylsalicortin (\(Y = 78.5 - 3.3X, R^2 = 0.41, p < 0.001\)). These negative correlations suggest that hybrids may not be able to produce high concentrations of both phenolic glycosides and condensed tannins (see also Orians and Fritz, 1995). Interestingly, many F1 hybrids contained low concentrations of both condensed tannin and salicortin (Fig. 2).

4. Discussion

Most secondary chemicals are under polygenic control and inherited in a quantitative fashion (Berenbaum and Zangerl, 1992), as we have found for both salicortin and...
Fig. 1. The proportional deviation from the parental midpoint. (A) Values above the midpoint indicate that condensed tannin concentrations deviate toward *S. eriocephala*. (B) and (C) Values above the midpoint indicate salicortin and 2'-cinnamoylsalicortin concentrations deviate toward *S. sericea*. NS: not significant (* p < 0.05; ** p < 0.01.

2'-cinnamoylsalicortin (Orians et al., 1996). As a consequence most parental chemicals are found in hybrids (Table 2; Rieseberg and Ellstrand, 1993). However, the concentration in the hybrids can be quite variable (Harborne and Turner, 1984). Our results show that on average F1 hybrids contain intermediate concentrations of condensed tannin (equal to the parental midpoint), suggesting predominantly additive inheritance or a lack of dominance of this defensive chemical from the parental species. In contrast, the concentration of the phenolic glycosides, salicortin and 2'-cinnamoylsalicortin, is lower than the parental midpoint, indicating incomplete dominance over production. This incomplete dominance suggests that one or more alleles that control phenolic glycoside production, especially 2'-cinnamoylsalicortin, are recessive in the F1 hybrid. Altman et al. (1990) also found recessive control of the production of the terpenoid raimondal in cotton hybrids; compared to the parental taxon, the concentration in hybrids was 9–12%.

There have been a number of studies evaluating the genetics of secondary chemical production by F1 hybrids of contrasting species. These studies have shown that the
patterns of secondary chemical expression is variable. Of the chemicals present in both parents and hybrids, the concentration of 38% were similar to one or both of the two parental taxa (dominance or co-dominance), 29% were intermediate to the two parental taxa (incomplete dominance or no dominance), 19% were greater than either parent (over-dominance), and 14% were less than either parent (underexpression) (Fahselt and Ownbey, 1968; Belzer and Ownbey, 1971; McMillan et al., 1975; Harborne and Turner, 1984; Spring and Schilling, 1990; Buschmann and Spring, 1995; Orians and Fritz, 1995). Parental chemicals are not always expressed in the F1 hybrids. Current estimates suggest that parental chemicals are found only 68% of the time, lost 27% of the time and novel 5% of the time in the hybrids (Rieseberg and Ellstrand, 1993).

However, none of the above-cited studies looked at variation among F1 hybrids. We found extensive variation among F1 hybrid families (Table 2). Some hybrid families produced concentrations higher or lower than the midpoint (incomplete dominance), concentrations similar to one of the parental taxa (dominance, Group VII), or levels indistinguishable from the parental midpoint (no dominance). Furthermore, there was extensive variation among hybrid families. For example, the concentration of salicortin in one family was over twice that of a second family (Group VI vs. IV, Table 3). Parental production explained some of the differences in production of condensed tannin and 2'-cinnamoylsalicortin by hybrids families; there was a positive correlation between related parent and hybrid families. Surprisingly, the
concentration of condensed tannin in hybrids was not correlated with *S. eriocephala*, but with *S. sericea*, the parental taxon that produces low concentrations. It is not clear why this occurred. No correlation was observed for related parents and hybrids for salicortin. Although genetic differences among parental individuals are the most likely explanation for the variation in concentration, non-genetic maternal factors could be important as well. Further work is required to differentiate between genetic and maternal effects.

The negative correlation between condensed tannin and phenolic glycosides in hybrids suggests that F1 hybrid offspring are not able to produce high concentrations of both condensed tannin and phenolic glycosides (Fig. 2). We reported a similar trade-off previously (Orians and Fritz, 1995). The basis of this trade-off between tannins and phenolic glycosides remains unknown. There are two possible explanations. First, there may be a resource allocation trade-off such that only a certain amount of carbon is allocated to these carbon-based defenses. Production of one would limit the production of the other. However, this explanation does not explain the low production of both chemicals in some hybrids. Second, there may be a genetic tradeoff. If specific alleles control the production of both chemicals, one chemical may be produced at the expense of the other (= negative pleiotropy). Again, this explanation does not explain the reduced production of both chemical types in some individuals. Perhaps the reduced production is due to heterozygosity in some parents. This could lead to low production of one or both chemicals in some hybrids.

Additional work is required to determine if this trade-off is due to resource allocation constraints or to genetic constraints. The analysis of later generation hybrids could provide critical information. Unless there is strong genomic selection preventing reorganization (Rieseberg et al., 1995, 1996), F2 recombinants could contain all the alleles responsible for the production of both chemicals. These individuals would be able to produce high concentrations of both chemicals, unless there is a resource allocation constraint. We are currently designing experiments with F2 recombinants to test these alternative hypotheses. Furthermore, if we do identify F2 recombinants that produce high concentrations of both, we could examine the costs of chemical production (defense). Reduced growth of these individuals relative to those with lower concentrations would suggest a trade-off between growth and defense.

4.1. Implications

This study clearly documents extensive variation among F1 hybrids, and this variation may affect the hybrid survival. Phenolic glycosides, for example, are important deterrents of herbivores, especially generalist herbivores (Rowell-Rahier, 1984; Tahvanainen et al., 1985a; Orians et al., 1997). Slugs and Japanese beetles, for example, are dominant generalist herbivores of seedlings and adults, respectively, and both are inhibited by phenolic glycosides (Orians et al., 1997; Fritz et al., unpublished data). Therefore, selection may favor hybrids that produce higher concentrations of phenolic glycosides.
Unfortunately, few studies have evaluated the importance of secondary chemistry. Previously, we showed the phenolic glycoside chemistry of naturally occurring hybrids was not significantly different than the midpoint of the two parental taxa (Orians and Fritz, 1995; Orians, unpublished data). In contrast, in this study, we show that the concentration of phenolic glycosides in F1 hybrids was lower on average than the midpoint. Also, the concentrations reported here are lower than in our previous studies of naturally occurring hybrids (Orians and Fritz, 1995; Orians, unpublished data). Taken together, these results suggest that recombinant hybrids that produce higher concentrations of phenolic glycosides may be more likely to survive. If true, it would be the first example of selection on hybrid chemistry. We are currently experimentally testing the role of chemistry in the resistance of hybrids.

Are herbivores generally selective filters on the fitness of hybrids? Unfortunately, there is little empirical work on this question. Our work and that of others has demonstrated extensive variation in the secondary chemistry of hybrid plants, and we believe that such variation can affect hybrid fitness.

Overall, our understanding of the effects of hybridization on plant–herbivore interactions is in its infancy. There is debate over the role of herbivores in plant hybridization, and the effects of hybridization on community structure, host range expansion, and evolution of herbivore virulence (Whitham, 1989; Floate and Whitham, 1993; Fritz, 1999; Pilson, 1999; Whitham et al., 1999). Secondary chemistry is unquestionably an important determinant of plant–herbivore interactions, and we believe more careful attention to chemistry will help predict the ecological and evolutionary consequences of hybridization.

Acknowledgements

We give thanks to Len and Ellie Sosnowski for permitting us to conduct experiments on their property, Rachel Samberg for help with the chemical analyses, and Diana Pilson for her thoughtful discussion of the results. This research was supported by the National Science Foundation (Grant No. DEB 92-07363), a Science Education Grant from the Howard Hughes Medical Institute, the Faculty Research Awards Committee (Tufts University). This work was completed while CMO was a Mellon Grant Research Fellow at Tufts University.

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