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Interspecific hybridization of plants and resistance to herbivores: hypotheses, genetics, and variable responses in a diverse herbivore community

Abstract We studied the morphology, molecular genetics, and herbivory of two species of willows (*Salix sericea* and *S. eriocephala*) and their interspecific hybrids to test four alternative hypotheses concerning the effects of hybridization on plant resistance. Individually marked plants were identified using morphological traits in the field and measurements of stipule and leaf pubescence were made and compared using Canonical Discriminant Function Analysis. DNA was extracted from the leaves of a sample of the marked plants and RAPD-PCR analysis was performed to establish the genetic status of parental and hybrid plants. RAPD band analysis generally verified the genetic status of parental plants. Hybrid plants were usually correctly identified in the field with a few exceptions. However, the hybrid plants were a heterogeneous group of plants made up of most plants that appear to be F_1s and a few plants that appear to be backcrosses to *S. sericea*. Morphological variables were useful for distinguishing *S. sericea* from *S. eriocephala* and hybrids, but were not as dependable in distinguishing between *S. eriocephala* and hybrids. We compared the densities of 11 herbivore species and the infection by a leaf rust pathogen (*Melampsora* sp.) on the leaves and stems of two parents and the hybrids in the field. We found support for the Additive hypothesis (3 species), the Dominance hypothesis (2 species) and the Hybrid Susceptibility hypothesis (7 species, 6 herbivores and the *Melampsora* rust). We found no evidence for the Hybrid Resistance hypothesis. Guild membership was not a good predictor of similar responses of species to hybrid versus parental plants. A Canonical Discriminant Function Analysis showed discrete separation of the taxa based on herbivore densities, illustrating different community structures on hybrid and parental plants. This study demonstrates the diversity of responses of phytophages in response to interspecific hybridization.

Key words Hybridization · Herbivores · RAPD Host plant resistance · Willow

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Introduction

Natural plant hybridization presents unique opportunities for studying the processes of speciation and adaptation (Stebbins 1950, 1959; Grant 1981; Barton and Hewitt 1985; Hewitt 1988; Rieseberg and Brunsfeld 1992). The extent of hybridization between plant species and the commonness of hybrid individuals in some populations and locations indicates that interactions of hybrid plants with communities of herbivores and pathogens could be a key element of plant-phytophage ecology and evolution. Analysis of herbivory on hybrid plants in natural populations is recently gaining attention (Whitham 1989; Keim et al. 1989; Paige et al. 1991; Boecklen and Spellenberg 1990; Whitham et al. 1991; Aguilar and Boecklen 1992; Whitham et al. 1994), and it may be important for understanding plant-herbivore interactions (Whitham 1989). To assess the significance of interspecific hybridization of plants for plant-herbivore interactions it will be important to know how interspecific hybridization of plants influences: 1) the resistance of hybrid plants 2) the distribution of herbivores among pure parents and hybrids, and 3) the evolution of herbivore virulence and thus the process of coevolution.

There are several alternative hypotheses of phytophage response to hybrid plants. We specifically conceive of these hypotheses as comparing pure parent species to F_1 hybrids. However, the patterns observed in other systems may apply to a heterogeneous group of hybrids or to advanced generation backcrosses. The

Fig. 1A–D Hypothesized patterns that would result from the Additive Hypothesis (**A**), the Dominance Hypothesis (**B**), the Hybrid Susceptibility Hypothesis (**C**), and the Hybrid Resistance Hypothesis (**D**). Imaginary densities (Susceptibility) are plotted on the Y-axis for each parent species and their F_1 interspecific hybrid
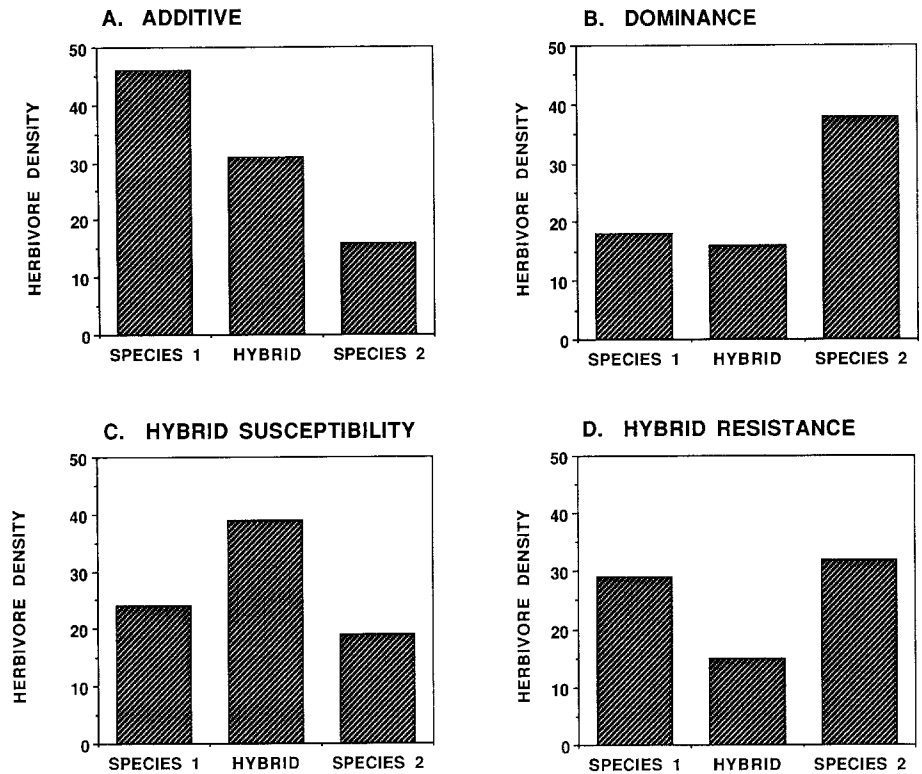
reason we focus on F_1 hybrids first, is that this comparison could suggest: 1) how resistance mechanisms are being inherited from the parental species and 2) how these traits may be expressed upon backcrossing. Boecklen and Spellenberg (1990) stated that a null hypothesis is that of no difference in susceptibility between hybrid and parental taxa. We believe that their hypothesis is not a null hypothesis but, rather, is a special case of a more general hypothesis that we call the Additive hypothesis (Fig. 1A). This hypothesis predicts that hybrids will not differ from the mean of the resistances of the two parents. Thus F_1 hybrids are intermediate between the resistance of the parental species. This suggests that hybrid resistances are due to the additive inheritance of resistance traits from both parents.

The second hypothesis, not discussed by previous authors, we call the Dominance hypothesis (Fig. 1B). If hybrid resistance differs significantly from the mean resistance of both parents, but it does not differ significantly from that of one of the parents the Dominance hypothesis would be supported. Hybrid resistance could resemble that of either the more resistant or the more susceptible parent. (Dominance in this context refers to phenotypic similarity between a parent and hybrids, not necessarily genetic dominance, though it may imply dominant inheritance of resistance traits.)

The third hypothesis is the Hybrid Susceptibility hypothesis (the Hybrids-as-Sinks hypothesis of Whitham 1989; see also Keim et al. 1989; Boecklen and Spellenberg 1990). This hypothesis predicts higher herbivore densities and/or higher herbivore performance on hybrids compared to parental taxa (Fig. 1C). Whitham's

(1989) hypothesis refers especially to advanced generations of backcrossed hybrids, which distinguishes it from our hypothesis. For the Hybrid Susceptibility hypothesis to be distinguished from the Dominance hypothesis the susceptibility of the hybrid must exceed that of the most susceptible parent. In mixed stands of parental and hybrid plants, this hypothesis predicts that a larger than expected fraction of the herbivore population would reside on hybrid individuals (Drake 1981; Whitham 1989; Floate et al. 1993). Whitham (1989) referred to highly susceptible hybrid poplars as being an ecological sink for a galling aphid, but in terms of metapopulation dynamics, unless herbivore fitness on hybrids was also much lower, hybrid plants could function as a "source" rather than a sink, although hybrids might serve both as sinks and sources (but see Paige and Capman 1993 who suggest that backcross hybrids are no more susceptible than one of the parents).

Our final hypothesis, the Hybrid Resistance hypothesis, predicts that hybrid plants will be more resistant than either parent, resulting in lower herbivore densities on hybrid plants (Fig. 1D). This hypothesis requires that hybrids be more resistant than the most resistant parent. Boecklen and Spellenberg (1990) found support for this hypothesis for herbivore communities in an oak hybrid zone. Hybrids supported lower densities and diversities of herbivores than the mean of the parental species, but they did not distinguish between the fit of their data to the Hybrid Resistance hypothesis or the Dominance hypothesis. Since ultimately one function of studies of hybrid plant-herbivore interactions could be to infer the genetic mechanisms of resistance, we present

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and discuss these hypotheses in genetic terms. Support of any of these hypotheses may suggest a probable underlying mechanism of inheritance of resistance and thus testing these hypotheses can be a useful first step in investigation the genetics of resistance.

It is not clear a priori why one of these hypotheses should be more likely than the other. Because of the divergent results of Whitham (1989) and Boecklen and Spellenberg (1990) a controversy has developed over the perceived prevalence of these alternative hypothesized responses of herbivores to hybrids. If the Hybrid Susceptibility hypothesis is prevalent, as Whitham (1989) suggests, and herbivory reduces plant fitness, then herbivores could be important in maintaining distinct plant species by limiting the fitness of hybrids. Furthermore, if hybrids commonly act as "evolutionary sinks" to pests (Whitham 1989), then hybrid-herbivore interactions may be critical to understanding why plants seem to maintain pest resistance in the face of a greater evolutionary potential for virulence in pests and in understanding the evolution of pest virulence. (The "evolutionary sink" refers to the condition where herbivore fitness could be sufficiently higher on hybrids compared to parents that there is selection for specialization on the hybrids, thereby preventing selection for herbivore virulence on the parental species.) Boecklen and Spellenberg (1990) contend that Whitham's suggestion of a general model of hybrid susceptibility is premature. They found evidence for the Hybrid Resistance and for our Additive hypothesis. Boecklen and Spellenberg (1990) argue that geographic range of hybrid zones, environmental gradients, genetic status of hybrids (e.g. – F_1's vs backcrosses), directionality of backcrossing, age of the hybrid zone, and chemical and morphological similarity of parental species are all factors that will influence herbivore response to hybrid plants. We suggest that, additionally, the genetic mechanisms of inheritance of resistance mechanisms will determine the expression of resistance in hybrids. So far, genetic mechanisms of resistance have only gotten scant attention in this literature. Resolution of the patterns of herbivore responses to hybrid plants and the causes of these patterns could contribute significantly to understanding important ecological and evolutionary aspects of plant-phytophage interactions. Distribution patterns of herbivores among hybrid and parental taxa may be an important part of understanding the ubiquitous phenotypic variation of herbivore abundances among plants (Fritz 1992).

The purposes of this paper are to document hybridization between two willow species in nature, to present a preliminary RAPD analysis of the genetic status of the naturally occurring hybrids, to investigate the responses of a number of individual herbivore species from different guids to parental and hybrid willows. This paper will show that different herbivores, even those in the same guild, vary dramatically in their response to hybrid versus parental taxa but that a number of herbivore species fit the pattern suggested by the Hybrid Susceptibility hypothesis.

Material and methods

The system

The system we study has several advantages for studying herbivore-hybrid plant interactions. First, the two willow species that hybridize often co-occur in the same habitats with their hybrids, eliminating, to a certain extent, the confounding of environmental variation across naturally occurring hybrid zones with herbivore resistance variation among hybrids and parents (e.g. – Whitham 1989; Boecklen and Spellenberg 1990; Paige and Capman 1993). Second, willows have a number of herbivore species in different guilds, including gall-formers, leaf miners, leaf tiers, and chewers, that are well characterized and that have been studied extensively on one of the willow species in this system. Finally, willows can be cloned and crossed for more explicit tests of genetics of resistance of hybrids and parents.

Plants

Salix sericea Marshall and S. eriocephala Michx. co-occur in swamps and along streams in central New York. S. sericea is a 0.5–4 m high shrub that has lanceolate leaves with densely sericeous hairs on the lower leaf surface. Stipules are small, lanceolate, and usually absent on older leaf nodes (i.e. – stipules are deciduous). It predominantly occurs in swamps from Canada south through the northeastern U.S. and along the Appalachian range to Georgia (Argus 1986). Salix eriocephala is a shrub that reaches 6 m in height and its leaves are lanceolate to narrowly oblong and glabrous beneath. Stipules are large, persistent, half ovate, and half cordate at the base. The species frequently occurs along streams and its range extends from Canada south as far as Virginia and west to Missouri (Argus 1986). The ranges of the two species are broadly sympatric, and at the study site both species co-occur and intermingle. Species can be easily distinguished in the field based on leaf, stipule, and bud characteristics (Argus 1986).

These willow species hybridize to form plants that are usually distinctive from each parent. Large persistent, half ovate stipules (a S. eriocephala trait) and a sericeous (hairy) lower leaf surface (a S. sericea trait) serve as morphological markers to identify S. sericea × S. eriocephala hybrids in the field (Table 1). The natural hybrids at our study site do not appear to be extraordinary; S. eriocephala and S. sericea have been reported to hybridize throughout their range (Argus 1974, 1986; Mosseler and Papadopol 1989). We have found naturally occurring hybrids between these species at eight different sites in upstate New York. Specimens of parental species and some hybrid individuals have been confirmed by Dr. George W. Argus, National Museum of Natural Sciences, Ottawa, Canada. Both species are diploid (2n = 38, Dorn 1976, Brunsfeld unpublished). Flowering phenology may explain the occurrence of hybridization between S. sericea and S. eriocephala (Argus 1974; Mosseler and Papadopol 1989), which differ by only a few days in the onset of flowering (foliage phenology is also similar) (Fritz, pers. obs.).

Table 1 Traits used to distinguish parental and hybrid willow plants in the field

Trait	S. eriocephala	Hybrid	S. sericea
Stipule Size	Large	Large	Minute
Stipule Persistence	Persistent	Persistent	Deciduous
Leaf Pubescence	Glabrous	Hairy	Hairy

Herbivores and pathogen

Many herbivores attack the parental and hybrid willows. Some species attack primarily *S. eriocephala* and others attack primarily *S. sericea*, but with these few exceptions most species attack both parents and hybrids. Gall-forming sawflies (Hymenoptera: Tenthredinidae) include *Phyllocolpa nigrita, Phyllocolpa* sp. nov., *Phyllocolpa terminalis*, and *Pontania* sp. (leaf galls). Gall-forming flies (Diptera: Cecidomyiidae) include the stem gallers *Rabdophaga rigidae*, the beaked willow gall; *R. salicisbrassicoides*, willow cabbage gall; and a leaf galler, *Iteomyia salicifolia*. The other common leaf galler species is the gall mite *Aculops tetanothrix* (Acarina: Eriophyidae). There are two species of leaf miners, *Phyllonorycter salicifoliella* and *Phyllocnistis* sp. (Lepidoptera: Gracillariidae) that commonly form mines on willow leaves.

There are two frequently seen leaf folder species (Lepidoptera) that have distinctive leaf folds, but have not yet been successfully reared or identified. One species folds over and sews the leaf margin to the lamina with silk. The other species forms a tube of the leaf tip sewing the edges together. In this paper we do not consider damage by leaf chewers, but experimental results of beetle choice tests of parental and hybrid willows will be presented elsewhere.

In addition to the herbivores, a leaf rust pathogen (*Melampsora* sp.) forms orange-yellow uredia on the lower leaf surface from the middle to end of the growing season on hybrids an occasionally on parents. Infections spread quickly on plants from older to younger leaves via urediospores with heavy infections causing stunted shoot growth and death of large numbers of leaves (Jokela 1966). Resistance of *Populus deltoides* and willows to *Melampsora* has been shown to be heritable (Jokela 1966; Widin and Schipper 1976; Verwijst 1990).

Methods

These studies were performed during 1991 at the Sosnowski site 3 km west of Milford, NY along County Route 44. To document the morphological differences among hybrids and parents we measured stipule persistence (proportion of nodes on each shoot with stipules remaining) on 5 shoots per plant on 11 *S. eriocephala*, 7 *S. sericea*, and 19 hybrids. We then removed one stipule from node 6 on each shoot and measured stipule length, width, and distance from the point of attachment to the shoot to the widest part of the base of the stipule. Stipules on *S. eriocephala* and hybrids are wide and cordate on one side whereas stipules on *S. sericea* are lanceolate. We calculated the ratio of length to width as a new shape variable. We selected five leaves of the same age from different shoots on each plant and counted the densities of leaf hairs in 5 squares of a 100 square grid of an optical grid in a dissecting microscope. These data were used in a Canonical Discriminant Function Analysis to determine the degree of separation between hybrids and parents.

Because morphological data is likely to be unreliable in distinguishing F_1 hybrids from backcrosses, and also may not indicate whether parents are free of introgression, we performed a genetic analysis of 45 plants using Randomly Amplified Polymorphic DNA (RAPD), following the methods of Williams et al. (1990) and Welsh and McClelland (1990). RAPD markers are dominant markers, since heterozygotes cannot be distinguished from homozygotes. Total DNA was extracted from fresh leaf tissue using a modified CTAB method previously described (Brunsfeld et al. 1992). Eighty 10-mer primers of random sequence (Operon Technologies, Kits A, B, C, D) were tested using a subset of the parental DNA samples. Primers producing clear markers that distinguished the species were further tested on a larger sample of parents, including several from different populations distant from the study site. Finally 20 putative hybrids were tested using only primers that produce diagnostic markers. Markers that were polymorphic within species were not used in this analysis. Each plant was scored for presence or absence of each parental marker. Hybrid index values (distances from *S. sericea*) were calculated as

follows: "pure" *S. sericea* were given a score of 0; the presence of any *S. eriocephala* marker or the lack of any *S. sericea* marker increased the index value up to a maximum of 1 for "pure" *S. eriocephala*. F_1 plants would theoretically have an index of 0.5 and possess all markers from both parents. Backcrosses are expected to lack a portion of the markers from one species. The presence of unique markers from both parents identifies the presence of a hybrid, however, with the few markers (12) that we used in this analysis the exact genetic classification of hybrid plants is less certain (i.e. – F_1 versus backcross 1(BC1)). The absence of markers from another species, however, does not confidently establish that the plant being considered is "pure". The "pureness" of our parents is certain only within the limits of the number of markers we have identified. More markers are being sought to increase our confidence in the status of parents.

Censuses of herbivores species on the marked on 13 *S. eriocephala*, 29 *S. sericea*, and 22 hybrids were conducted during late July and early August 1991. Galls, leaf mines, or leaf folds were counted on 50 shoots per plant for hybrids and *S. eriocephala* and on 300 shoots for *S. sericea*. (The larger number of shoots censused on *S. sericea* is because these plants are part of a longer term analysis of herbivore population dynamics.) Because leaf folds of *Phyllocolpa nigrita* and *Phyllocolpa* sp. nov. were not distinguished on *S. sericea* we combined them for these analyses. All other species densities are considered separately. We performed multivariate and univariate ANOVAs on $\log_e$ transformed mean densities per 300 shoots. We first performed Pearson correlations among densities of herbivores on hybrids an parents separately $((11 \times 10)/2 = 55$ correlations per taxon) to determine if there are significant correlations of herbivore responses to willow taxa. We found, using sequential Bonferroni analysis (Rice 1989) of the correlations within the willow taxa (we considered taxa to be independent), that only one of 135 possible correlations was significant. The significant correlation was between *Phyllocolpa* spp. and *Phyllocolpa terminalis* on *S. sericea*. Therefore species densities were considered statistically independent among plants within taxa, and we applied species-wide significance tests rather than tablewide significance tests. We performed only two orthoganol contrasts to avoid overparameterization of the analysis, since only three taxonomic groups were present (SAS Institute, 1985). We first tested the significance of the hybrid-parent contrast using sequential Bonferroni analysis at $P \leq 0.05/2 = 0.025$. This is a test of the Additive hypothesis. If this test was significant (i.e. – we rejected the Additive hypothesis) we then tested the contrast between herbivore density on hybrids and density on the numerically closest parent at $P \leq 0.05/1 = 0.05$. This contrast tested if there was a significant departure from the Dominance hypothesis. If the Dominance hypothesis was rejected we inspected the means to determine if the Hybrid Susceptibility or Hybrid Resistance hypothesis was supported.

Results

Genetic analysis of hybrid and parental DNA using RAPD

Our preliminary studies indicate that RAPDs are powerful tools for characterizing hybrids between *S. sericea* and *S. eriocephala*. In our survey of 80 primers we detected numerous markers unique to each parent. However, many of these markers are polymorphic within species (particularly those in *S. eriocephala*), limiting their usefulness in quantifying the genetic composition of putative hybrids. For this preliminary analysis, we chose 10 primers that produce 12 unambiguous marker loci: 8 are present in "pure" *S. sericea* and absent in "pure" *S. eriocephala*, and 4 are present in "pure" *S.*
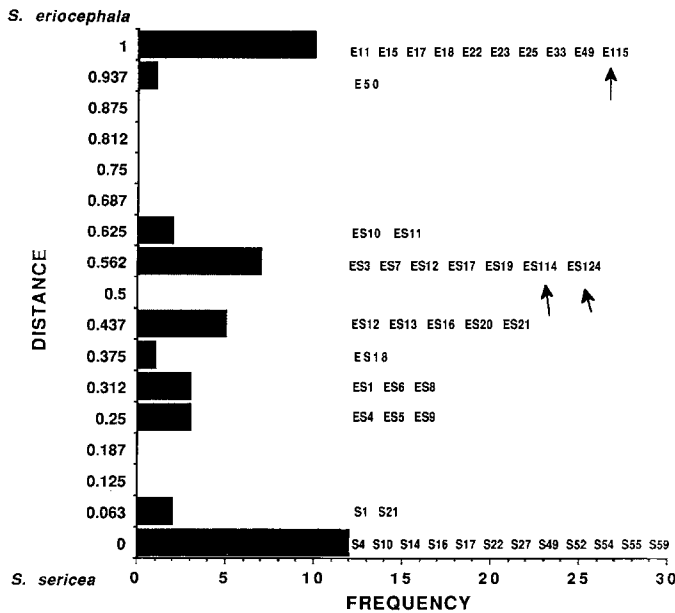
Fig. 2 Frequency distribution of individuals versus the hybrid index derived from the RAPD band data. The numbers of the individual plants are given to the right for each plant that was evaluated using RAPD. Note the arrows which indicate where plants bearing E or ES numbers had been misidentified in the field. The figure shows clusters of the pure or nearly pure parents (index = 0 or 1), a cluster of probable F_1 hybrids (index = 0.437–0.562), and probable backcrosses to *S. sericea* (index = 0.250–0.312). Other intermediate plants may be complex backcrosses, F_2's, or were not discretely classified because of having too few RAPD bands to classify

Fig. 3 Plot of canonical variables 1 and 2 resulting from a canonical discriminant function analysis of morphological data of the hybrids and parents

eriocephala and absent in "pure" *S. sericea*. Based on these markers, 45 plants analyzed from the Sosnowski site were given hybrid index scores and are plotted on Fig. 2. Because there are twice as many *S. sericea* markers, each was given half the weight of a *S. eriocephala* marker.

RAPD analysis corroborated the field identification of twelve plants as "pure" *S. sericea* – all were uniform with respect to the 12 genetic markers (Fig. 2). Two additional plants (S1, S21) differ only in lacking one of the eight *S. sericea* markers, neither had *S. eriocephala* markers. S21 is noteworthy because it had herbivore densities similar to a hybrid, see below. This plant does not possess any *S. eriocephala* markers, but has a number of other genetic differences that warrant additional study. Nine plants identified in the field as *S. eriocephala* also appear to be pure based on RAPDs. One additional plant (E50) has a single *S. sericea* marker but is otherwise identifiable as *S. eriocephala*.

No plant exhibited perfect marker additivity expected in an F_1, but twelve plants deviated by only one character (a distance from *S. sericea* of 0.437 to 0.562) and are thus interpreted as F_1-types. Three other plants deviate from F_1 by only two characters (0.375–0.625, Fig. 2). We also classify these plants as F_1-types, given the limited number of markers in this preliminary analysis. These plants might be segregating offspring from F_2 crosses, but an alternative explanation is that these
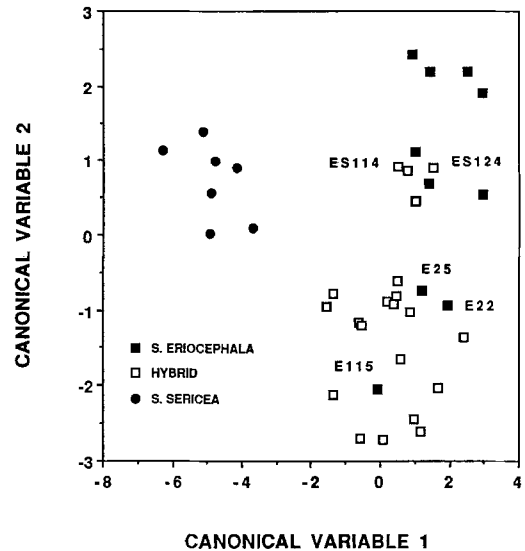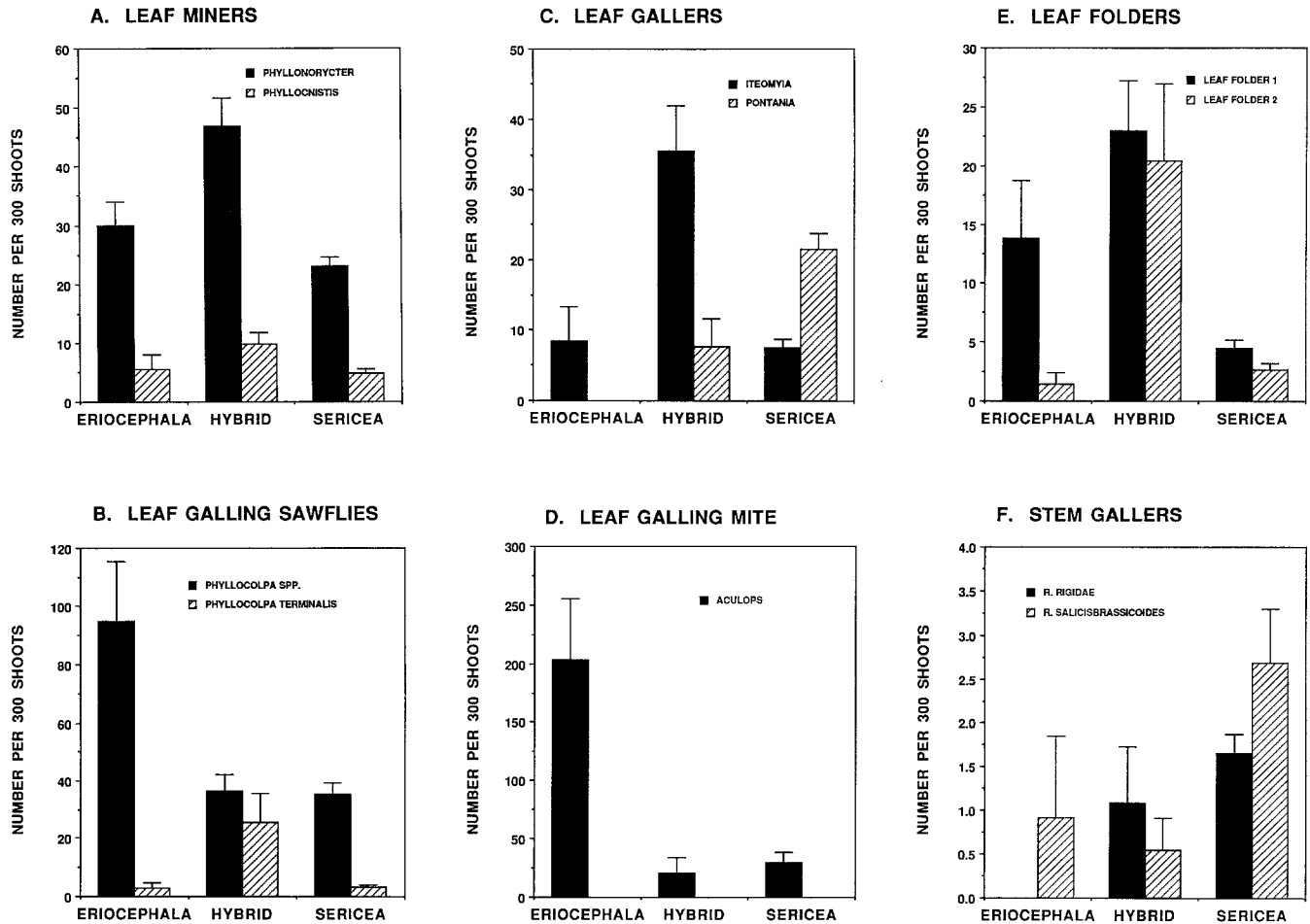
marked loci could have been heterozygous in the parents, and that the dominant marker allele was not inherited in the hybrid. Six plants (Fig. 2) have a distance of 0.250–0.312 from *S. sericea*, and are here interpreted as backcrosses (probably BC_1).

Two plants (ES114, ES124) were identified as *S. eriocephala* in the field, but appear to be F_1 based on RAPD analysis. Both plants have morphological characters similar to *S. eriocephala* (Fig. 3), but ES124 has an herbivore community characteristic of a hybrid. For further analyses these two plants are treated as hybrids.

Morphological analysis of hybrids and parents

Using stipule traits and leaf hair densities the Canonical Discriminant Function Analysis (Fig. 3) showed fair separation between parental species and between the hybrids and each parent. *S. sericea* formed a distinct cluster which was primarily separated along canonical variable 1, which is influenced by having a more lanceolate stipule and by having leaf hairs. *S. eriocephala* and the hybrids are separated mostly along canonical variable 2, which is influenced mostly by stipule width and degree of asymmetry of stipule shape. Between *S. eriocephala* and the hybrids there was considerable overlap (Fig. 3). Four hybrid plants were on the edge of the *S. eriocephala* cluster. Two of these plants ES114 and ES124 had originally been marked as *S. eriocephala* in the field, but RAPD analysis and subsequent field examination (Fritz pers. obs.) confirmed that they were hybrid plants. Three *S. eriocephala* (E25, E22, and E115) are located in the main cluster of hybrid plants. These plants appear to be pure *S. eriocephala* based on RAPD data. E115 was originally marked as an hybrid but RAPD analysis and subsequent field examination (Fritz

A. LEAF MINERS

B. LEAF GALLING SAWFLIES

C. LEAF GALLERS

D. LEAF GALLING MITE

E. LEAF FOLDERS

F. STEM GALLERS

pers. obs.) confirmed that it was a *S. eriocephala*. These data show that there is only a fair level of confidence in identifying hybrids and *S. eriocephala* properly using stipule traits but that some plants were originally misclassified or were misidentified in the field.

Fig. 4A–F Plots of the mean densities ($\pm$1SE) of herbivores on plants classified as *S. eriocephala*, *S. sericea*, or hybrids based on morphological criteria in 1991. Species pairs are grouped by guild. **A** Leaf miners, **B** Leaf galling sawflies, **C** Leaf gallers, **D** Leaf galling mite, **E** Leaf folders, and **F** Stem gallers. See Table 2 for results of orthogonal contrasts from ANOVA for significant differences between bars

Herbivore and pathogen responses to hybrids and parents

Because the RAPD analysis demonstrated that hybrids plants were heterogeneous group of F1-types and backcrosses, we first tested whether there were significant differences in the densities of herbivores between the F_1-types and backcrosses. ANOVA showed that there were no significant differences between F_1-types and backcrosses in herbivores densities. Furthermore, there were no significant differences in the subsequent contrasts between hybrids and parents whether backcrosses were included or not. Therefore, were combined all of the hybrid plants together. There was one exception to this finding, however. Density of *P. terminalis* was significantly lower on the 6 backcross plants than on the F_1-types ($F_{1,60} = 17.23$, $P < 0.001$). None of the backcross plants had any *P. terminalis*. Because the mean density of *P. terminalis* was significantly higher on hybrids than

on either parent, even with the 6 backcross plants included, we combined all hybrid plants together, which resulted in a more conservative test of the other hypotheses.

Multivariate ANOVA showed a highly significant contrast between hybrids and the parent species (Table 2), thus, overall, hybrids differed significantly from the mean density of the two parents. This is, therefore, a rejection of the Additive hypothesis. Univariate ANOVA's showed 7 of 11 significant hybrid vs. parents contrasts at $P < 0.025$. Contrasts for *Phyllocolpa* spp. (Fig. 4B), *Pontania* sp. (Fig. 4C), and *R. rigidae* (Fig. 4F) were clearly not significant, therefore these species support the Additive hypothesis. *Phyllocnistis* sp. (Table 2) had a P-value close to significant ($P = 0.037$) marginally indicating a departure from the Additive hypothesis.

Five of the seven species that showed significant hybrid vs. parent contrasts also had densities on hybrids

Table 2 Summary of multivariate and univariate ANOVAs of herbivore densities on *S. sericea*, *S. eriocephala*, and hybrid field plants. Degrees of freedom and F-values for the multivariate ANOVAs are based on Wilks' criterion (other criteria gave identical results)

Species Guild	Hybrid vs. parents		Hybrid vs nearest parent	
	$F_{(1,61)}$	P	$F_{(1,61)}$	P
Multivariate (df = 11,51)	11.22	0.001	–	–
Leaf Miners				
Phyllonorycter salicifoliella	12.27	0.001	6.41	0.014
Phyllocnistis sp.	4.57	0.037	5.04	0.028
Leaf Gallers				
Phyllocolpa spp.	3.60	0.063	0.77	0.384
Phyllocolpa terminalis	5.44	0.023	6.18	0.016
Pontania sp.	0.80	0.375	15.03	0.001
Iteomyia salicifolia	17.68	0.001	17.77	0.001
Aculops tetanothrix	8.84	0.004	1.09	0.302
Leaf Folders				
Leaf folder 1	9.53	0.003	4.45	0.039
Leaf folder 2	20.80	0.001	10.11	0.002
Stem Gallers				
Rabdophaga rigidae	0.82	0.370	2.18	0.145
Rabdophaga salicisbrassicoides	5.63	0.021	0.01	0.932
Total Herbivores (with mites)	0.16	0.694	3.93	0.052
Total Herbivores (without mites)	13.98	0.001	3.94	0.052

that exceeded that of the numerically highest parent (Table 2), therefore these species support the Hybrid Susceptibility hypothesis. *Phyllocnistis* sp., which had a marginally significant hybrid vs. parents contrast, also had a mean density on hybrids that was greater than that of the nearest parent. Therefore, this species can be considered on balance to support the Hybrid Susceptibility hypothesis. *Phyllonorycter salicifoliella* and *Phyllocnistis* sp. (Fig. 4A), *Phyllocolpa terminalis* (Fig. 4B), both leaf folders (Fig. 4E), and *Iteomyia salicifolia* (Fig. 4C) had significantly higher densities on hybrids than on the parental species. These species represent three different guilds (leaf gallers, leaf miners, and leaf folders). The leaf miner and leaf folder guilds had both species represented, and four of the six species are moths, which might suggest a taxonomic similarity in response to hybrids. Within the leaf galler guild, however, two species (*Phyllocolpa* spp. and *Pontania* sp.) supported the Additive hypothesis, two species (*Iteomyia salicifolia* and *Phyllocolpa terminalis*) supported the Hybrid Susceptibility hypothesis, and one species (*Aculops tetanothrix*) supported the Dominance hypothesis. The leaf galler guild therefore does not demonstrate a consistent pattern of herbivore responses to hybrids.

Two herbivores had densities on hybrids that did not differ from those on one of the parents, and therefore, the resistances of hybrids to these species supported the Dominance hypothesis. These species are unrelated (*Aculops tetanothrix* (Fig. 4D) and *Rabdophaga salicisbrassicoides* (Fig. 4F)), are in different guilds (leaf gall vs. stem gall), and the densities on the hybrids matched that

of a different parent. That is to say, densities of *R. salicisbrassicoides* on hybrids was similar to densities on *S. eriocephala* (Fig. 4F), while densities of *A. tetanothrix* on hybrids were similar to densities on *S. sericea* (Fig. 4D). These data demonstrate three very different responses of herbivores to hybrids versus parental plants, supporting three of the four hypotheses. Only the Hybrid Resistance hypothesis was not supported by our data.

To visualize the overall response of herbivores to hybrids vs. parents we summed densities of herbivores on each plant and reanalyzed the responses. When mites were included in the analysis there was no significant hybrid vs. parent contrast (Table 2). Figure 5 shows that total density was almost exactly intermediate between the densities on the parents, and therefore these data support the Additive hypothesis. Because mite densities on *S. eriocephala* were equal to the sum of all other species combined we removed mites from the data and reran the analysis to see if it altered the result. When mites were excluded there was a highly significant hybrid vs. parent contrast (Table 2), and the comparison between hybrids and *S. eriocephala* was also significant. Thus, with mites removed, total herbivore load on hybrids supports the Hybrid Susceptibility hypothesis (Fig. 5). This latter result seems consistent with the multivariate result (Table 2) showing a significant overall hybrid vs. parents contrast.

Melampsora rust infection was far greater on hybrids than on either parent (Fig. 6), supporting the Hybrid Susceptibility hypothesis. Rust infection (percentage of leaves with rust uredia) was nearly 15% on hybrid
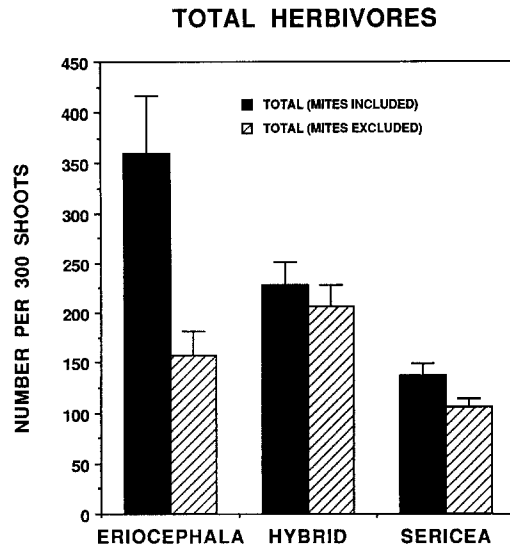
TOTAL HERBIVORES

Fig. 5 Plot of the total densities of herbivores with mites included and excluded. See Table 2 for results of orthogonal contrasts from ANOVA for significant differences between bars
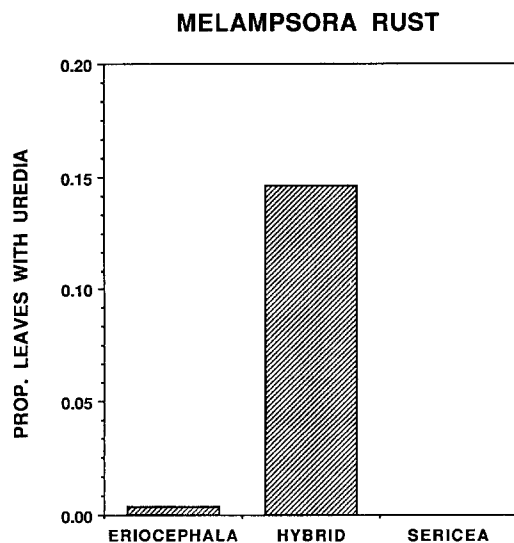
MELAMPSORA RUST

Fig. 6 Plot of the proportion of leaves with uredia of *Melampsora* rust on *S. eriocephala*, *S. sericea*, and hybrids

Table 3 Standardized canonical coefficients for each herbivore species for canonical variables 1 and 2, and eigenvalues and proportion variance explained by the two variables

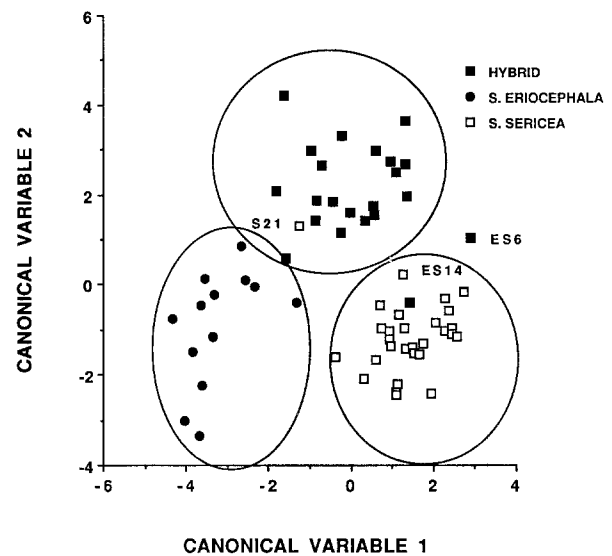| Herbivore Species	Canonical Variable 1	Canonical Variable 2
Phyllocolpa spp.	−0.3181	−0.7376
Phyllocolpa terminalis	−0.0400	−0.3201
Pontania sp.	1.4020	−0.2724
Iteomyia salicifolia	0.4174	0.5908
Aculops tetanothrix	−0.6985	−0.5081
Rabdophaga rigidae	0.1238	−0.5218
Rabdophaga salicisbrassicoides	0.1494	−0.3913
Phyllonorycter salicifoliella	−0.1138	0.3565
Phyllocnistis sp.	0.0871	0.0114
Leaf folder 1	−0.1608	0.6418
Leaf folder 2	0.3453	0.6290
Eigenvalues	3.1325	2.3544
Proportion	0.571	0.429

Fig. 7 Plot of canonical variables 1 and 2 resulting from a canonical discriminant function analysis of herbivore density data of the hybrid and parent plants in the field

plants, but was virtually zero on both parents. All hybrid plants contained some degree of rust infection, whereas most parents had no uredia on their leaves.

The communities of herbivores on the three taxa differed markedly (Fig. 7). The Canonical Discriminant Function analysis showed nearly complete separation among the taxa using species' densities. Separation between *S. eriocephala* and *S. sericea* along canonical variable 1 was due to positive coefficients for *Pontania* sp, and *Iteomyia* sp., and negative coefficients for mites, and *Phyllocolpa* spp. (Table 3). The separation along canonical variable 2 was due to positive coefficients for *Iteomyia*, and leaf folders and negative coefficients for mites, *Phyllocolpa* spp., and the stem gallers (Table 3).

Both variables in the discriminant analysis contributed substantially to the separation among taxa in community structure. About 57% of the variation was explained by variable 1 and about 43% of the variation was explained by variable 2 (Table 3). As expected from the differing responses of the herbivores to hybrids versus parents, community structure differs substantially due to interspecific hybridization.

There were three interesting exceptions to the generally excellent overall separation between communities among the three taxa (Fig. 7). One hybrid (ES14) fell in the range of most of the *S. sericea* values. S21 fell within the range of the hybrids; this plant had most of the *S. sericea* markers based on the RAPD analysis, but it appears genetically different based on other polymorphic loci not scored in this analysis. Since we did not collect leaf samples for DNA extraction and RAPD

analysis from ES14 and it subsequently died in 1992, we cannot determine if there were mistakes in identifying this plant.

Discussion

Although *S. sericea*, *S. eriocephala* and their hybrids can usually be readily recognized in the field, RAPD analysis revealed considerable complexity in the genetic structure of the intermingled parental and hybrid willow populations. *Salix sericea* is by far the most abundant willow at the study site, and all of the samples we analyzed appear genetically "pure" or nearly so. *Salix eriocephala* also occurs at the site, and many of our samples appear to be free of introgression or nearly so. All but one of the plants identified in the field as hybrids were confirmed as such by RAPD analysis. However, hybrids were found to be heterogeneous in their collection of parental markers. Although no plant had the complete additivity of markers expected in an F_1, many of the hybrids had a majority of the 12 parental markers. We interpret these plants as F_1-types, recognizing that the heterogeneity in these plants may result from segregation of parental markers that are possibly heterozygous or from interbreeding among hybrids. Our future studies will increase the number of RAPD markers employed to identify genotypes, which will improve our ability to classify plants based on their genetic composition. Future analysis of progeny from controlled crosses should also provide further insights into the interpretation of natural hybrids, especially if there is evidence of heterozygosity of RAPD marker loci.

Given the large number of *S. sericea* at the site, backcrossing of hybrids to this parent would be expected. Indeed, RAPD analysis revealed that six of the hybrids have an assemblage of markers that could be the result of F_1's backcrossing to *S. sericea*. Alternatively, these plants could have arisen from an F_2 cross.

RAPD data also revealed evidence of hybridization not suspected from morphology. Two plants considered to be *S. eriocephala* based on their stipules and pubescence were found to be close to F_1's in the genetic analysis. This appears to illustrate segregation of the few morphological traits that distinguish the species, and indicates that morphology may not necessarily reflect the genetic contribution of the two parental species. Our future work will explore the relationship between genetic data and the chemical composition of hybrids, which may be important in determining herbivory patterns.

The goals of this study were to examine the densities of members of a diverse community of phytophages on hybrid and parent willows. The main conclusions that we draw from our results are: 1) for many of the herbivore species there are highly significant differences in resistance of hybrids compared to parental species, 2) there is no uniform response of all herbivores to hybrids (three different hypotheses were supported), and 3) guild membership did not necessarily lead to similar responses to hybrids and parents. The hypotheses of Fig. 1 have as an assumption that hybrids are F_1's. The plants that were censused are predominantly F_1's based on our RAPD analysis (Fig. 2), but 6 plants are most closely identified as backcrosses to *S. sericea*. However, with only one exception, there were no differences in the herbivore densities on the F_1's and backcrosses. With this genetic heterogeneity as a caveat we will interpret our census results in terms of their fit to the hypotheses.

The Hybrid Susceptibility hypothesis was supported by six herbivore species from various taxa and by the rust pathogen (Fig. 4A–C, E, and Fig. 6). Also, when densities of the herbivores were combined, with the exception of mites, then total herbivore density supported the Hybrid Susceptibility hypothesis. Thus, Whitham's hypothesis is supported in part by our results. However, densities of three species did not differ from expectations of the Additive hypothesis (Table 2). In these cases densities of herbivores on hybrids were intermediate to those of both parents. The Dominance hypothesis was supported by the a stem galler (Fig. 4F) and the leaf galling mite (Fig. 4D). For these two species densities on the hybrid resembled the resistant parent in both cases (but this was a different species in each case).

The Dominance hypothesis has not been presented and tested previously (e.g. – Boecklen and Spellenberg 1990). However, this hypothesis is particularly plausible because of the inheritance of defensive chemicals in hybrids of several crop and wild plants. For *Lotus* (O'Donoughue et al. 1990), *Nicotiana* (Huesing et al. 1989), and *Papaver* (Levy and Milo 1991) chemical defense mechanisms have been shown to be inherited as dominant traits in hybrids. If the parent possessing the high levels of defensive chemical is resistant then F_1 hybrids should also be resistant. Thus, the suggestion by Boecklen and Spellenberg (1990) that differences between hybridizing species in chemical defenses should affect the responses of herbivores to hybrids seems justified. Paige and Capman (1993) showed that hybrid breakdown in resistance to *Pemphigus betae* only occurs in backcrosses of *Populus* hybrids to the susceptible parent, and F_1 hybrids are as resistant as the resistant *P. fremontii*. This illustrates the Dominance hypothesis in the cottonwood-aphid system.

Guild membership was not a reliable indicator of similar responses of herbivores to hybrids and parents. Among the leaf galling guild three different hypotheses were supported, and the stem gallers responded differently to the parents with two hypotheses being supported (Table 2, Fig. 4F). Leaf folders and leaf miners did show similar responses to hybrids and parents. Our results cast doubt on the advisability of a priori combining the densities of species in the same guild for hybrid-parent comparisons (e.g. – Boecklen and Spellenberg 1990; Aguilar and Boecklen 1992). Hypothetically, two species that showed opposite responses to hybrids and parents (one with Hybrid Susceptibility and the other with Hybrid Resistance) might seem to support the Additive hypothesis if densities were combined.

Our results suggest that interspecific hybridization of plants does not lead to a uniform pattern of breakdown of hybrid resistance. It does suggest that there are numerous resistance traits of plants that might have different mechanisms of inheritance and/or that different resistance factors could affect different herbivores (results of Maddox and Root 1990 support a similar conclusion). A dominantly inherited trait from one parent would be fully expressed in hybrids and could result in a pattern of herbivore resistance that supported the Dominance hypothesis. Backcrossing to the susceptible parents, as seems to have occurred in cottonwoods, could lead to the loss of one or more dominant resistance genes, thereby resulting in susceptibility of the backcross progeny. Paige and Capman (1993) suggest that there is more than one dominant gene involved in resistance to aphids in the cottonwood system. Additively inherited, polygenic traits could result in intermediate dosages of resistance traits in hybrids. Depending on the sensitivity of particular herbivores to the resistance factor Additive or the Hybrid Susceptibility hypothesis could be supported. Herbivores that responded linearly to dosage of some resistance trait, like a chemical, could show an Additive response to hybrids in relation to parental species. Herbivores that require a threshold amount of a chemical defense could have higher densities on hybrids (Hybrid Susceptibility) if the dosage was less than the threshold amount. Hybrids with dominant inheritance of different resistance traits from each parent could be more resistant than either parent (i.e. – Hybrid Resistance). These alternative mechanisms suggest that various outcomes of interspecific hybridization on resistance are likely and that mechanisms of inheritance of resistance need to be investigated in hybrid systems. This could result in a better understanding of resistance mechanisms in plants.

A major difference between this study and those of Whitham (1989), Paige et al. (1990, 1991) and Keim et al. (1989) is the genetic status of the hybrids. Our hybrid plants are mostly F_1-types (although not exclusively), whereas the susceptible hybrid plants in the cottonwood system are from the backcross 2 or later generations (Paige and Capman 1993). The difference in genetic status of hybrids in these studies has important implications for the generality of altered resistance of hybrid plants in nature and in suggesting the possible genetic mechanisms of hybrid breakdown in resistance. First, while all cases of hybridization begin with the formation of F_1 hybrids, backcrossing and introgression will not occur in all groups due to F_1 sterility, F_2 breakdown, or ecological factors (e.g. *Quercus*; Nason et al. 1992). Interspecific and intergeneric hybridization appears to be extremely common; Knobloch (1972) was able to list 23,675 putative cases. In only a small fraction of these cases, however, has introgression been rigorously documented (Rieseberg and Wendel 1993). Thus the full extent of introgression is still uncertain. If, however, hybrid breakdown in resistance routinely occurs in F_1 plants then the phenomenon could be widespread, and if herbivores reduce plant fitness then herbivores may be important in maintaining species differences among plants.

Second, hybrid breakdown in resistance in F_1 plants supports a hypothesis of additively inherited resistance traits. Monogenic and polygenic resistance traits where allele effects on traits are additive will have half the dosage of alleles in hybrids compared to parents, unless parents have similar defense mechanisms determined by the same loci. There is evidence from willows supporting additive inheritance of phenolic glycosides, tannins, and morphological defenses (Soetens et al. 1991; Meier et al. 1989; and Nichols-Orians and Fritz, unpublished data). How the altered dosage of defensive traits influences herbivores will depend on the sensitivity of the herbivores to the resistance trait and the presence of other resistance traits inherited from species that the herbivore might not normally use as a host. For two species of *Pontania* on willow (Soetens et al. 1991, and this study) gall numbers are intermediate on hybrids (supporting the Additive hypothesis) and gall numbers are greatest on the parent species with the highest concentration of phenolic glycosides. Studies on sawflies on birches (Hanhimäki et al. 1994), beetles on elms (Hall and Townsend 1987), and beetles on willow (Soetens et al. 1991; and Fritz unpublished) also indicate that hybrids are intermediate in suitability for herbivore growth. Mosseler (pers. comm.) reports that in his garden of F_1 hybrids and parents of seven *Salix* species, that hybrids were highly susceptible to *Melampsora* rust but that parents were immune. This observation shows, at least for *Melampsora*, that F_1's appear to show breakdown of resistance, consistent our results and with the additive inheritance model of hybrid breakdown described above.

A further consequence of additive inheritance of resistance is that backcrossing should result in a reconstitution of defense rather than a breakdown of resistance as found by Whitham (1989) and Paige and Capman (1993) for a resistance trait that is apparently dominantly inherited. The resistances of backcross individuals should more closely resemble that of the recurrent parent as more parental genes are reincorporated into the genome. Whitham et al. (1994) found evidence for this in work on eucalypts in Tasmania. When F_1-type hybrids were compared with backcross plants and parents, often backcross plants were intermediate between F_1's and parents. We did not find this to be true for this study, where 6 backcross plants were compared to the F_1-type plants. Whitham et al. (1994) propose the "phenotypic affinity hypothesis" to describe the predicted increased similarity of backcross and parental phenotypes, which is a simple correlary of the Additive hypothesis.

Much of the current interest in herbivory on hybrid plants has been generated because of studies in hybrid zones. To one degree or another in these studies herbivore densities on plants were sampled over an environmental gradient and environmental variation could di-

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rectly or indirectly have affected herbivore abundance on hybrid and parent plants. Whitham (1989) reported densities of galling aphid over a 60 km elevational transect along the Weber River in northern Utah. Environmental effects due to elevation could have contributed to some of the variation in aphid response to hybrids and parents. Paige and Capman (1993) show that some of the variation in susceptibility of hybrid cottonwoods to aphids is due to environment and they suggest that environmental effects were more important than genetic differences between hybrids and parents. Boecklen and Spellenberg (1990) studied herbivore density on oak hybrids and parents across two hybrid zones. One hybrid zone had a narrow zone of overlap and the other hybrid zone had a broad zone of overlap. It was only for the oaks with the narrow hybrid zone (on a 45 degree slope) where they found significantly lower densities of herbivores on hybrids versus parents. In the broad hybrid zone, where hybrid and parent plants were sampled from the same area, they found no significant differences in density between parents and hybrids. Environmental gradients could have been directly responsible for different densities on herbivores on hybrids compared to parents by restricting some herbivores from the narrow range where hybrids were located. Hybrids often occur in habitats that are marginally suitable for parents (Grant 1981). Hybrid plants occurring in this narrow overlap zone could be under stress, which could either make them less suitable for herbivores or could make them more suitable depending on herbivore responses to plant stress. Whitham et al. (1994) was able to study eucalypt hybrids and parents in close proximity where is was less likely that environmental variation was important.

S. eriocephala and *S. sericea* have commonly share the same habitat with their interspecific hybrids. Thus, we are not studying herbivore responses in a typical hybrid zone. At our field site each hybrid plant was found adjacent to *S. eriocephala* and *S. sericea*. At seven other field sites we have also found hybrids coexisting with both parents. Therefore, it seems unlikely that environmental variation is either directly or indirectly responsible for the differences in herbivore densities and pathogen incidence on these willow hybrids compared to parents. Ultimately, it will be important for experiments to be performed where hybrid plants of known genetic status (identified with molecular markers or from crosses) are grown in common gardens with parents and exposed to herbivory. Hanhimäki et al. (1993) have studied herbivore performance on hybrid and parental birches that grew in a common plantation in Finnish Lapland. Of the 14 herbivore species studied (13 sawflies and a moth), 11 species either had no difference in growth rate on hybrid versus parental leaves or if growth differed between parents then hybrids were intermediate. Those results fit the Additive hypothesis of no differences of herbivore performance on hybrids versus the mean of the parents. For three species, the moth and two sawflies, performance on hybrids did not differ

on that of one parent but did differ from the other parent, supporting the Dominance hypothesis.

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