Transgenerational effects of herbivory in a group of long-lived tree species: maternal damage reduces offspring allocation to resistance traits, but not growth

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Summary

1. Numerous studies have explored plant strategies of resource allocation to growth and/or resistance traits within a single generation. In contrast, exceedingly little is known about whether such patterns hold *across* generations; that is, in seedlings of plants that experienced maternal herbivory.

2. In a common garden study with clonally replicated genotypes of three cottonwood taxa (*Populus angustifolia*, *Populus fremontii* and their F_1 hybrids), we examined transgenerational response to maternal herbivory in terms of half-sibling seedling offspring (i) germination and growth and (ii) constitutive vs. transgenerational plastic allocation to resistance (measured as both phytochemical content and concentration). Two major results emerged.

3. First, we found that taxa (and often genotypes within a taxon) significantly differed in their constitutive allocation to both growth and resistance. Fremont (*P. fremontii*) seedlings grew up to seven times more rapidly than did narrowleaf (*P. angustifolia*) seedlings and had higher or similar content of two key phytochemical resistance traits. Overall, this led to a dilution effect in Fremont relative to narrowleaf, whereby concentrations of two key phytochemical resistance traits were more than 50% lower.

4. Secondly, maternal herbivory by cottonwood leaf beetle larvae on foliage adjacent to developing seeds did not significantly alter offspring growth, but did decrease offspring phytochemical content by 10–55% relative to offspring of maternal control (undamaged) trees. As a result, concentrations of offspring phytochemical resistance traits were reduced by 10–18% in seedlings with maternal herbivory, relative to maternal control seedlings, across all three taxa. These patterns suggest an allocational trade-off, whereby maternal damage results in maintenance of offspring seed size and growth traits at the expense of phytochemical defences in the next generation.

5. *Synthesis:* This is the first instance in which transgenerational effects of herbivory on growth and defence traits have been described in long-lived, woody plant species. *Populus* differs substantially from herbaceous plant species or short-lived animals in which transgenerational plasticity of resistance has been examined, in terms of life history (time from germination or hatching to reproductive maturity) and/or in the lag time between generations. These differences may influence the ecological and evolutionary relevance of transgenerational plasticity in defence.

Key-words: cottonwood, herbivory, plant–herbivore interactions, plasticity, *Populus*, resistance, transgenerational

Introduction

Herbivores impose strong selective pressures on plants, influencing plant reproductive fitness, distribution and abundance.

A variety of plant defence mechanisms, including multiple forms of resistance, have evolved in response to herbivory (e.g. Marquis 1992; Mauricio & Rausher 1997). If resources are limiting, allocation of resources to resistance is expected to carry a cost, insofar as those resources are necessarily diverted away from growth, reproduction or numerous other

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plant functions (Mauricio 1998; Purrington 2000; Koricheva 2002; Strauss *et al.* 2002; Ivey, Carr & Eubanks 2009).

Plasticity of resource allocation varies in accordance with plant life history and defence strategy. A low level of constitutive resistance, with the capacity for induced resistance, allows plants to allocate the maximum amount of resources to growth and differentiation-related processes unless resistance is necessary (Stamp 2003). The following conditions should promote the evolution of induced responses to herbivory: genetic variation in induction, decreases in plant fitness due to herbivory, reduction in herbivory levels that outweigh costs of induced resistance and cues that are reliable predictors of future herbivory (Karban & Adler 1996; Karban *et al.* 1999). In contrast, response to herbivory may involve induced susceptibility (reduced resistance) if plants invest resources in (re)growth or reproduction rather than resistance (Underwood 1998; Strauss & Agrawal 1999; Simonsen & Stinchcombe 2007).

Allocational costs of resistance may be magnified in seedlings, which initially depend on the reserves stored in their seeds for growth, or in juvenile and other rapidly growing developmental stages, which have greater limitations on the amount of resources that they can acquire and store (Herms & Mattson 1992; Hanley *et al.* 2004; Boege & Marquis 2005; Goodger, Choo & Woodrow 2007; Elger *et al.* 2009; but see Barton & Koricheva 2010). For this reason, seedlings might utilize induced resistance more often than constitutive (McKey 1979; Zangerl & Berenbaum 1990; Karban & Baldwin 1997; Agrawal & Karban 1999; Cipollini, Purrington & Bergelson 2003; Zangerl 2003). Likewise, tolerance has been predicted to be lowest in seedlings and highest in mature plants, but empirical evidence to date does not necessarily support these predictions (Barton & Koricheva 2010).

Our knowledge of allocational patterns to growth and resistance to herbivory is generally limited to the context of a single plant generation, although documentation of transgenerational effects of herbivory (or simulated herbivory) is becoming increasingly common (Agrawal 2001, 2002; Holeski 2007; Steets & Ashman 2010; Herrera & Bazaga 2011; Scoville *et al.* 2011; Rasmann *et al.* 2012; Verhoeven & van Gurp 2012). Transgenerational effects of herbivory, where the offspring of damaged plants have altered levels of defence and/or resources relative to the offspring of control plants (i.e. transgenerational plasticity), transcend the traditional discrete boundaries of constitutive vs. induced defences, and introduce several new considerations to the dynamic theory of the ecology and evolution of defences (Bonduriansky & Day 2009; Jablonka & Raz 2009; Herman & Sultan 2011; Holeski, Jander & Agrawal 2012). In plants, mother-offspring transgenerational effects may involve mechanisms such as resource partitioning in seeds (Roach & Wulff 1987; Galloway 1995; Sultan, Barton & Wilczek 2009) or transmission of epigenetic state (Jaenisch & Bird 2003; Richards 2006; Scoville *et al.* 2011; Rasmann *et al.* 2012).

With transgenerational plasticity, if the cues are reliable (i.e. parent and offspring experience similar herbivore pressures), offspring circumvent the lag time that would occur if they had to first experience herbivory and then respond.

Avoidance of lag time in defence production may be particularly crucial to seedlings, which generally have the highest mortality rate of any plant developmental stage (Harper 1977; Fritz *et al.* 2001). The reliability of the cue takes on added importance in transgenerational induction; if the cue is unreliable and defence is costly, resource-limited offspring inherit the costs but not the benefits of (unnecessary) defences. Cue reliability should depend in part on the time period between generations, which differs greatly among plant species. Herbaceous plants often exhibit seed dormancy and may have a year or more between seed dispersal and seedling germination, thus induction cues must be reliable *across* seasons. In contrast, seeds of some *Populus* species are, in natural conditions, viable for only 1–2 weeks after dispersal (Braatne, Rood & Heilman 1996). For *Populus*, then, transgenerational induction cues must be reliable across only a short time span (similar to the time span involved in within-plant induction).

In this study, we describe transgenerational patterns of resource allocation to resistance and growth in multiple genotypes of three cottonwood taxa (*Populus* spp.). To our knowledge, this is the first instance of transgenerational effects of herbivory on growth and resistance traits that has been described in a long-lived, woody plant species. In addition to differences between reproductively mature woody and herbaceous species in within-generation defence strategy (e.g. resistance vs. growth and/or reproduction, constitutive vs. induced defences), *Populus* is only the second plant species (or group of species) with no seed dormancy in which transgenerational plasticity in defence has been examined. The first of such plant species to be examined was dandelion, *Taraxacum officinale*, although transgenerational plasticity in defence has also been investigated in the animal model *Daphnia cucullata*, which can also have an analogously short time between generations (Agrawal, Laforsch & Tollrian 1999; Verhoeven *et al.* 2010). Using the offspring of cottonwood trees grown in a common garden (maternal trees are genotype replicates with and without herbivore damage), we address the questions:

1 Does foliar herbivory on maternal trees alter offspring germination, growth or offspring phytochemical resistance traits relative to offspring of maternal control (undamaged) trees?

2 If so, is transgenerational plasticity in growth and/or resistance traits genetically variable among genotypes within a species, and across species and their hybrids?

Materials and methods

We used clonally replicated cottonwood genotypes, grown in an 18-year-old common garden, to test the effects of maternal tree foliar herbivory (near open-pollinated, maturing seeds) on germination, growth and resistance traits of the resulting offspring. We assigned each replicate maternal tree within each genotype to either 'control' or 'herbivory' treatment, imposed treatment, collected mature seeds and grew the seedlings in a common garden environment for trait measurements. Throughout this report, these families of half-sibling offspring are referred to by their maternal genotype.

The trees used in this study were a subset of a cottonwood common garden at the Ogden Nature Center (ONC) in Ogden, Utah. The common garden trees were originally propagated from

cuttings of trees of known taxa and genotypes (Keim *et al.* 1989; Martinsen *et al.* 2001) growing along the Weber River in Utah; cuttings were established in the ONC in 1991. Fremont cottonwoods (*P. fremontii*) naturally occur in the lower elevations (below *c.* 1400 m) of the Weber River, narrowleaf cottonwoods (*Populus angustifolia*) occur in the higher elevations (above *c.* 1500 m), and F$_1$ hybrids of the two species occur in a hybrid zone at elevations between the two species zones (Keim *et al.* 1989; Whitham 1989; Martinsen *et al.* 2001). Hereafter, we refer to the two species and their F$_1$ hybrids as 'taxa'. Each of these cottonwood taxa is dioecious.

The cottonwood leaf beetle, *Chrysomela scripta* (Coleoptera: Chrysomelidae) and its congeners (e.g. *Chrysomela confluens*) are common herbivore species on *Populus* (Waltz & Whitham 1997). In the Weber River valley in Utah, adult cottonwood leaf beetles emerge in April, feed on developing leaves, mate and lay eggs (a single female may lay multiple clutches of up to 40 eggs). First and second instars skeletonize leaves, while later instars eat all leaf tissues (including secondary veins); cottonwood leaf beetle larvae can completely defoliate small trees (Floate & Whitham 1994). We used second instar *C. scripta* larvae, obtained from a laboratory-maintained colony, ancestors of which were collected in Georgia several months earlier.

We conducted this experiment with female trees, after natural pollination was complete (flowers were desiccated on all male trees in the region). We had four female genotypes within both the narrowleaf and F$_1$ taxa and one within the Fremont. For each genotype, we used between two and seven replicate trees, for a total of 39 maternal trees. Our sample size for the number of genotypes within a taxon and the number of replicate trees within a genotype was limited by the available female genotypes/replicates. Within each genotype, each replicate tree was arbitrarily assigned to receive *one* of two treatments, herbivory or control. For trees receiving herbivory, we used a paintbrush to transfer 10–20 second instar cottonwood leaf beetle larvae onto a leaf on a branch containing three fertilized catkins. We then bagged both herbivory branches and control branches (on their respective trees) using bags sewn from no-see-um mesh, both to keep leaf beetle larvae localized on the treatment branch and to exclude other herbivores. Bagged branches were 3–6 m from the ground, and we attempted to minimize variation in environmental factors, such as light, within and across treatments. There was no previous herbivore damage visible on either damage or control branches prior to being bagged. Because our goal was to standardize the amount of leaf area removed by the leaf beetle larvae, rather than measure leaf palatability or leaf beetle performance, we monitored larval herbivory on each 'herbivory' tree, and supplemented mesh bags as needed with additional larvae in the same instar as those on the branch at the time. At least 50% of five leaves was damaged on each herbivory-treatment branch by the time seed was mature. We also monitored each bagged control branch at least once every 3 days, to insure that no herbivores had accessed the bagged leaves.

We collected mature seeds from our experimental trees 21–24 days after treatments were initiated. Seeds from all experimental trees reached maturity within the same 3-day time span. We transported the seeds in paper bags to the University of Wisconsin, where we germinated them within 20 days of their removal from the trees. Seeds were kept at room temperature (20–25 °C) between collection and germination.

GERMINATION

We weighed 30 seeds from each maternal tree prior to germination. We soaked each group of 30 seeds in distilled water, then soaked them in a 10% commercial bleach solution (90 mL distiled water, 10 mL bleach) with 1 mL Tween® 20 (a surfactant) to disinfect the seed surface. We then rinsed seeds three times with distiled water, and placed them on sterile, moist filter paper (Fisherbrand P5; Fisher Scientific, Waltham, MA, USA) in clear, covered petri dishes. Petri dishes with seeds were placed in a growth chamber (25 °C, 18 h light/day). We counted the number of seeds that had germinated by the fifth day (all seeds that germinated did so by this day).

SEEDLING GROWTH

Within 5 days of germination, seedlings were transplanted into 98-well potting trays filled with sieved, damp Fafard 3B potting soil (Conrad Fafard Inc., Agawam, MA, USA). There were 149/118 seedlings for the narrowleaf maternal herbivory/maternal control, 169/137 for the F$_1$ and 44/24 for the Fremont. Seedlings were given a constant source of bottom water and were kept in a growth chamber (18-h days; 24–29 °C day temperature, 18 °C night temperature) for 13 days. The location of each seedling in each 98-well tray was haphazardly chosen, seedlings of a particular parent tree were distributed across the trays, and the trays were rotated daily within the growth chamber. Seven days after the final seedling was transplanted, seedlings were fertilized via their bottom water with soluble J.R. Peters fertilizer (Allentown, PA; 21:8:18 N-P-K; 2.5 mL per 1 L water) for one hour. Thereafter and until harvest, seedlings were fertilized every 7 days, first through bottom water, then by top-watering when seedlings grew large enough to withstand slight disturbance to the soil surface.

After 13 days in a growth chamber, we moved the seedlings to the greenhouse (same day length, temperature regime as growth chamber; supplemental high pressure sodium lighting for cloudy days and/or after dark). Fifteen days later (28 days after seedlings germinated), we transplanted seedlings into D-pots (5-cm diameter; 30-cm depth) with a 30% sand, 70% soil (MetroMix, Conrad Fafard Inc., Agawam, MA, USA) mixture. Seedlings were top-watered as necessary (initially every day, then once every 2 days). Forty-eight days after seedlings germinated, we moved them outside.

SEEDLING HARVEST

Eighty days after germination, we harvested all unfurled leaves on each seedling by cutting them off at the leaf base (without petiole attached). For each seedling, we recorded leaf number and total leaf area, then flash-froze (liquid nitrogen), vacuum-dried and weighed the leaves. Leaves were finely ground using a Wiley mill (mesh size #40) and stored at −20 °C. Total leaf mass for each seedling was assessed using dried leaves. We measured stem basal diameter and stem height for each seedling, then harvested the stems and roots. Roots were thoroughly washed with water. Both stems (including leaf petioles) and roots were then dried in a drying oven at 50 °C and weighed.

CHEMICAL ANALYSES

We analysed leaf samples for condensed tannins and phenolic glycosides (salicin, salicortin and HCH-salicortin). Condensed tannins and phenolic glycosides are the main secondary metabolites in *Populus* (Palo 1984; Lindroth, Hsia & Scriber 1987; Rehill *et al.* 2005, 2006). These compounds provide resistance to beetles and other insect herbivores (e.g. Hemming & Lindroth 1995; Hwang & Lindroth 1998; Donaldson & Lindroth 2004; Rehill *et al.* 2006). To

assess condensed tannin content, we used the acid butanol assay (Porter, Hrstich & Chan 1986) with purified narrowleaf cottonwood condensed tannins as standards, as described by Rehill *et al.* (2006). We quantified phenolic glycosides (salicin, salicortin, and HCH-salicortin) using capillary HPLC (methods described in Holeski *et al.* 2012). Our salicin standard was obtained from Sigma-Aldrich (St. Louis, MO, USA), while salicortin and HCH-salicortin standards were purified from Fremont and narrowleaf cottonwood tissue in our laboratory.

We report the results of each chemical assay as total foliar content (mg of foliar phytochemical compound per individual seedling) and concentration [per cent (%) mass]. Content is a more accurate measure of absolute chemical synthesis than is concentration, as concentration can be strongly influenced by differences in size (mass) among plants. Content is thus a more appropriate trait to use when assessing allocational trade-offs such as those between growth and phytochemical production (Baldwin & Karb 1995; Gebauer, Strain & Reynolds 1998; Koricheva 1999). Concentration, however, remains the more relevant trait from an herbivore perspective, as it is the measure of plant quality actually experienced by the herbivore (Koricheva 1999). We use graphical vector analysis (GVA) to evaluate the relationship between foliar phytochemical synthesis, foliar phytochemical concentration and foliar biomass (Timmer & Stone 1978; Haase & Rose 1995; Gebauer, Strain & Reynolds 1998; Koricheva 1999; Veteli *et al.* 2007).

GRAPHICAL VECTOR ANALYSIS

We used GVA to examine the mechanistic relationships behind differences in phytochemical production among taxa, as well as between the offspring of maternal control vs. maternal herbivory treatments (see Appendix S1 in Supporting Information for detailed GVA construction methods). Relationships observed between content, concentration and biomass via GVA generally fall into one of six categories (Fig. S1; Koricheva 1999; Couture 2011). (i) Steady-state increase: concentrations do not change because content and biomass increase in parallel. (ii) Steady-state decrease: concentrations do not change because content and biomass decrease in parallel. (iii) Dilution effect: concentrations decrease because content increases at a slower rate than does biomass. (iv) Concentration effect: concentrations increase because content decreases at a slower rate than does biomass. (v) Enhanced synthesis: concentration and content increase, with little change in biomass. Biomass increases at a slower rate than does content or decreases. (vi) Reduced synthesis: concentration and content decrease, with little change in biomass.

MOLECULAR GENETICS

We observed maternal herbivory × genotype interactions for offspring phytochemistry only within the F_1 hybrids (see the 'Genetic variation for transgenerational plasticity' section in the Results). To confirm that maternal treatment and paternal taxon were not confounded in the offspring of these trees, we conducted a paternity analysis for each F_1 hybrid offspring family. Total genomic DNA was extracted from dried leaves using a DNeasy plant mini kit (Qiagen, Valencia, CA, USA) and standardized to 20 ng ul^{-1}. All offspring and 38 reference genotypes (four F_1 mothers and the 34 potential father genotypes) were genotyped at seven randomly chosen simple sequence repeat (SSR) loci (Table S1) from the *Populus* genome (Smulders *et al.* 2001; Tuskan *et al.* 2004). Additional molecular genetics methodology is described in Appendix S1.

STATISTICAL ANALYSES

To meet assumptions of normality, we log-transformed condensed tannin data, and arcsine square root transformed seed germination and survivorship data, prior to statistical analysis. Statistical tests of the effects of taxa (fixed factor), maternal treatment (fixed factor) and genotype (nested within taxon, random factor) on each trait were conducted using a series of General Linear Model analyses of variance (ANOVAs, Minitab 14; State College, PA, USA). We added the effects of total leaf biomass (dry mass) as a covariate (random effect) in all of the ANOVAs investigating variation in phytochemical content. Fremont cottonwood data were removed from all statistical analyses except for the tests for an effect of taxon, because of Fremont's limited replication at the genotype and maternal herbivory levels. Nonetheless, we occasionally provide graphical or narrative descriptions of these data, for baseline comparisons.

To visualize the bivariate phytochemistry data (condensed tannins and total phenolic glycosides), we created a Euclidean distance-based similarity matrix and used nonmetric multidimensional scaling (NMDS; Kruskal 1964; Bangert *et al.* 2006). Two-dimensional stress levels across several NMDS runs were consistently less than 0.10, suggesting the data fit the ordination well. The error bars on NMDS plots represent the variance of the NMDS scores in the x and y directions across tree replicates (e.g. for each taxon and/or maternal treatment). NMDS axes do not have associated quantitative units.

To assess possible paternal effects on phytochemistry in the offspring of F_1 hybrid mothers (see the 'Genetic variation for transgenerational plasticity' section in the Results), the taxon of offspring individual was calculated using the program NewHybrids (Anderson & Thompson 2002). Offspring were assigned to a particular hybrid class (i.e. F_2, F_1 backcross to Fremont or F_1 backcross to narrowleaf cottonwood) if the probability of assignment was greater than 0.75. Analysis was performed using all 165 offspring and 38 reference genotypes from the ONC garden and burn-in of 10^4 iterations followed by 10^5 iterations. Offspring taxon was verified using paternity analysis and the true taxon of F_1 mothers/34 potential cottonwood fathers located in the ONC garden (Martinsen *et al.* 2001). Paternity analysis was carried out using the 'MSW' method that allows for genotyping error (Wang 2004) and was performed using the Master-Bayes package (Hadfield, Richardson & Burke 2006) in the statistical program R (R Core Team 2011). Paternal taxon (fixed factor) was included in the GLM ANOVAs for each phytochemical trait.

Results

GERMINATION AND SURVIVORSHIP

Maternal herbivory did not have significant overall effects on any measured aspect of seedling germination or survivorship (Tables 1 and 2). Taxa also showed a uniform lack of response to maternal herbivory (no significant interactions for taxon × maternal herbivory, Tables 1 and 2).

In contrast, across maternal treatments, we found genetic variation for offspring seed mass, the proportion of seeds that germinated, and the proportion of seeds sown that survived to harvest (significant taxon and/or genotype nested within taxon effects, Table 1). Offspring of F_1 hybrid mothers had the highest seed mass, followed by narrowleaf and then Fremont cottonwood (Table 2). Seed mass did *not*, however, have a

Table 1. Results of GLM anovas for germination and growth variables. Above ground mass includes leaves and stems. The term 'taxon' refers to the two cottonwood species (*Populus angustifolia*, *Populus fremontii*) and their F_1 hybrids

Response variable	Factor	*F* statistic	d.f.	*P*-value
Average mass of one seed	**taxon**	7.04	2,32	0.003
	genotype(taxon)	3.37	6,22	0.016
	maternal herbivory	3.68	1,22	0.068*
	taxon * mat. herbivory interaction	0.08	1,22	0.786
Germination	**taxon**	5.60	2,34	0.008
	genotype(taxon)	1.07	6,24	0.409
	maternal herbivory	0.48	1,24	0.495
	taxon * mat. herbivory interaction	0.64	1,24	0.433
Survivorship (seed to harvest)	**taxon**	6.11	2,34	0.005
	genotype(taxon)	1.18	6,24	0.351
	maternal herbivory	0.02	1,24	0.897
	taxon * mat. herbivory interaction	2.45	1,24	0.131
Stem basal diameter	**taxon**	35.23	2,324	< 0.001
	genotype(taxon)	2.69	6,287	0.015
	maternal herbivory	0.70	1,287	0.402
	taxon * mat. herbivory interaction	0.30	1,287	0.585
Stem height	**taxon**	40.89	2,324	< 0.001
	genotype(taxon)	0.27	6,287	0.952
	maternal herbivory	0.41	1,287	0.525
	taxon * mat. herbivory interaction	1.07	1,287	0.302
Diameter^2 * height (D^{2}H)	**taxon**	37.5	2,324	< 0.001
	genotype(taxon)	1.7	6,287	0.114
	maternal herbivory	0. 1	1,287	0.747
	taxon * mat. herbivory interaction	1.7	1,287	0.198
Total leaf area	**taxon**	108.18	2,334	< 0.001
	genotype(taxon)	2.79	6,294	0.012
	maternal herbivory	1.47	1,294	0.226
	taxon * mat. herbivory interaction	0.10	1,294	0.749
Total leaf mass	**taxon**	102.59	2,336	< 0.001
	genotype(taxon)	2.48	6,296	0.024
	maternal herbivory	1.47	1,296	0.227
	taxon * mat. herbivory interaction	0.03	1,296	0.865
Root mass	**taxon**	106.00	2,331	< 0.001
	genotype(taxon)	2.36	6,291	0.030
	maternal herbivory	0.76	1,291	0.384
	taxon * mat. herbivory interaction	0.23	1,291	0.630
Above ground mass	**taxon**	92.94	2,330	< 0.001
	genotype(taxon)	2.72	6,290	0.014
	maternal herbivory	2.31	1,290	0.130
	taxon * mat. herbivory interaction	0.21	1,290	0.649

Factors in bold print had a significant effect on the particular variable of interest.
*Non-significant trend – seeds of trees with maternal herbivory had equivalent or slightly higher seed mass relative to those of maternal control trees.

significant effect on germination ($R^2 = 0.03$; $P = 0.35$) or on seedling survivorship ($R^2 = 0.02$; $P = 0.49$) in our growth chamber environment.

GROWTH

Offspring of maternal herbivory trees were similar to offspring of control trees for all measured aspects of seedling growth (i.e. no significant differences due to maternal herbivory treatment, Tables 1 and 2 and Fig. 1). At seedling harvest, taxon and/or genotype nested within taxon influenced basal stem diameter, stem height, d^2 h (stem basal diameter^2 × height, which is a common metric of tree size), total leaf area, total leaf dry mass and above ground and below ground biomass (regardless of maternal treatment; Tables 1 and 2). For each of these traits (regardless of maternal treatment), Fremont had the highest growth (often up to 3 × greater than narrowleaf), followed by the F_1 hybrids and narrowleaf cottonwood (Table 2).

PHYTOCHEMISTRY

Across all taxa, total phenolic glycoside *content* was 11–35% lower in the seedlings of maternal herbivory trees than in those of maternal control trees (Fig. 2a; $F_{1,271} = 4.88$; $P = 0.028$). Likewise, total phenolic glycoside *concentrations* were c. 10% lower in the seedlings of maternal herbivory trees, relative to maternal control trees (Fig. S2,
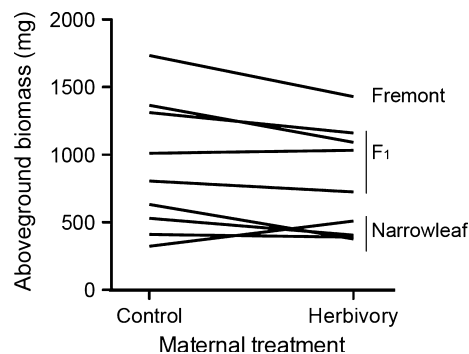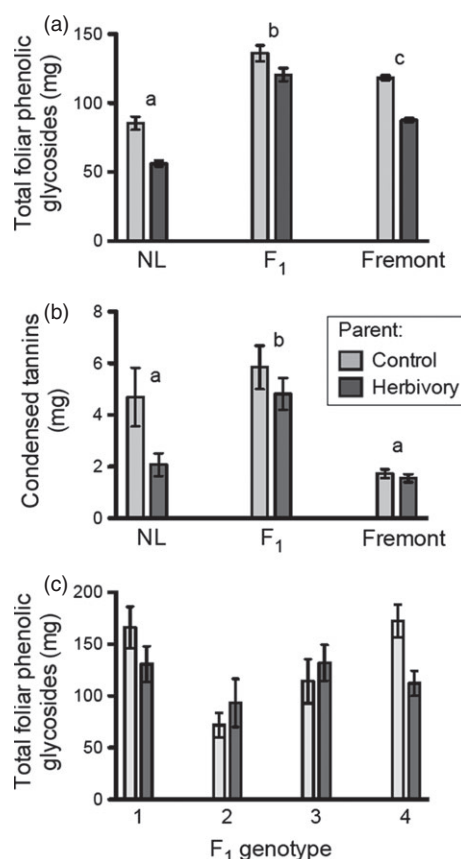
Table 2. Taxon means and standard deviations for seedling germination, survivorship and growth

Trait	Taxa	Mean	SD	*Post hoc* comparisons
Seed mass (mg)	Narrowleaf	0.65	0.14	A
	F_1	0.82	0.09	B
	Fremont	0.55	0.27	A
Germination	Narrowleaf	0.48	0.32	A
	F_1	0.80	0.22	B
	Fremont	0.83	0.06	B
Survivorship	Narrowleaf	0.25	0.20	A
	F_1	0.45	0.10	B
	Fremont	0.41	0.12	AB
Stem basal	Narrowleaf	2.1	1.2	A
diameter (mm)	F_1	3.0	0.8	B
	Fremont	3.4	0.9	B
Stem height (cm)	Narrowleaf	14.0	6.0	A
	F_1	18.6	5.9	B
	Fremont	23.3	5.2	C
Diameter^2*	Narrowleaf	910	1413	A
height (mm^3)	F_1	1956	1342	B
	Fremont	3024	1510	C
Total leaf	Narrowleaf	55	45	A
area (cm^2)	F_1	129	63	B
	Fremont	196	82	C
Total leaf	Narrowleaf	291	261	A
mass (mg)	F_1	762	406	B
	Fremont	1042	428	C
Root mass (mg)	Narrowleaf	149	177	A
	F_1	536	372	B
	Fremont	1088	671	B
Above ground	Narrowleaf	437	382	A
mass (mg)	F_1	1111	585	B
	Fremont	1558	626	C

Statistical tests for differences among taxa for each trait are in Table 1; whilst results of *post hoc* comparisons of taxa are indicated by letters (taxa with different letter assignments are significantly different from one another). Values were averaged across offspring of both herbivory and control maternal plants, as the maternal herbivory treatment had no effect on any of these traits. Germination refers to the proportion of seeds that germinated, while survivorship refers to the proportion of seeds surviving at seedling harvest.

$F_{1,302} = 4.41$; $P = 0.037$). Taxa responded equivalently to maternal herbivory in terms of both content and concentrations (non-significant taxon × maternal herbivory interactions; $F_{1,270} = 1.82$; $P = 0.178$ and $F_{1,272} = 0.19$; $P = 0.660$, respectively).

Across maternal treatments, offspring total phenolic glycoside content and concentrations differed among taxa ($F_{2,302} = 49.45$; $P < 0.001$, and $F_{2,302} = 45.95$; $P < 0.001$, respectively). Total phenolic glycoside content was highest in the F_1, followed by Fremont and narrowleaf (Fig. 2a). In contrast, total phenolic glycoside concentration was highest in narrowleaf and lowest in Fremont (Fig. S2), due to the narrowleaf producing a much lower leaf mass per tree than the other taxa (Table 2). These taxon patterns for phenolic glycoside concentrations generally correspond with patterns documented for adult trees (Rehill *et al.* 2006; Holeski *et al.* 2012), although overall levels of phenolic glycosides in the seedlings are higher than those found in adult trees (Table

Fig. 1. Above ground mass (mg) of seedlings from herbivory and control maternal trees. Each line represents a genotype mean.

Fig. 2. Panel (a) Total foliar phenolic glycoside content per seedling (mg) in foliage of seedlings from maternal herbivory or maternal control trees, averaged over genotypes within each taxon. The term 'taxa' refers to the two cottonwood species (*Populus angustifolia*, *Populus fremontii*) and their F_1 hybrids. NL is narrowleaf. Fremont is displayed for comparison purposes only and was not included in the associated statistics (see Materials and methods for more information). Error bars on all panels represent ± 1 standard error (SE) from the mean. Panel (b) Foliar condensed tannin content (mg) in seedlings of maternal herbivory vs. control trees within each taxon. Panel (c) Genotype × maternal herbivory interactions for total phenolic glycoside content (mg) within the F_1 hybrids.

S2). Across maternal treatments and within the narrowleaf and F_1 taxa, there was no overall effect of genotype on either total phenolic glycoside content or concentration

($F_{6,272} = 0.81$; $P = 0.563$ and $F_{6,274} = 1.11$; $P = 0.355$, respectively).

Across all taxa, seedlings of maternal trees that experienced herbivory produced 10–55% lower content of condensed tannins than did seedlings of control trees, although this trend was marginally non-significant (Fig. 2b, $F_{1,209} = 3.67$; $P = 0.057$). Concentrations of condensed tannins were also reduced in the seedlings of maternal herbivory trees relative to maternal control trees (c. 18% reduction in both the narrowleaf and F_1; Fig. S3; $F_{1,210} = 4.97$; $P = 0.027$). Taxa responded to maternal herbivory equivalently, both in terms of their condensed tannin content and concentrations (non-significant taxon × maternal herbivory interactions; $F_{1,208} = 0.03$; $P = 0.858$ and $F_{1,210} = 0.34$; $P = 0.562$, respectively).

Across maternal treatments, we found genetic variation in condensed tannin content and concentration. Seedling condensed tannin content differed among taxa and among genotypes nested within taxon ($F_{2,242} = 12.24$, $P = 0.006$; $F_{2,242} = 3.01$, $P = 0.007$), with the F_1 producing slightly higher content than the Fremont and narrowleaf (Fig. 2b). Seedling condensed tannin concentration also varied among taxa and among genotypes nested within taxon ($F_{2,246} = 6.49$; $P = 0.032$; $F_{6,246} = 3.39$; $P = 0.003$), with narrowleaf and F_1 hybrid seedlings containing concentrations of condensed tannins that were at least 75% greater than Fremont. As with total phenolic glycosides, our findings with seedling condensed tannin concentrations correspond with the results of previous studies of condensed tannins in adult cottonwood trees (Table S2; Rehill *et al.* 2006; Holeski *et al.* 2012). All seedlings (those with and without maternal herbivory) had very low concentrations of condensed tannins relative to those found in adult trees.

Non-metric multidimensional scaling illustrated differences in the bivariate resistance phenotype (combining total phenolic glycoside and condensed tannin concentrations for each individual; Fig. 3). Data points near each other in the NMDS plots are more similar than points further apart. The NMDS plot shows differences in the resistance phenotype both among taxa and among maternal herbivory treatments within taxon (Fig. 3), with differences in the former generally greater than differences in the latter.

VARIATION IN TRANSGENERATIONAL PLASTICITY

Within narrowleaf cottonwood, we found no significant effects of genotype, maternal tree treatment or their interaction on any aspect of phenolic glycoside content or concentration. When only the F_1 taxon was included, genotypes within this taxon responded to maternal damage differently in terms of their content and concentration of total phenolic glycosides (Fig 2c, $F_{3,156} = 2.67$; $P = 0.050$ and $F_{3,137} = 3.90$; $P = 0.010$). Differential paternity partially influences this interaction. Paternity substantially affected both content and concentration of total phenolic glycosides across all genotypes and treatments, and altered the significant genotype × maternal treatment interactions for content but not for concentration of these resistance traits (Appendix S1, Molecular genetics

results and discussion; Table S3). Individually, neither maternal genotype nor maternal tree herbivory had significant overall effects on total phenolic glycoside concentrations within the F_1 hybrids (Table S3).

We had insufficient statistical power to nest maternal tree within maternal treatment, for a more decisive confirmation that the observed pattern in the F_1 taxon is not driven by environmental heterogeneity across the maternal common garden. However, a GLM ANOVA including paternity and nesting maternal tree (random factor) within maternal genotype and treatment (fixed factor) had a non-significant effect of mother tree, indicating that nesting may not be necessary.

ALLOCATION TO GROWTH AND RESISTANCE

In terms of constitutive total phenolic glycosides, narrowleaf exhibits a strong 'concentration effect' relative to Fremont; phenolic glycoside content decreases in the narrowleaf at a slower rate than does biomass, relative to Fremont (Fig. 4a). In the F_1, total phenolic glycoside content is higher than that of Fremont, while biomass is lower, resulting in higher concentrations of phenolic glycosides in the F_1, relative to Fremont (an 'enhanced synthesis' effect; Fig. 4a). Both the narrowleaf and the F_1 hybrids demonstrate an 'enhanced synthesis' effect relative to Fremont in terms of condensed tannins (Fig. 4b). As with F_1 phenolic glycosides, narrowleaf and the F_1 produce higher condensed tannin content than does Fremont, while their biomass is lower. This results in higher
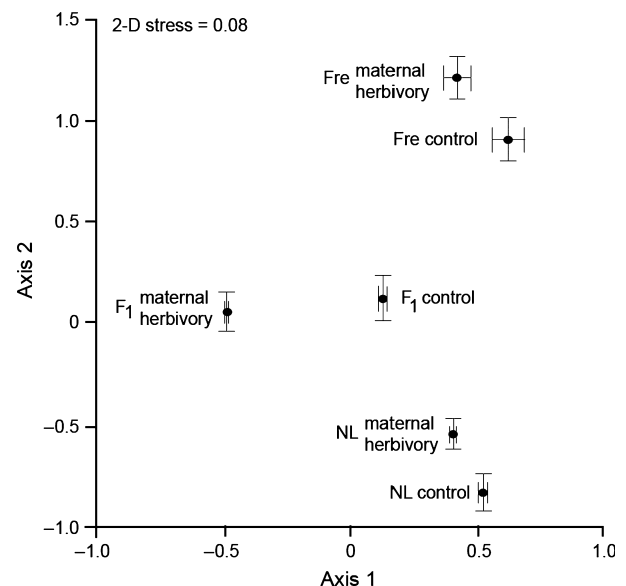

Fig. 3. Bray–Curtis non-metric multidimensional scaling (NMDS) ordination plot comparing the bivariate resistance phenotype (total phenolic glycosides and condensed tannin concentrations) among three cottonwood taxa, narrowleaf (NL), Fremont (Fre) and their F_1 hybrids, and between maternal treatments within a particular taxon. Each point represents the mean of the NMDS scores of multiple trees sampled for each taxon. Data points near each other in the NMDS plots are more similar than points further apart. Standard error bars represent the phenotype variance for each axis of the two-dimensional solution [± 1 standard error (SE)]. Axes have no inherent meaning.
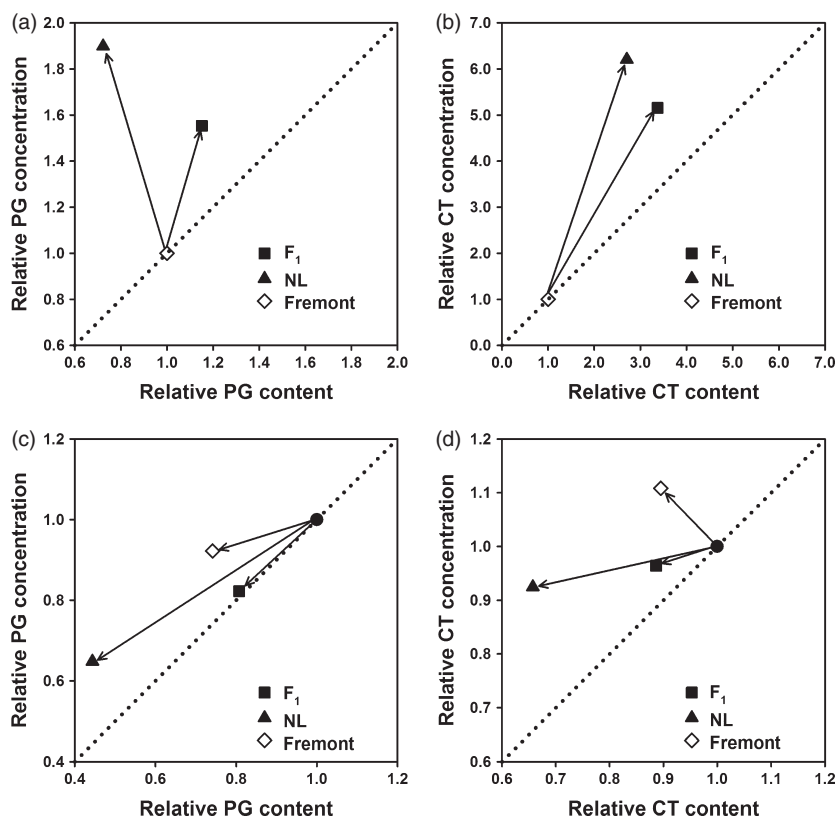
Fig. 4. Graphical vector analysis for seedling phytochemical content, concentration and foliar biomass. Panels a and b show constitutive results (offspring of maternal control trees only), as the F_1 and narrowleaf taxa standardized relative to Fremont. Panels c and d show seedling relative response to maternal herbivory treatment (standardized relative to maternal control plants (dark circle)). 'PG' represents total phenolic glycosides, while 'CT' indicates condensed tannins.

concentrations of condensed tannins in narrowleaf and the F_1, relative to Fremont (Fig. 4b).

For both total phenolic glycosides and condensed tannins, all taxa exhibited some form of 'reduced synthesis' effects of maternal herbivory treatment, relative to maternal controls (Fig. 4c,d). For total phenolic glycosides in all three taxa, seedling biomass of maternal herbivory trees remained close to constant relative to maternal control trees, while relative phytochemical content, thus concentration, decreased (Fig. 4c). A similar pattern is observed with condensed tannins in narrowleaf and the F_1, while Fremont exhibits a marginal 'concentration effect', where biomass decreases slightly more than does content, resulting in a marginally higher concentration of condensed tannins (Fig. 4d).

Discussion

Numerous studies describe or predict plant strategies of resource allocation to resistance and/or growth within a single generation (e.g. Herms & Mattson 1992; Mauricio, Rausher & Burdick 1997; Sampedro, Xoaquin & Zas 2011). In contrast, exceedingly little is known about whether such patterns hold *across* generations, in seedlings of plants that experienced maternal herbivory. In our common garden study with replicated genotypes of three cottonwood taxa (*P. angustifolia*, *P. fremontii* and their F_1 hybrids), two major findings

emerged. First, independent of maternal herbivory-treatment effects, we found that offspring taxa (and genotypes within a taxon) generally differed significantly in their constitutive allocation to growth and resistance (both content and concentration) traits. Secondly, maternal herbivory by cottonwood leaf beetle larvae on foliage adjacent to developing seeds did not significantly alter offspring growth traits relative to controls, but reduced content of offspring phytochemicals by 10–55%, and reduced concentrations of offspring phytochemical resistance traits by 10–18%, across taxa.

CONSTITUTIVE DEFENCES

Seedlings of different taxa diverged in their constitutive allocation patterns to growth vs. resistance (Fig. 4a,b). For example, narrowleaf cottonwood seedlings had the lowest total phenolic glycoside content of all the taxa (Fig. 2a), but also grew less rapidly than did the Fremont and F_1 seedlings (Table 2). As a result, narrowleaf seedlings had substantially higher concentrations of phenolic glycosides than did the other taxa (Fig. S2). In addition to providing resistance to generalist insect herbivores (Hemming & Lindroth 1995; Hwang & Lindroth 1998), phenolic glycosides deter mammalian browsers (Iason 2005; Wooley *et al.* 2008), which can exert strong selection pressures on young *Populus* (Kay & Bartos 2000). For condensed tannin production, taxa exhibited

a similar, although less pronounced pattern to that of phenolic glycoside production (Fig. 2b, Table 2, Fig. S3).

Investment in growth could be an alternative mechanism of resistance to mammalian browsers, via escape, if seedlings grow to a height above the browse line before being eaten. The trade-offs in allocation to growth vs. defence trait concentrations that we found *between* taxa are analogous to genotypic variation within quaking aspen (*Populus tremuloides*); young aspen trees may allocate resources to produce relatively high concentrations of resistance traits such as phenolic glycosides even at the expense of slower growth, when they grow in resource-limited conditions (Osier & Lindroth 2006; Stevens, Waller & Lindroth 2007). The overall low levels of condensed tannins observed in our study correspond with general trends in cottonwood and aspen, where concentrations of these compounds increase over a tree's lifetime (Table S2; Donaldson *et al.* 2006; Rehill *et al.* 2006; Holeski *et al.* 2012). However, the selective pressures leading to this pattern are not well understood (Ayres *et al.* 1997; Donaldson *et al.* 2006).

TRANSGENERATIONAL PLASTICITY IN DEFENCES

Transgenerational effects of herbivory led to decreased offspring resistance across taxa in our study. Offspring of maternal herbivory trees had consistent, moderate (10–55%) reductions in total phenolic glycoside and/or condensed tannin content and concentrations, relative to offspring of maternal control trees (Figs 2a,b, S2 and S3). Seed mass and seedling growth, however, were similar in offspring of maternal herbivory and maternal control trees (Tables 1 and 2 and Fig. 1). Moreover, any marginal differences in seed mass between the two maternal treatments (Table 1) were in the opposite direction than would be expected if they were to influence the differences in phytochemistry between maternal treatments via resource partitioning. Published studies to date on the transgenerational effects of maternal herbivory have found genetic variation in offspring resistance in response to maternal herbivory, general increases in resistance in response to maternal herbivory (Agrawal 2002), and both increases (Holeski 2007; Verhoeven & van Gurp 2012) and decreases (Agrawal 2002) in resistance in response to simulated maternal herbivory. Studies that evaluate seed traits and/or offspring growth are more common (Obeso 1993; Steets & Ashman 2010; Herman & Sultan 2011; Verhoeven & van Gurp 2012). Maternal herbivory can increase seed size or seed mass (Wulff 1986; Dickson & Mitchell 2010), resulting in offspring with increased growth rates relative to offspring of control mothers (Thalmann *et al.* 2003; Steets & Ashman 2010). Alternatively, maternal herbivory can decrease seed viability and mass (Obeso 1993), resulting in offspring with lower growth rates relative to controls (Mueller *et al.* 2005). We know of only one other study to date that examined the relationship between plant growth and resistance in offspring of maternal plants that experienced herbivory (Agrawal 2002). In wild radish (*Raphanus raphanistrum*), the effect of maternal herbivory on seed mass and offspring growth varied among families (with the effect on growth ranging from positive to neutral to negative; Agrawal 2002).

In our study, seedlings with maternal herbivory may have invested more in growth and other, unmeasured fitness traits than in phytochemical resistance, analogous to within-generation compensatory growth and induced susceptibility involved with tolerance to herbivory (Karban & Baldwin 1997; Strauss & Agrawal 1999; Simonsen & Stinchcombe 2007). Another possibility is a transgenerational scenario that is analogous to within-generation rapid response induction followed by delayed susceptibility. Within a generation of soybean (*Glycine max*), Underwood (1998) found that induced resistance decayed into increased susceptibility only 20 days after herbivore damage. Finally, seedling response could be specific to the maternal herbivore. Cottonwood leaf beetles in Utah are univoltine, but adults emerge in time to feed on new seedling foliage (Floate, Kearsley & Whitham 1993). Cottonwood leaf beetles are specialist herbivores on tissue containing phenolic glycosides, at least one of which they convert to salicylaldehyde and use in their own defence against predators (Pasteels *et al.* 1983; Kearsley & Whitham 1992). Relatively lower levels of phenolic glycosides might thus decrease cottonwood leaf beetle relative preference for seedlings.

In addition to the transgenerational plasticity in resistance across all three taxa, we observed potential within taxon genetic variation for this plasticity, in concentrations of phenolic glycosides within the F_1 hybrids (Fig. 2c). Our finding of putative genetic variation in transgenerational plasticity in resistance is consistent with prior studies of herbaceous plants (Agrawal 2002; Holeski 2007), one of which showed transgenerational induction in recombinant inbred lines and in one but not both of their parent populations (Holeski 2007). In our study, putative genetic variation was found in interspecies hybrids, but not in the narrowleaf parent species. A larger sample size for all three taxa is necessary to confirm that the F_1 hybrids contain more genetic variation for transgenerational plasticity than the two parent taxa; if that is indeed the case it could help explain the long-term evolutionary persistence of hybrids between these particular cottonwood taxa (Arnold & Hodges 1995; Rieseberg, Archer & Wayne 1999).

ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

The patterns of transgenerational plasticity in resistance traits that we observed in our study could be a result of traditional maternal effects (Roach & Wulff 1987; Galloway 1995; Sultan, Barton & Wilczek 2009) or transmission of epigenetic state (Richards 2006; Herman & Sultan 2011; Scoville *et al.* 2011; Rasmann *et al.* 2012). The maternal damage treatment was applied while seeds were developing (rather than prior to fertilization), thus any epigenetic signals would necessarily be passed from vegetative tissue to the developing seed (Mosher & Melnyk 2011; Rasmann *et al.* 2012). We saw no evidence of traditional maternal effects in terms of seed provisioning (e.g. differences in seed size or mass between maternal treatments in our study), indicating that epigenetic effects or a

combination of traditional maternal effects and epigenetic effects are likely.

Numerous differences in life history exist between *Populus* and the other species in which transgenerational plasticity in resistance has been examined. These could play a contributing role to differences in patterns of transgenerational plasticity among species. In general, two key life history differences likely to play a critical role in the ecological impact and evolution of transgenerational plasticity are generation time (germination or hatching to reproductive maturity) and the lag time between parent and offspring generations (time from seed production to seed germination). To date, most studies of transgenerational plasticity of defences have occurred in species with at least some degree of seed dormancy or analogous time-lag between generations (with the exception of dandelion and daphnia; see Table 1 in Holeski, Jander & Agrawal 2012). *Populus*, in contrast, has non-dormant seeds; the lag time between generations in these plants is much more similar to the lag time for within-generation plasticity. More studies of both annual and long-lived species are necessary to assess the extent to which life-history differences may lead to differential patterns of transgenerational plasticity.

The ecological impact of transgenerational plasticity of defences is contingent upon the effect of the organism's phenotype on its community. The degree to which transgenerational plasticity is similar in effect to within-generation plasticity rests upon plant and herbivore life history, population dynamics and the generality or specificity of the cues and defence responses.

Acknowledgements

References

Agrawal, A.A. (2001) Transgenerational consequences of plant responses to herbivory: an adaptive maternal effect? *American Naturalist*, **157**, 555–569.

Agrawal, A.A. (2002) Herbivory and maternal effects: mechanisms and consequences of transgenerational induced plant resistance. *Ecology*, **83**, 3408–3415.

Agrawal, A.A. & Karban, R. (1999) Why induced defenses may be favored over constitutive defenses in plants. *The Ecology and Evolution of Inducible Defenses* (eds R. Tollrian & C.D. Harvell), pp. 45–61. Princeton University Press, Princeton, NY.

Agrawal, A.A., Laforsch, C. & Tollrian, R. (1999) Transgenerational induction of defenses in plants and animals. *Nature*, **401**, 60–63.

Anderson, E.C. & Thompson, E.A. (2002) A model-based method for identifying species hybrids using multilocus genetic data. *Genetics*, **160**, 1217–1229.

Arnold, M.L. & Hodges, S.A. (1995) Are natural hybrids fit or unfit relative to their parents? *Trends in Ecology and Evolution*, **10**, 67–71.

Ayres, M.P., Clausen, T.P., MacLean, S.F. Jr, Redman, A.M. & Reichardt, P.B. (1997) Diversity of structure and antiherbivore activity in condensed tannins. *Ecology*, **78**, 1696–1712.

Baldwin, I.T. & Karb, M.J. (1995) Plasticity in allocation of nicotine to reproductive parts in *Nicotiana attenuata*. *Journal of Chemical Ecology*, **21**, 897–909.

Bangert, R.K., Allan, G.J., Turek, R.J., Wimp, G.M., Meneses, N., Martinsen, G.D., Keim, P. & Whitham, T.G. (2006) A genetic similarity rule determines arthropod community structure. *Molecular Ecology*, **15**, 1379–1392.

Barton, K.E. & Koricheva, J. (2010) The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. *American Naturalist*, **175**, 481–493.

Boege, K. & Marquis, R.J. (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends in Ecology and Evolution*, **20**, 441–448.

Bondurianskyy, R. & Day, T. (2009) Nongenetic inheritance and its evolutionary implications. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 103–125.

Braatne, J.H., Rood, S. & Heilman, P.E. (1996) Life history, ecology, and conservation of riparian cottonwoods in North America. *Biology of Populus* and its Implications for Management and Conservation (ed. R.F. Steller), pp. 57–85. National Research Council of Canada, NRC Research Press, Ottawa, ON.

Cipollini, D., Purrington, C.B. & Bergelson, J. (2003) Costs of induced responses in plants. *Basic and Applied Ecology*, **4**, 79–89.

Couture, J.J. (2011) *Impacts of elevated carbon dioxide and ozone on community herbivory in a northern temperate forest*. PhD Thesis, University of Wisconsin, Madison.

Dickson, T.L. & Mitchell, C.E. (2010) Herbivore and fungal pathogen exclusion affects the seed production of four common grassland species. *PLoS ONE*, **5**, e12022.

Donaldson, J.R. & Lindroth, R.L. (2004) Cottonwood leaf beetle (Coleoptera: Chrysomelidae) performance in relation to variable phytochemistry in juvenile aspen (*Populus tremuloides* Michx.). *Environmental Entomology*, **33**, 1505–1511.

Donaldson, J.R., Stevens, M.T., Barnhill, H.R. & Lindroth, R.L. (2006) Age-related shifts in leaf chemistry of clonal aspen (*Populus tremuloides*). *Journal of Chemical Ecology*, **32**, 1415–1429.

Elger, A., Lemoine, D.G., Fenner, M. & Hanley, M.E. (2009) Plant ontogeny and chemical defence: older seedlings are better defended. *Oikos*, **118**, 767–773.

Floate, K.D., Kearsley, M.J.C. & Whitham, T.G. (1993) Elevated herbivory in plant hybrid zones- *Chrysomela confluens*, *Populus*, and phonological sinks. *Ecology*, **74**, 2056–2065.

Floate, K.D. & Whitham, T.G. (1994) Ant-aphid interaction reduces Chrysomelid herbivory in a cottonwood hybrid zone. *Oecologia*, **97**, 215–221.

Fritz, R.S., Hochwender, C.G., Lewkiewicz, D.A., Bothwell, S. & Orians, C.M. (2001) Seedling herbivory by slugs in a willow hybrid system: developmental changes in damage, chemical defense, and plant performance. *Oecologia*, **129**, 87–97.

Galloway, L.F. (1995) Response to natural environmental heterogeneity: maternal effects and selection on life-history characters and plasticities in *Mimulus guttatus*. *Evolution*, **49**, 1095–1107.

Gebauer, R.L.E., Strain, B.R. & Reynolds, J.F. (1998) The effect of elevated CO_2 and N availability on tissue concentrations and whole plant pools of carbon-based secondary compounds in loblolly pine (*Pinus taeda*). *Oecologia*, **113**, 29–36.

Goodger, J.Q.D., Choo, T.Y.S. & Woodrow, I.E. (2007) Ontogenetic and temporal trajectories of chemical defense in a cyanogenic eucalypt. *Oecologia*, **153**, 799–808.

Haase, D.L. & Rose, R. (1995) Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. *Forest Science*, **41**, 54–66.

Hadfield, J.D., Richardson, D.S. & Burke, T. (2006) Towards unbiased parentage assignment: combining genetic, behavioral, and spatial data in a Bayesian framework. *Molecular Ecology*, **15**, 3715–3730.

Hanley, M.E., Fenner, M., Whibley, H. & Darvill, B. (2004) Early plant growth: identifying the end of the seedling phase. *New Phytologist*, **163**, 61–66.

Harper, J.L. (1977) *Population Biology of Plants*. Academic Press, London.

Hemming, J.D.C. & Lindroth, R.L. (1995) Intraspecific variation in aspen phytochemistry – effects on performance of gypsy moths and forest tent caterpillars. *Oecologia*, **103**, 79–88.

Herman, J.J. & Sultan, S.E. (2011) Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. *Frontiers in Plant Science*, **2**, 1–10.

Herms, D.A. & Mattson, W.J. (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, **67**, 283–335.

Herrera, C.M. & Bazaga, P. (2011) Untangling individual variation in natural populations: ecological, genetic and epigenetic correlates of long-term inequality in herbivory. *Molecular Ecology*, **20**, 1675–1688.

Holeski, L.M. (2007) Within and between generation phenotypic plasticity in trichome density of *Mimulus guttatus*. *Journal of Evolutionary Biology*, **20**, 2092–2100.

Holeski, L.M., Hillstrom, M.L., Whitham, T.G. & Lindroth, R.L. (2012) Relative importance of genetic, ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype in a foundation tree species. *Oecologia*, **170**, 695–704.

Holeski, L.M., Jander, G. & Agrawal, A.A. (2012) Transgenerational defense induction and epigenetic inheritance in plants. *Trends in Ecology and Evolution*, **27**, 618–626.

Hwang, S.Y. & Lindroth, R.L. (1998) Consequences of clonal variation in aspen phytochemistry for late season folivores. *Ecoscience*, **5**, 508–516.

Iason, G. (2005) The role of plant secondary metabolites in mammalian herbivory: ecological perspectives. *Proceedings of the Nutrition Society*, **64**, 123–131.

Ivey, C.T., Carr, D.E. & Eubanks, M.D. (2009) Genetic variation and constraints on the evolution of defense against spittlebug (*Philaenus spumarius*) herbivory in *Mimulus guttatus*. *Heredity*, **102**, 303–311.

Jablonka, E. & Raz, G. (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *The Quarterly Review of Biology*, **84**, 131–176.

Jaenisch, R. & Bird, A. (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nature Genetics*, **33**, 245–254.

Karban, R. & Adler, F. (1996) Induced resistance to herbivores and the information content of early season attack. *Oecologia*, **107**, 379–385.

Karban, R. & Baldwin, I.T. (1997) Induced defense and the evolution of induced resistance. *Induced Responses to Herbivory* (eds R. Karban & I.T. Baldwin), pp. 167–204. The University of Chicago Press, Chicago, IL.

Karban, R., Agrawal, A.A., Thaler, J.S. & Adler, L.S. (1999) Induced plant responses and information content about risk of herbivory. *Trends in Ecology and Evolution*, **14**, 443–447.

Kay, C.E. & Bartos, D.L. (2000) Ungulate herbivory on Utah aspen: assessment of long-termexclosures. *Journal of Range Management*, **53**, 145–153.

Kearsley, M.J.C. & Whitham, T.G. (1992) Guns and butter: a no cost defense against predation for *Chrysomela confluens*. *Oecologia*, **92**, 556–562.

Keim, P., Paige, K.N., Whitham, T.G. & Lark, K.G. (1989) Genetic analysis of an interspecific Hybrid swarm of *Populus*: occurrence of unidirectional introgression. *Genetics*, **123**, 557–565.

Koricheva, J. (1999) Interpreting phenotypic variation in plant phytochemistry: problems with the use of concentrations. *Oecologia*, **119**, 467–473.

Koricheva, J. (2002) Meta-analysis of sources of variation in fitness costs of plant anti-herbivore defenses. *Ecology*, **83**, 176–190.

Kruskal, J.B. (1964) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika*, **29**, 1–26.

Lindroth, R.L., Hsia, M.T.S. & Scriber, J.M. (1987) Seasonal patterns in the phytochemistry of 3 *Populus* species. *Biochemical Systematics and Ecology*, **15**, 681–686.

Marquis, R.J. (1992) Selective impact of herbivores. *Plant Resistance to Herbivores and Pathogens* (eds R.S. Fritz & E.L. Simms), pp. 301–325. University of Chicago Press, Chicago, IL.

Martinsen, G.D., Whitham, T.G., Turek, R.J. & Keim, P. (2001) Hybrid populations selectively filter gene introgression between species. *Evolution*, **55**, 1325–1335.

Mauricio, R. (1998) Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana*. *American Naturalist*, **151**, 20–28.

Mauricio, R. & Rausher, M.D. (1997) Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution*, **51**, 1435–1444.

Mauricio, R., Rausher, M.D. & Burdick, D.S. (1997) Variation in the defense strategies of plants: are resistance and tolerance mutually exclusive? *Ecology*, **78**, 1301–1311.

McKey, D. (1979) The distribution of secondary compounds within plants. *Herbivores: Their Interaction with Secondary Plant Metabolites* (eds G.A. Rosenthal & D.H. Janzen), pp. 56–134. Academic Press, Orlando, FL.

Mosher, R.A. & Melnyk, C.W. (2011) siRNAs and DNA methylation: seedy epigenetics. *Trends in Plant Science*, **15**, 204–210.

Mueller, R.C., Wade, B.D., Gehring, C.A. & Whitham, T.G. (2005) Chronic herbivory negatively impacts cone and seed production, seed quality, and seedling growth of susceptible pinyon pines. *Oecologia*, **143**, 558–565.

Obeso, J.R. (1993) Does defoliation affect reproductive output in herbaceous perennials and woody plants in different ways? *Functional Ecology*, **7**, 150–155.

Osier, T.L. & Lindroth, R.L. (2006) Genotype and environment determine allocation to and costs of resistance in quaking aspen. *Oecologia*, **148**, 293–303.

Palo, R.T. (1984) Distribution of birch (*Betula* spp.), willow (*Salix* spp.), and poplar (*Populus* spp.) secondary metabolites and their potential role as chemical defense against herbivores. *Journal of Chemical Ecology*, **10**, 499–520.

Pasteels, J.M., Rowellrahier, M., Braekman, J.C. & Dupont, A. (1983) Salicin from host plant as precursor of salicylaldehyde in defensive secretion of Chrysomeline larvae. *Physiological Entomology*, **8**, 307–314.

Porter, L.J., Hrstich, L.N. & Chan, B.G. (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, **25**, 223–230.

Purrington, C.B. (2000) Costs of resistance. *Current Opinion in Plant Biology*, **3**, 305–308.

R Core Team (2011) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rasmann, S., De Vos, M., Casteel, C.L., Tian, D.L., Halitschke, R., Sun, J.Y., Agrawal, A.A., Felton, G.W., Jander, G. (2012) Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiology*, **158**, 854–863.

Rehill, B.J., Clauss, A., Wieczorek, L., Whitham, T.G. & Lindroth, R.L. (2005) Foliar phenolic glycosides from *Populus fremontii*, *Populus angustifolia*, and their hybrids. *Biochemical Systematics and Ecology*, **33**, 125–131.

Rehill, B.J., Whitham, T.G., Martinsen, G.D., Schweitzer, J.A., Bailey, J.K. & Lindroth, R.L. (2006) Developmental trajectories in cottonwood phytochemistry. *Journal of Chemical Ecology*, **32**, 2269–2285.

Richards, E.J. (2006) Inherited epigenetic variation-revisiting soft inheritance. *Nature*, **7**, 395–401.

Rieseberg, L.H., Archer, M.A. & Wayne, R.K. (1999) Transgressive segregation, adaptation, and speciation. *Heredity*, **83**, 363–372.

Roach, D.A. & Wulff, R.D. (1987) Maternal effects in plants. *Annual Review of Ecology and Systematics*, **18**, 209–235.

Sampedro, L., Xoaquin, M. & Zas, R. (2011) Costs of constitutive and herbivore-induced chemical defences in pine trees emerge only under low nutrient availability. *Journal of Ecology*, **99**, 818–827.

Scoville, A.G., Barnett, L.L., Bodbyl-Roels, S., Kelly, J.K. & Hileman, L.C. (2011) Differential regulation of a MYB transcription factor is correlated with transgenerational epigenetic inheritance of trichome density in *Mimulus guttatus*. *New Phytologist*, **191**, 251–263.

Simonsen, A.K. & Stinchcombe, J.R. (2007) Induced responses in *Ipomoea hederacea*: simulated mammalian herbivory induces resistance and susceptibility to insect herbivores. *Arthropod-Plant Interactions*, **1**, 129–136.

Smulders, M.J.M., Van Der Schoot, J., Arens, P. & Vosman, B. (2001) Trinucleotide repeat microsatellite markers for black poplar (*Populus nigra* L.). *Molecular Ecology Notes*, **1**, 188–190.

Stamp, N. (2003) Out of the quagmire of plant defense hypotheses. *The Quarterly Review of Biology*, **78**, 23–55.

Steets, J.A. & Ashman, T.-L. (2010) Maternal effects of herbivory in *Impatiens capensis*. *International Journal of Plant Sciences*, **171**, 509–518.

Stevens, M.T., Waller, D.M. & Lindroth, R.L. (2007) Resistance and tolerance in *Populus tremuloides*: genetic variation, costs, and environmental dependency. *Evolutionary Ecology*, **21**, 829–847.

Strauss, S.Y. & Agrawal, A.A. (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution*, **14**, 179–185.

Strauss, S.Y., Rudgers, J.A., Lau, J.A. & Irwin, R.E. (2002) Direct and ecological costs of resistance to herbivory. *Trends in Ecology and Evolution*, **17**, 278–285.

Sultan, S.E., Barton, K. & Wilczek, A.M. (2009) Contrasting patterns of transgenerational plasticity in ecologically distinct congeners. *Ecology*, **90**, 1831–1839.

Thalmann, C., Freise, J., Heitland, W. & Bacher, S. (2003) Effects of defoliation by horse chestnut leafminer (*Cameraria ohridella*) on reproduction in *Aesculus hippocastanum*. *Trees-Structure and Function*, **17**, 383–388.

Timmer, V.R. & Stone, E.L. (1978) Comparative foliar analysis of young balsam fir fertilized with nitrogen, phosphorous, potassium, and lime. *Soil Science Society of America Journal*, **42**, 125–130.

Tuskan, G.A., Gunter, L.E., Yang, Z.K., Yin, T., Sewell, M.M. & Difazio, S.P. (2004) Characterization of microsatellites revealed by genomic sequencing of *Populus trichocarpa*. *Canadian Journal of Forest Research*, **34**, 85–93.

Underwood, N.C. (1998) The timing of induced resistance and induced susceptibility in the soybean-Mexican bean beetle system. *Oecologia*, **114**, 376–381.

Verhoeven, K.J.F. & van Gurp, T.P. (2012) Transgenerational effects of stress exposure on offspring phenotypes in apomictic dandelion. *PLoS ONE*, **7**, e38605.

Verhoeven, K.J.F., Jansen, J.J., van Dijk, P.J. & Biere, A. (2010) Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytologist*, **185**, 1108–1118.

Veteli, T.O., Mattson, W.J., Niemelä, P., Julkunen-Tiito, R., Kellomäki, S., Kuokkanen, K. & Lavola, A. (2007) Do elevated temperature and CO_2 generally have counteracting effects on phenolic phytochemistry of boreal trees? *Journal of Chemical Ecology*, **33**, 287–296.

Waltz, A.M. & Whitham, T.G. (1997) Plant development affects arthropod communities: opposing impacts of species removal. *Ecology*, **78**, 2133–2144.

Wang, J.L. (2004) Sibship reconstruction from genetic data with typing errors. *Genetics*, **166**, 1963–1979.

Whitham, T.G. (1989) Plant hybrid zones as sinks for pests. *Science*, **244**, 1490–1493.

Wooley, S.C., Walker, S., Vernon, J. & Lindroth, R.L. (2008) Aspen decline, aspen chemistry, and elk herbivory: are they linked? *Rangelands*, **30**, 17–21.

Wulff, R.D. (1986) Seed size variation in *Desmodium paniculatum*. 1. Factors affecting seed size. *Journal of Ecology*, **74**, 87–97.

Zangerl, A.R. (2003) Evolution of induced plant responses to herbivores. *Basic and Applied Ecology*, **4**, 91–103.

Zangerl, A.R. & Berenbaum, M.R. (1990) Furanocoumarin induction in wild parsnip: genetics and populational variation. *Ecology*, **71**, 1933–1940.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Methods and results.

Table S1. Simple sequence repeat (SSR) genetic markers used in this study.

Table S2. Phenolic glycoside and condensed tannin concentrations in different developmental stages and among taxa in cottonwood.

Table S3. Results of GLM ANOVAs for total phenolic glycoside content and concentration in seedlings with F_1 hybrid mothers.

Figure S1. Illustration and interpretation of six common vector endpoints in graphical vector analysis.

Figure S2. Phenolic glycoside concentrations in foliage of seedlings from maternal herbivory or maternal control trees within each taxon.

Figure S3. Condensed tannin concentrations in foliage of seedlings of maternal herbivory vs. control trees within each taxon.