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Ecosystem implications of genetic variation in water-use of a dominant riparian tree

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Abstract Genetic variation in dominant species can affect plant and ecosystem functions in natural systems through multiple pathways. Our study focuses on how genetic variation in a dominant riparian tree (*Populus fremontii*, *P. angustifolia* and their natural F₁ and backcross hybrids) affects whole-tree water use, and its potential ecosystem implications. Three major patterns were found. First, in a 12-year-old common garden with trees of known genetic makeup, hybrids had elevated daily integrated leaf-specific transpiration (E_{tl} ; $P=0.013$) and average canopy conductance (G_c ; $P=0.037$), with both E_{tl} and G_c ~30% higher in hybrid cross types than parental types. Second, $\delta^{13}\text{C}$ values of leaves from these same trees were significantly more negative in hybrids ($P=0.004$), and backcross hybrids had significantly more negative values than all other F₁ hybrid and parental types ($P < 0.001$). Third, in the wild, a similar pattern was found in leaf $\delta^{13}\text{C}$ values where both hybrid cross types had the lowest values ($P < 0.001$) and backcross hybrids had lower $\delta^{13}\text{C}$ values than any other tree type ($P < 0.001$). Our findings have two important implications: (1) the existence of a consistent genetic difference in whole-tree physiology suggests that whole-tree gas and water exchange could be another pathway through which genes could affect ecosystems;

and (2) such studies are important because they seek to quantify the genetic variation that exists in basic physiological processes—such knowledge could ultimately place ecosystem studies within a genetic framework.

Keywords Extended phenotype · Intrinsic water-use efficiency · Intraspecific genetic variation · *Populus* · Sap flow

Introduction

Recent research has demonstrated that differences among (Binkley and Giardina 1998; Giardina et al. 2001) and within dominant plant species or hybridizing complexes (Driebe and Whitham 2000; Treseder and Vitousek 2001; Madritch and Hunter 2002) can influence ecosystem processes such as decomposition and nutrient cycling. Such studies are important because they begin to separate the genetic and environmental components of ecosystem-level processes and to place ecosystem studies within a genetic framework (Whitham et al. 2003). Interestingly, these studies have focused solely on genetic effects on plant litter quality and nutrient cycling despite the wide array of pathways through which gene×environment responses can affect ecosystems (Chapin 2003).

Studies with silviculturally important tree species have also shown that plant genotype affects plant water use, carbon uptake, intrinsic water use efficiency, and resilience to changing environmental conditions at the individual level (e.g., Bassman and Zwier 1991; Osorio and Pereira 1994; Benowicz et al. 2001). Other researchers have found evidence for ecotypic or family differences in physiologic parameters such as leaf conductance, photosynthesis, and stomatal size/density in naturally occurring trees (Hogan et al. 1994; Zhang et al. 1994; Fan et al. 1997; Tognetti et al. 1997; Kundu and Tigerstedt 1999; Wang et al. 2000; Dunlap and Stettler 2001; Marshall et al. 2001). Additionally, some studies have noted differences in rates of leaf gas exchange and $\delta^{13}\text{C}$ ratios in hybrid taxa

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compared to parental species (McArthur et al. 1998; Williams and Ehleringer 2000).

No studies have examined the degree of coupling between genetic change and whole-tree water relations in natural systems. This coupling could have important implications at the ecosystem scale if whole-tree physiology scales predictably, and would represent an unexplored pathway through which genetic composition could affect ecosystem function. Establishing the existence of significant natural genetic variation in tree-level water use rates was our goal as a first step in understanding if genetics could affect ecosystems through water-use dynamics pathways.

We assessed how natural genotypic differences between *Populus* trees of pure and hybrid status influenced whole-tree physiology in a 12-year-old common garden environment and $\delta^{13}\text{C}$ values in the garden and natural stand environments. Earlier work (Schweitzer et al. 2002) documented that backcross hybrid cottonwood trees asexually produced more ramets than parental trees without sacrificing seed production. This strategy would likely incur a greater carbon cost. If hybrid trees had higher stomatal conductance and altered water-use efficiency with the same photosynthetic capacity, it could allow them to assimilate a greater amount of carbon than parents, and possibly direct it towards greater reproduction. Similarly, silvicultural studies have found that hybrids can be more productive and more water-use efficient than parentals (e.g. Braatne et al. 1992). Hence, we hypothesized that leaf-specific transpiration and canopy conductance values would be higher in hybrids than their parental taxa (Fremont cottonwood *Populus*

fremontii S. Wats. and narrowleaf cottonwood *P. angustifolia* James). Such genetically based physiological differences could potentially affect water use and gas exchange within *Populus*-dominated riparian ecosystems where hybrids are common and in plantations where hybrids predominate (e.g., Stettler et al. 1996). This last point is dependent on scaling relationships from tree to stand and a lack of genetic by environment (G×E) interactions. Thus, we sought to make no direct inferences about the magnitude of genetic effects on ecosystems through physiological pathways. However, we argue that the existence of large natural genetic-based variation in plant physiology potentially represents an unexplored pathway through which plant genetics can affect ecosystems through direct and indirect mechanisms. Additionally, the existence of genetic-based differences in traits that can affect ecosystems would suggest that ecosystem function may be subject to changes in gene frequencies through evolution. Plant hybrid zones have been widely recognized as centers of plant evolution (Rieseberg et al. 2000). They also are areas where genetic variation is high, and represent ideal systems for the study of genetic effects on ecosystems because selection strength is proportional to genetic variability (Fisher 1958).

Materials and methods

Plant material

Natural hybrid cottonwood zones are found on virtually every river in the western USA (Eckenwalder 1984a, 1984b). They range in size from small isolated relics to large hybrid swarms over 1,000 km in

Table 1 Selected characteristics of trees within the common garden used in this study. Clone codes serve as markers for individual genotypes, but otherwise have no relation to cross type. Cross types

are *A* (*Populus. angustifolia*), *B* (backcross hybrids), *F₁* (*F₁* hybrids), and *F* (*Populus fremontii*)

Clone	Diameter at the base of the live crown (cm)	Height (m)	Fraction of Fremont to total markers	Cross type	Leaf area (m ²)	Sapwood area (cm ²)	Leaf area: sapwood area (m ² /cm ²)	Fraction of sapwood area to total basal area
1008	12.4	8.6	0.00	A	64	109	0.59	0.78
1008	12.8	9.2	0.00	A	47	79	0.59	0.75
t-15	26.5	10.4	0.00	A	161	402	0.40	0.72
wc-5	23.5	9.0	0.00	A	105	126	0.83	0.71
rm-2	23.0	12.8	0.00	A	100	242	0.42	0.63
996	31.0	12.4	0.03	B	205	496	0.41	0.71
1012	11.5	8.8	0.03	B	33	64	0.52	0.78
996	13.4	9.4	0.03	B	41	87	0.47	0.77
1017	41.5	16.2	0.03	B	464	912	0.51	0.61
3200	20.0	14.4	0.05	B	62	207	0.30	0.70
989-3	41.9	16.4	0.50	F ₁	392	680	0.58	0.57
1997	9.7	9.2	0.50	F ₁	22	62	0.35	0.98
989-3	48.7	18.0	0.50	F ₁	459	1005	0.46	0.56
989-3	35.0	16.0	0.50	F ₁	216	481	0.45	0.65
Kscr-1	25.3	13.2	1.00	F	175	255	0.69	0.67
Kscr-1	23.0	14.8	1.00	F	86	125	0.69	0.69
17	27.3	21.0	1.00	F	225	284	0.79	0.71
0031	27.0	15.2	1.00	F	169	346	0.49	0.59

length (T.G. Whitham, unpublished data), and cottonwood hybrids are also found in the fossil record (Eckenwalder 1984c). Along the Weber River watershed in north-central Utah, USA, higher elevation riparian habitat is dominated by *Populus angustifolia*, the lower elevation riparian habitat is dominated by *P. fremontii*, and in a 13-km zone at their boundaries both parental species and abundant hybrids are found (Keim et al. 1989).

Studies on hybridization along the Weber River (Keim et al. 1989; Martinsen et al. 2001) show that the degree of hybridization can be accurately characterized using the fraction of species-specific molecular markers of *P. fremontii* found in each tree (Table 1). Extensive previous genetic work using restriction fragment length polymorphism (RFLP) methods has been conducted on all trees in the common garden and more than 350 trees in the wild. We used these data to establish the hybrid and genetic status for all common garden trees used in this study (see Martinsen et al. 2001).

In 1991, 350 clones, representing 81 naturally occurring individuals of both parental species, F_1 and backcross hybrids, were planted randomly in a common garden at the Ogden Nature Center in Ogden, Utah, (elevation 1,370 m; 41°11'N, 111°56'W). Each genotype was represented by 1–10 replicates and genetic status was determined using RFLP methods (Martinsen et al. 2001). Trees were planted 4 m apart. The common garden is located at the bottom end of the 13 km hybrid zone along the Weber and Ogden Rivers and was home to native cottonwood-dominated gallery forests prior to clearing in the mid-twentieth century. The soil at the common garden is in the Entisol Soil Taxonomic order, and is composed of ~60% sand, ~30% silt, and ~10% clay (e.g., Schweitzer 2002). The site receives approximately 440 mm of precipitation annually, with an average of 14 mm in July. The mean annual air temperature is 10.4°C, and the mean daily air temperature is 24.4°C for the month of July (Western Regional Climate Center 2001). During our study, 0.4 mm of precipitation was recorded and the average daytime air temperature was 25.2°C. Supplemental water of 32 l per week (8 h per week, 4 l per hour) was supplied to each tree during the summer months by drip irrigation.

Study protocol

Sap flux density (Granier 1987) was measured at the base of the live crown in 18 trees (Table 1) using the Granier sap flow method (Granier and Loustau 1994; Granier et al. 1996; Ewers and Oren 2000). We measured sap flux density in four *P. angustifolia*, two F_1 hybrids, four backcross hybrids, and three *P. fremontii* genotypes. Some genotypes were represented by multiple clones, thus although 18 trees were measured, this only represented 13 genotypes. Sensors were placed across the hydroactive xylem of each tree at 0–2 cm, 2–4 cm, and 4–6 cm depths. In all cases, an attempt was made to measure the entire length of the hydroactive xylem from the bark to the heartwood, and values were corrected when sensors were in contact with non-conductive tissue (Clearwater et al. 1999). Measurements were collected hourly using Campbell Scientific CR10X dataloggers and AM416 multiplexers (Logan, Utah, USA). Measurements were taken continuously from day of year (DOY) 196 to 202, 2001 to capture the peak of the summer drought season in northern Utah.

Sap flux estimates were scaled to the whole-tree by multiplying sap flux values by the 2 cm thick bands of sapwood associated with each probe as in Granier (1987). Leaf specific transpiration (E_l) was calculated by integrating whole-tree sap flux values over the daylight hours (assumed to be between 0600 and 2100 hours) and then dividing this value by projected leaf area of the tree (see below).

Projected leaf area (LA) was estimated for all trees using allometric equations based on branch diameter (see Gielen et al. 2001; Fischer et al. 2002). We developed the equations by removing three branches from three stratified canopy positions on six trees (stratified over cross type) at the end of our study. All leaves were removed, dried (72 h at 70°C) and weighed, except for a subsample from each branch used to determine specific leaf area using an Agvis

Imaging System (Decagon Devices, Pullman, Wash., USA). After the areas of these leaves were measured, they were also dried at 70°C to determine oven-dry mass. Dried leaf weights from each branch were multiplied by specific leaf area to get an estimate of projected leaf area. An equation was then constructed for estimation of projected leaf area (cm^2) based on the diameters (cm) of removed branches ($R^2=0.81$, $P<0.0001$, leaf area = $1,990.49 \times [\text{branch diameter}]^2 + 3,115.30 \times [\text{branch diameter}]$). Branch diameters from every branch on every tree in the study were measured after sap flux measurements were completed, and the application of this allometric equation yielded an estimate of projected leaf area for each tree. Sapwood area was estimated using tree-cores for each tree and visually distinguishing between light colored sapwood and dark colored heartwood.

Projected leaf area-scaled sap flux was divided by vapor pressure deficit (D) to produce estimates of canopy conductance (G_c , $\text{mmolH}_2\text{O m}^{-2} \text{s}^{-1}$; Ryan et al. 2000) using the equation:

$$G_c = E_l / (D/A_p), \quad (1)$$

where E_l is leaf specific transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{LA s}^{-1}$), D is the vapor pressure deficit (kPa) calculated from air temperature and humidity measured on-site (Montieth and Unsworth 1990; see below), and A_p is the mean atmospheric air pressure (kPa) for the study site. We assumed lag effects did not affect our measurement of G_c based on tests of time lagged E_l versus D (data not shown; Fischer et al. 2002). G_c data with D lower than 1.0 kPa were excluded from analysis due to high error associated with low D values and our calculation of G_c (see Ewers and Oren 2000 for extended analysis on this subject). Only E_l values collected during daylight hours were used for G_c calculation.

Vapor pressure deficit was calculated from air temperature and humidity measured 1.5 m above the ground in a canopy gap (>1 m from nearest tree) midway between sap flux measurement trees using a Campbell Scientific CS5000 air temperature and humidity measurement probe (Logan, Utah, USA). The canopy within the garden is characteristically rough and open; thus, we felt the 1.5 m measurement height was appropriate for our purposes. However, our measurements would ideally have been higher in the canopy (Table 1) and this could be a source of error in G_c measurement. Weather data were collected every minute using Campbell Scientific CR10X dataloggers (Logan, Utah, USA), and these values were then averaged hourly.

Between 20 and 30 sun-exposed leaves were collected from mid-canopy in each garden tree used for sap flow measurements on DOY 171, 2002 for stable isotope ratio ($\delta^{13}\text{C}$) and nitrogen (N) concentration analyses. Sun-exposed leaves were also collected from mid canopy in trees of known parental and cross type growing in six natural stands where *P. fremontii*, F_1 hybrids and backcross hybrids all co-occur. For these trees, 20 leaves were collected from each of 10 *P. fremontii* trees, 6 F_1 hybrid trees and 13 backcross hybrids growing along a greater than 13 km stretch of the Weber River, UT on DOY 225 in 2002. These field trees were chosen at random from a subset of randomly selected trees previously used by Martinsen et al. (2001) for RFLP analysis and Young et al. (unpublished data collected in 2001; W.P. Young, University of Idaho) for AFLP analysis. For the field trees, pure *P. angustifolia* leaves were not used because they are rare in the hybrid zone and so did not co-occur with other tree types in sufficient numbers. All leaves were cut at the base of the petiole and immediately removed and dried at 70°C for 48 h. Dried leaves were ground in a Wiley Mill (Thomas Scientific, Swedesboro, N.J., USA) through a 40 mesh screen and analyzed for carbon isotope composition. Carbon isotope ratios and total N concentrations were determined using an elemental analyzer (CE Elantech Model NC 2100, Lakewood, N. J., USA) coupled to a continuous flow mass spectrometer (Thermo Finnegan Delta XL, Bremen, Germany) at the Colorado Plateau Stable Isotope Facility, Northern Arizona University, Flagstaff, Ariz., USA. Carbon isotope ratios are expressed in parts per mil (‰), where $\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000$, using PDB as the standard. For leaves with equivalent photosynthetic capacities, as

suggested by the similarities in their leaf N concentration, a more negative leaf $\delta^{13}\text{C}$ value indicates a greater stomatal aperture during carbon uptake, and hence a lower intrinsic water-use efficiency (Ehleringer and Cooper 1988; Ehleringer et al. 1990).

Tests for significance of parent and cross type as a factor for sap flux data were run in two separate ways. The two ways were chosen to capture potential variability in results due to scale. For example, patterns observed in averages from a single day may not be reflected in the averages across multiple days and vice versa. First, daily average G_c and E_{tl} were compared across parental and cross types for each day independently using ANOVA followed by least squared means (LSM) contrasts. Second, averages were calculated from daily E_{tl} and G_c for all genotypes across all days. An ANOVA was then performed with parent/cross type as the only factor followed by LSM contrasts for specific hypotheses. Repeated clones within each parental and cross type were averaged, and the average was treated as an individual clonal representative of the cross type in all analyses. ANOVA and LSM contrasts were also used to evaluate differences in leaf $\delta^{13}\text{C}$ values among trees of different parental and cross type status in the common garden and in the wild. All tests and regressions were run using JMP Discovery statistical package (SAS, Cary, N.C., USA). Our alpha level for significance was set at $P < 0.05$.

Results

Sapwood depth and leaf area

Mean sapwood depth across all study trees within the common garden was $5.3 \text{ cm} \pm 0.5 \text{ cm}$ (1 SE). There were no significant differences or trends in sapwood depth among parental and cross types ($P > 0.1$). Sapwood area ranged from 55.6 to 97.6% of total basal area (Table 1). These high sapwood percentages are not surprising considering the young age of these trees (11 years), their open-grown nature, and the tendency for cottonwood trees to carry large quantities of foliage. The mean percent of basal area as sapwood was $69.9 \pm 2.3\%$ (1 SE).

When pooled across parental and cross types, strong correlations were found for all trees between total projected leaf area and independently measured sapwood

area at the base of the live tree crown ($R^2=0.94$, $p < 0.001$, $[\text{LA} (\text{m}^2)] = 0.48 \times [\text{SA} (\text{cm}^2)] + 3.90$). Total tree projected leaf area ranged from 21.8 to 464.2 m^2 . Mean projected leaf area per tree was not significantly different among parental and cross types ($P=0.363$) and averaged $168.1 \pm 33.2 \text{ m}^2$ (1 SE) across all study types.

Leaf specific trends

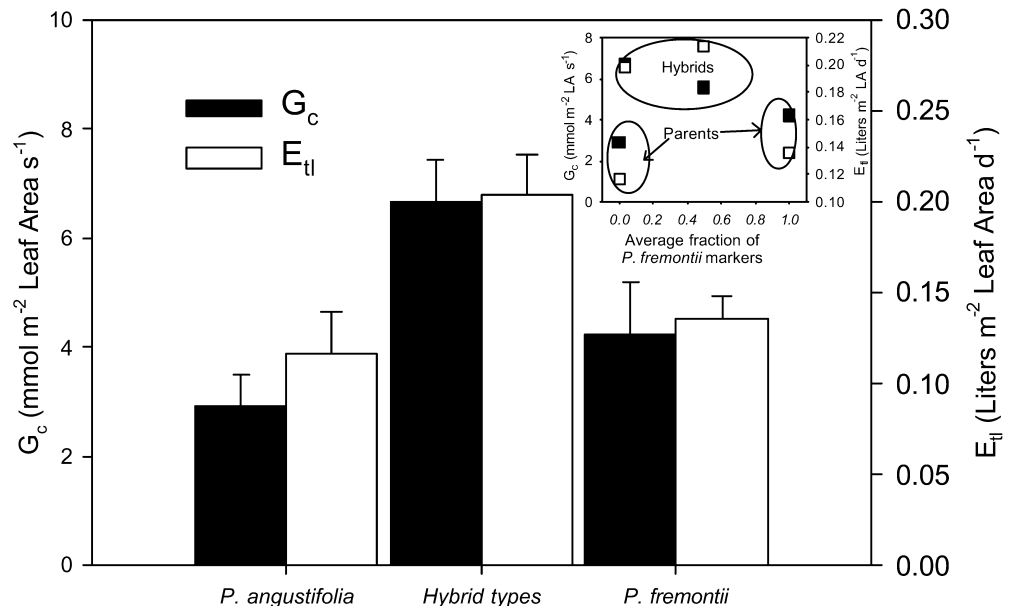
ANOVA was significant for analysis on both average E_{tl} ($P=0.034$, $F=4.86$) and average G_c ($P=0.049$, $F=4.13$). Hybrids (backcross and F_1 hybrids pooled) were significantly different from parent types in daily total leaf-specific transpiration and mean daily canopy conductance (E_{tl} : $P=0.013$, $F=9.07$; G_c : $P=0.037$, $F=5.82$; Fig. 1), but backcross hybrids were not significantly different from F_1 hybrids (E_{tl} : $P=0.828$; G_c : $P=0.953$).

Both E_{tl} and G_c were significantly higher in hybrids compared to parent types on every day except DOY 201 (Fig. 2). Thus, analysis conducted on different temporal scales yielded similar results. Within the hybrid class, there were no obvious differences between F_1 and backcross hybrids measured during our sap flux study (Figs. 1 inset, 2). However, due to the small sample size for F_1 hybrids ($n=2$) and the relatively short time over which sap flux-based measurements were conducted, we are not confident that differences do not exist (risk of Type II error is very high).

Leaf $\delta^{13}\text{C}$

$\delta^{13}\text{C}$ values in the common garden generally matched the results from sap flow measurements (see Fig. 3) suggesting that high canopy conductance was the result of high stomatal conductance and this produced correspondingly low $\delta^{13}\text{C}$ values. Hybrids had lower $\delta^{13}\text{C}$ than parents

Fig. 1 Comparison of canopy conductance (G_c) and leaf specific transpiration rates (E_{tl}) between hybrid and pure cross types show that hybrids have significantly greater G_c and E_{tl} compared to parents (G_c : $P=0.049$, E_{tl} : $P=0.035$). Bars are means ± 1 SE. Inset shows G_c and E_{tl} for different cross types displayed on the x-axis as fractions of *P. fremontii* markers (0 = *P. angustifolia*, 0.034 = Backcross hybrids, 0.5 = F_1 Hybrids, 1 = *P. fremontii*)



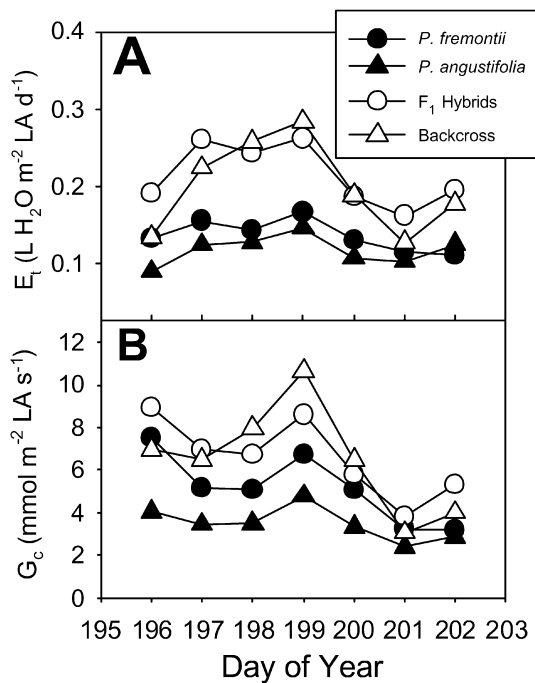


Fig. 2 **A** Average leaf specific transpiration (E_t ; Liters m^{-2} Leaf area day^{-1}) for each cross type across measurement dates. **B** Average canopy conductance (G_c ; $\text{mmol H}_2\text{O m}^{-2}$ Leaf area s^{-1}) for each cross type across measurement dates

($P=0.004$), and backcross hybrids had significantly more negative $\delta^{13}\text{C}$ values than any other trees [$P < 0.001$, -30.3 ± 0.2 (1 SE)]. F_1 hybrid leaf $\delta^{13}\text{C}$ values were -28.9 ± 0.3 (1 SE). Those for *P. angustifolia* averaged -28.7 ± 0.1 (1 SE), and those for *P. fremontii*, -28.5 ± 0.3 (1 SE). The difference between hybrid classes (i.e., F_1 and backcross hybrids) was greater than the difference between the two parent species. This pattern was not detected in our sap flow study and suggests that this more time-integrated measure ($\delta^{13}\text{C}$) was better able to detect potential water-use differences between cross types.

Differences in leaf $\delta^{13}\text{C}$ values among one parental and two cross types for trees in natural stands followed a similar pattern as in the garden (Fig. 4). Hybrids had lower values [-28.7 ± 0.3 (1SE); $P < 0.001$] than *P. fremontii* [-26.6 ± 0.3 (1SE)], and backcross hybrids were lowest overall [-29.2 ± 0.3 (1SE); $P < 0.001$]. There was no significant difference in leaf N concentration among parental and cross types for common garden trees or trees in natural stands (common garden: $P=0.185$; natural stand trees: $P=0.424$). Average N values for common garden and field (wild) trees were 2.3 ± 0.1 (1SE) and 1.8 ± 0.04 (1SE) respectively.

Discussion

Genetic components to ecosystem processes

In our study, higher E_t and G_c values in hybrids suggest unique phenotypic expression of water use dynamics in

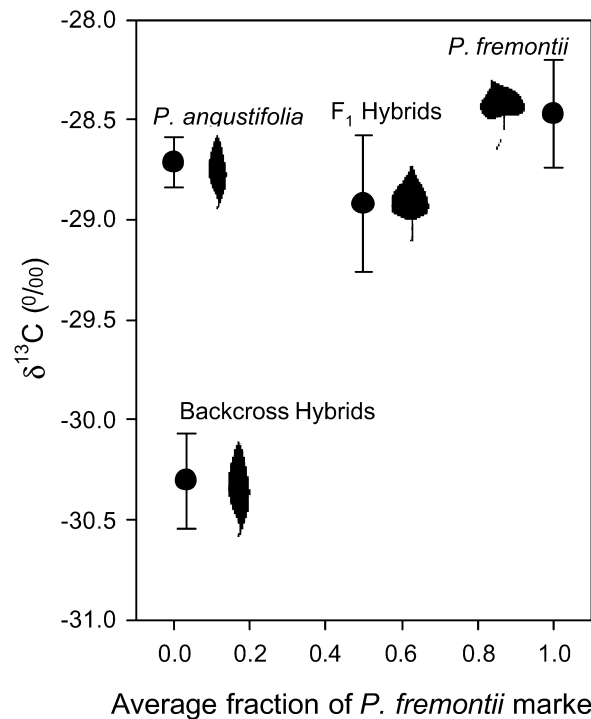


Fig. 3 Mean leaf $\delta^{13}\text{C}$ values versus fraction of *P. fremontii* markers. Vertical bars are ± 1 SE of the mean

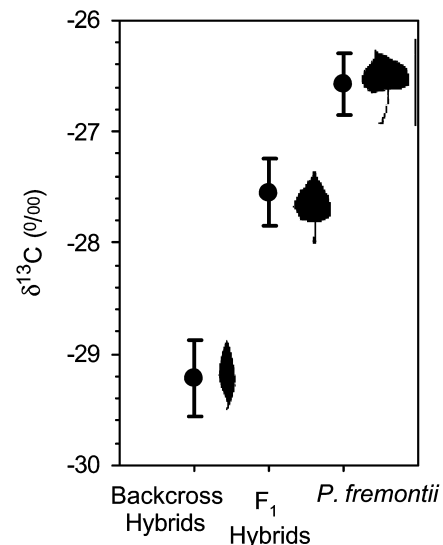


Fig. 4 $\delta^{13}\text{C}$ values for leaves from different *Populus* cross types collected from natural cottonwood stands along the Weber River, Utah, USA. Leaf $\delta^{13}\text{C}$ values for *P. angustifolia* were not determined as they are rare in the hybrid zone

these taxa. Lower intrinsic water use efficiency and higher stomatal conductance in hybrids was also supported by $\delta^{13}\text{C}$ values for trees in the common garden and natural stands. Positive relationships between conductance and photosynthetic rates have long been recognized (Pallardy and Kozlowski 1997; also see Wang et al. 2000 for a recent example in *Populus*). If differences in G_c values that we observed are sustained over the growing season, and photosynthetic capacity remains similar among groups,

then our results suggest hybrid leaf specific carbon assimilation may also be higher than parentals. This is also consistent with the widespread use of hybrids in plantations where maximizing yield for pulp production is emphasized (Stettler et al. 1996).

Why do hybrids have higher E and G ? Other research has shown that backcross *P. fremontii* × *angustifolia* hybrids invest significantly more energy into leaf-level tannins (Driebe and Whitham 2000). This is a well accepted sink for carbon and incurs a high energy cost (McNaughton 2001). Higher allocation to tannins would require higher rates of carbon gain if allocation of carbon to other organic constituents of the plant are not reduced. Differences in belowground allocation among the cross types are also possible (Jackson et al. 2001). For instance, Ibrahim et al. (1998) found higher belowground carbon allocation in *Populus* hybrids significantly associated with drought tolerance and leaf water potential values. Scarascia-Mugnozza et al. (1997) also found differences in root: shoot ratio among parental and hybrid *Populus* clones. In similar western cottonwood ecosystems of North America, higher rates of belowground carbon allocation in hybrids may result in higher water use rates and lower intrinsic water use efficiency. Schweitzer et al. (2002) showed that *Populus fremontii* × *angustifolia* hybrids were more likely to reproduce vegetatively than either parent, which may also be linked to greater belowground carbon allocation.

How might these results affect ecosystems? Altered water-use dynamics could affect ecosystems through direct and indirect mechanisms. Increased canopy-level transpiration could result in increased ecosystem transpiration if leaf area is held constant across ecosystems (e.g., Vertessy et al. 2001) and canopy boundary layer conditions support the ecosystem-level expression of canopy-level phenomena (Jarvis and McNaughton 1986). This is important to recognize in western intermountain watersheds of the USA, where water quality and quantity is a large social concern. At the local scale, increased tree transpiration could deplete surface soil water (i.e., Braatne et al. 1992; Oren et al. 1998), increase hydraulic lift (Horton and Hart 1998), and affect ecosystem boundary layer conditions (Montieth and Unsworth 1990). Schweitzer (2002) found that soil moisture is lower in *P. fremontii* × *angustifolia* hybrid zones along the Weber River than adjacent zones dominated by each parent species despite intermediate precipitation in the hybrid zone compared to the two parent-dominated zones. Thus, differences in transpiration among parental and cross types determined with sap flow methods are supported by observations of soil moisture in natural communities where the same parental and cross types are dominant. Scaling relationships from tree to stand clearly warrant more study in such systems.

Ecosystems may also be affected indirectly by different water-use dynamics between genotypes. For example, indirect influences on ecosystem function could include differential survival of genotypes with differing water-use dynamics during drought events. Differential dependence on mycorrhizae is also possible and different survival rates

could influence microbial biomass and microbial community structure, which in turn could affect nutrient dynamics. Differences in soil moisture dynamics could alter belowground root turnover and production dynamics (Stettler et al. 1996; Gill and Jackson 2000).

Contrasts with other ecosystems

Our research suggests that genetic controls over canopy water-use dynamics are possible in natural genotypes from a riparian ecosystem dominated by a single taxonomic group. The range of difference in E_{tl} we observed among hybrids and parents is similar to what Souch and Stephens (1998) found between clones of *P. trichocarpa* × *trichocarpa* and *P. trichocarpa* × *deltoides*. In their study, a *P. trichocarpa* × *deltoides* clone had >40% higher water-use rates than a *P. trichocarpa* × *trichocarpa* clone (determined using sap flow and mass-loss methods). Differences far smaller than 30% (as we report in the present study) in water-use parameters, such as canopy conductance, have been commonly observed among highly divergent tree taxa growing in mixed stands (Wullschlegger et al. 1998). For instance, Pataki et al. (1998) found differences of less than 30% between *Liquidambar styraciflua* and *Pinus taeda* in E_{tl} and G_c .

Differences of this magnitude also appear to be quite common in intraspecific comparisons. Here, we define intraspecific genetic variation as the genetic variation found in the largest taxonomic unit with significant gene flow and heritable transmission of traits from one generation to the next (Whitham et al. 2003). This distinction is important because such gene flow can affect ecologically important traits and have evolutionary consequences. Table 2 shows the results of a survey of the literature documenting intraspecific genetic variation in water-use and gas exchange traits. These studies are regularly interpreted in a tree-improvement context, but rarely in an ecosystem context. Studies on multiple tree taxa (both natural and artificially propagated) such as eucalypts, alder, birch, pine, and Douglas-fir, have demonstrated large intraspecific differences in plant water use, carbon uptake, intrinsic water use efficiency, and resilience to changing environmental conditions (e.g., Osorio and Pereira 1994; Benowicz et al. 2000, 2001; Marshall et al. 2001). Other studies have found both positive and negative affects of genetic variation on gas exchange in hybrid taxa compared to parental species (McArthur et al. 1998; Williams and Ehleringer 2000). These studies offer clues to how genetic variation could directly and indirectly affect ecosystem function through altering gas exchange dynamics in dominant trees, but have not been interpreted in this context. We suggest that strong genetic influences on natural ecosystem water and gas exchange parameters may be common in ecosystems dominated by single taxa. Additionally, patterns may be scale dependent. For example, clear differences between hybrid groups in $\delta^{13}C$ values (reflecting conductance throughout the growing season as well as stored carbon; Figs. 3, 4)

Table 2 Some recent studies of intraspecific differences in physiologic parameters which species or hybridizing complex that comprises the largest unit with significant gene flow and could have ecosystem-level gas exchange implications. We use the definition of intraspecific heritable transmission of traits from one generation to the next. (*N* nitrogen, *WUE* intrinsic genetic variation given in Whitham et al. (2003), i.e., the genetic variation found within a water use efficiency)

Study	Unit of comparison	Species	Common Garden (CG), Field (F), or Both (CGF)	Parameter	Maximum intra-taxonomic difference between units of comparison (%)
Tognetti et al. 1997	Ecotypic	<i>Pinus halepensis</i>	CG	Transpiration, stomatal conductance	Transpiration: 67%, stomatal conductance: 57%
Zhang et al. 1994	Families	<i>Larix occidentalis</i>	CG	$\Delta^{13}\text{C}$, stomatal conductance	$\Delta^{13}\text{C}$: 11.3%, stomatal conductance: 30.4%
Kundu and Tigerstedt 1997	Provenance	<i>Azadirachta indica</i>	CG	Photosynthesis, stomatal conductance, stomatal density	Photosynthesis: 46% stomatal conductance: 37% stomatal density: 29%
Fan et al. 1997	Intra-hybrid populations	<i>Picea sitchensis</i> × interior spruce (<i>P. glauca</i> × <i>P. engelmannii</i>)	CGF	Photosynthesis, stomatal density	Photosynthesis (under saturating light and drought): 12%, stomatal density: 55%
Wang et al. 2000	Natural clones	<i>Populus tremuloides</i>	CG	Photosynthesis, stomatal conductance	Photosynthesis: 24%, Stomatal conductance: 34%
Williams and Ehleringer 2000	Hybrids populations and parents	<i>Quercus turbinella</i> × <i>Quercus gambelli</i>	F	$\Delta^{13}\text{C}$, leaf N	$\Delta^{13}\text{C}$ varied 20% (4‰) between phenotypes resembling each parent in hybrid populations. This was positively correlated with leaf N concentration ($r^2=0.14$, $P=0.06$)
McArthur et al. 1998	Parent subspecies and subspecies hybrids	<i>Artemisia tridentata</i> ssp. <i>tridentata</i> × <i>A. tridentata</i> ssp. <i>vaseyana</i>	CG	Respiration, stem water potential	Subspecies differed in respiration by 14%. Hybrids were intermediate between parent subspecies
Osorio and Pereira 1994	Clones	<i>Eucalyptus globulus</i>	CG	$\delta^{13}\text{C}$, WUE, biomass production, leaf water potential	$\delta^{13}\text{C}$: ~9%, WUE: 15%, biomass production: ~43%, leaf water potential: 13%
Benowicz et al. 2000	Intraspecific	<i>Betula papyrifera</i>	CG	max. photosynthetic rate	max. photosynthetic rate: 16%
Benowicz et al. 2000	Intraspecific	<i>Alnus sinuata</i>	CG	max. photosynthetic rate	max. photosynthetic rate: 29%
Bassman and Zwier 1991	Parent and hybrid clones	<i>Populus trichocarpa</i> × <i>P. deltoides</i>	CG	Photosynthesis, transpiration, WUE, stomatal conductance	photosynthesis: 35%, transpiration: 25%, WUE: 19%, stomatal conductance: 42%

were apparent in our study, but our sap flow work (over a 7 day period; Figs. 1, 2) did not detect this difference. Thus, one level of resolution ($\delta^{13}\text{C}$; reflecting a longer time period) picked up a pattern that a finer scale measurement (sap flow measurement in August, 2001) could not detect. Similarly, patterns observed in common gardens may be reversed or confounded in the wild due to complex G×E interactions (J. Marshall, University of Idaho, personal communication 2002), and canopy boundary layer controls (Jarvis and McNaughton 1986), though differences retain a genetic basis.

The intraspecific differences in water use parameters we observed among cross types could potentially affect ecosystem water balance, gas exchange, and carbon allocation in natural populations similar to the influence of interspecific variation in other systems. Kaufmann (1984) found a 20% difference in stand transpiration between watersheds dominated by different tree species using modeling techniques. A recent sap flux study in a mixed deciduous forest also concluded that contributions of different species to whole stand water balance was influenced by species-specific differences in daily water use (Wullschlegel et al. 2001). In fact, intrinsic differences between species (Federer and Lash 1978; Pataki et al. 2000) are thoroughly investigated influences on ecosystem water balance. In some ecosystems (especially those dominated by single taxa), intraspecific differences may be similarly important.

Where one species is dominant in an ecosystem, most or all of the genetic variation in canopy water relations may be due to intraspecific variation. For example, cottonwoods dominate many western riparian ecosystems on North America where they contribute greatly to ecosystem transpiration (e.g., Schaeffer et al. 2000). Because these cottonwoods freely hybridize (Eckenwalder 1984a, 1984b) and genes introgress from one species to another (Martinsen et al. 2001), variation in genetic expression among parent and cross types may provide important variation in water use and carbon allocation throughout the riparian corridor. It is important to emphasize that the variation in $\delta^{13}\text{C}$ values between F₁ hybrids and backcross hybrids is far greater than the variation in $\delta^{13}\text{C}$ values between the parental species. Thus, all hybrids are not physiologically and phenotypically equal, and it is important to incorporate such variation among cross types in future studies.

Summary

Our study provides important new data on a genetic component to whole-tree physiology and suggests another pathway through which genes might effect ecosystems (Driebe and Whitham 2000; Treseder and Vitousek 2001; Madritch and Hunter 2002; Schweitzer 2002). The key findings of our study emphasize three main points. (1) Similar to some other more production-oriented studies, we found differences in physiological performance between hybrid types and hybrids compared to their

parent species. (2) Physiological patterns found in a common garden environment appear to also exist in the field, suggesting stability of genetic-based differences in multiple habitats. This statement is also supported by extensive work done by others (see Stettler et al. 1996). (3) The existence of widespread differences in gas and water flux dynamics between different genotypes, ecotypes, and hybrids, furthered by our study, suggests that genetic effects on ecosystems through gas flux dynamics pathways should be recognized and explored further as another mechanism by which genes affect ecosystems. Understanding broad-sense heritability of physiological parameters, and determining if patterns observed in our study scale up to the ecosystem level, will provide the next two steps in elucidating the influence of genes on these ecosystems through physiological pathways.

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