



Leaf hydraulic conductivity and photosynthesis are genetically correlated in an annual grass

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Summary

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- Comparative studies suggest that a positive correlation between xylem water transport and photosynthesis is adaptive. A requirement for the adaptive evolution of coordination between xylem and photosynthetic functions is the presence of genetic variation and covariation for these traits within populations.
- Here it was determined whether there was genetic variation and covariation for leaf blade hydraulic conductivity (K_W), photosynthetic rate (A), stomatal conductance (g_s), and time to flowering in a population of recombinant inbred lines of *Avena barbata*, a Mediterranean annual grass.
- Significant ($P < 0.05$) broad-sense heritabilities (H^2) were detected for K_W ($H^2 = 0.33$), A ($H^2 = 0.23$) and flowering time ($H^2 = 0.62$), but not for g_s . Significant positive genetic covariation between A and K_W was also observed. There was no other genetic covariation among traits.
- The first evidence of genetic variation for K_W within a species was obtained. These results also indicate that there is a genetic basis for the positive association between xylem water transport and photosynthesis. The presence of significant genetic variation and covariation for these traits in natural populations would facilitate correlated evolution between xylem and leaf functions.

Key words: *Avena barbata*, correlated evolution, drought adaptation, genetic variation, hydraulic conductivity, photosynthesis.

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Introduction

The maintenance of a stable water supply to leaves is a requirement for gas exchange and therefore carbon fixation in plants (Zimmermann, 1983; Tyree & Ewers, 1991; Maherali *et al.*, 2004). The physiological basis for this relationship is generally supported by empirical observations showing that xylem hydraulic conductivity is positively correlated with stomatal conductance, transpiration and photosynthetic capacity (Brodribb & Feild, 2000; Meinzer, 2002; Mencuccini, 2003; Maherali *et al.*, 2006; Brodribb *et al.*, 2007). The repeated observation of these correlations in comparative studies also implies that there is correlated evolution between xylem function and leaf gas exchange (Sperry, 2004). However, micro-evolutionary studies are required to test predictions of the

direction and speed of evolutionary change in plant water and carbon relations (Ackerly *et al.*, 2000). This is because adaptive evolution depends on genetic variation and covariation of traits within populations, as well as environment-specific natural selection on those traits (Lynch & Walsh, 1998; Geber & Griffen, 2003).

There is no clear consensus on the degree to which quantitative genetic variation constrains adaptive physiological evolution because reports of heritable genetic variation for physiology vary widely among species (Geber & Griffen, 2003). For example, genetic variation for photosynthetic rate and stomatal conductance is absent to low in some species (*Brassica campestris*, Evans, 1991; *Cakile edentula*, Dudley, 1996b; *Schiedea salicaria* and *Schiedea adamantis*, Culley *et al.*, 2006) but abundant in others (*Polygonum arenastrum*, Geber & Dawson, 1997;

Lobelia siphilitica, Caruso *et al.*, 2005). Some of this variation may be caused by the sensitivity of gas exchange to environmental variation during measurement, which would result in the underestimation of heritabilities for photosynthetic and stomatal traits (Arntz & Delph, 2001). Heritability estimates for plant hydraulic traits, however, may be less sensitive to environmental variation than estimates for gas exchange because measurements are made on excised tissues under controlled conditions. Although studies in agricultural species suggest that cultivars can differ in leaf xylem hydraulic conductivity (Neufeld *et al.*, 1992; Stiller *et al.*, 2003; Normand *et al.*, 2008; but see Cochard, 2002), no studies have examined whether there is heritable variation for this trait in natural populations, or how heritable variation for hydraulic traits compares with that for other physiological traits.

If natural selection favours coordination between xylem water supply and transpiration in order to prevent leaf desiccation (Mencuccini, 2003; Sperry, 2004), then there should be positive genetic covariation between gas exchange and hydraulic conductivity within populations (Conner, 2002). However, genetic covariation among these traits could also constrain future adaptive evolution (Arnold, 1987; Dorn & Mitchell-Olds, 1991; Caruso *et al.*, 2005). For instance, positive genetic covariation between photosynthetic rate (A) and stomatal conductance (g_s) (Farquhar & Sharkey, 1982; Geber & Dawson, 1990) would limit the evolution of increased water use efficiency (A/g_s) in a dry environment (Dudley, 1996a; Heschel *et al.*, 2002) because plants would be constrained from simultaneously maximizing A and minimizing g_s (Caruso *et al.*, 2005). Even though increased stomatal opening is a biophysical necessity for increasing the rate of diffusion of carbon dioxide to the inside of plant leaves (Wong *et al.*, 1979), genetic covariation between A and g_s is not universal. Results from the few species that have been examined suggest that this genetic correlation can be strong (Dudley *et al.*, 1996b; Geber & Dawson, 1997) or entirely absent (Caruso *et al.*, 2005; Culley *et al.*, 2006). There are no studies that have evaluated whether there is positive genetic covariation between leaf gas exchange and hydraulic conductivity in wild species.

We examined the genetic basis of leaf hydraulic conductivity and gas exchange traits in *Avena barbata*. We focused on *A. barbata* because of its history of use in the study of drought adaptation (Hamrick & Allard, 1972). The species exists as two distinct ecotypes in California, identified by contrasting multilocus allozyme genotypes associated with drought adaptation (Allard *et al.*, 1972). Individuals from dry sites (xeric ecotype) are fixed for one set of alleles at each of five loci, whereas individuals from more moist environments (mesic ecotype) are homozygous for the alternate allele at each locus (Hamrick & Allard, 1972). The ecotypes also differ for suite of quantitative traits including seed size, flowering time, root depth, competitive ability and fecundity (Hamrick & Allard, 1975; Latta *et al.*, 2004). Our objective was to determine if there was genetic variation for leaf hydraulic conductivity and gas exchange in

this species. If genetic variation for these traits was present, we predicted that leaf hydraulic conductivity and gas exchange would be positively genetically correlated.

Materials and Methods

Study species

Avena barbata Pott. ex. Link is a European annual grass that has invaded the Mediterranean region in the southwestern USA since its accidental introduction over 200 yr ago (Garcia *et al.*, 1989). Because *A. barbata* is highly selfing (> 95%), each parental ecotype is a genetically homogeneous monomorphic lineage (Johansen-Morris & Latta, 2006; Latta *et al.*, 2007). To examine genetic variation and covariation for physiology in this species, we used a population of recombinant inbred lines (RILs) derived from a cross of the mesic and xeric ecotypes. This cross, followed by generations of selfing, closely mimics a population of progeny that would derive from a hybridization event between the two parental ecotypes in regions where they overlap (Latta *et al.*, 2007). The cross between xeric and mesic genotypes and one generation of selfing produced 188 F_2 families. These plants were selfed for four generations through single seed descent, producing true-breeding hybrids with a different combination of the parental alleles. This procedure reduced variation within individual RILs and maximized variation between RILs, increasing our statistical power to detect heritable variation for physiological traits (Lynch & Walsh, 1998).

Experimental design and data collection

To examine genetic variation and covariation for physiology, we selected 26 RILs that were representative of the glasshouse fitness range of all 188 lines (Johansen-Morris & Latta, 2006; Sherrard & Maherali, 2006). To ensure that all physiological measurements were made on plants at the same life stage, we employed a randomized complete block design. We created three temporal blocks of 28 plants by planting one seedling from 26 family lines and the two parental ecotypes every 12 d. There were a total of 84 plants in the experiment, including three individuals of each of the 26 lines and the two parental ecotypes. Seeds were germinated in mid-February 2004 by removing the lemma and placing them on moist filter paper for 96 h at 4°C. After refrigeration, seeds were returned to room temperature and placed in the dark for 24 h. Each seedling was planted in a 4.1-l pot filled with Pro-Mix BX (Premier Tech, Riviere de Loupe, PQ, Canada) and placed on a glasshouse bench. Plants were watered daily to saturation and watered with an additional 100 ml of 20–20–20% nitrogen–phosphorus–potassium (NPK) fertilizer (Plant Products Inc, Brampton, ON, Canada) at a concentration of 2.5 g l⁻¹ every 2 wk. The fertilizer also contained the micronutrients boron (B; 0.02%), copper (Cu; 0.05%), iron (Fe; 0.1%), manganese

(Mn; 0.05%), molybdenum (Mo; 0.0005%) and zinc (Zn; 0.05%). Plants were provided with supplemental light from high-intensity discharge (HID) lamps to maintain the photoperiod at 16 h d⁻¹.

We measured light-saturated photosynthetic rate (A) and stomatal conductance (g_s) 110 d after germination on the youngest fully expanded leaf on the vegetative portion of all 84 plants. We randomly selected plants within each block for measurement on each day. Steady-state leaf gas exchange was measured at saturating irradiance (1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with an open gas exchange system (LI-6400; Li-Cor Inc., Lincoln, NE, USA) between 09:00 and 12:00 h Eastern Standard Time (EST). During measurements, incident irradiance was provided by red-blue light-emitting diodes and cuvette CO₂ concentration was maintained at 400 $\mu\text{mol mol}^{-1}$ to reflect prevailing ambient conditions. A Peltier cooling module maintained leaf temperature at approximately ambient conditions (25–30°C) during each measurement period. Gas exchange measurements were made at a leaf-to-air vapour pressure deficit (D) of 1.9–2.0 kPa. Following enclosure in the leaf cuvette, leaves reached steady-state values (when the coefficients of variation of CO₂ and H₂O within the chamber were < 0.25%) within 5 min. After measurements, leaf area was determined from leaf dimensions. Because gas exchange measurements were made when plants were relatively large, pot binding could have reduced carbon sink demand by roots, resulting in reduced photosynthesis. However, we made measurements on the vegetative leaves at the bottom of the canopy, where maximum photosynthetic capacity was more likely to be limited by light than by the carbon demand of roots. Photosynthesis of these leaves was lower than that of leaves on reproductive tillers at the top of the canopy (Sherrard & Maherali, 2006) but similar to that of vegetative leaves in the field (Jackson *et al.*, 1995).

Following gas exchange measurements, hydraulic conductivity was measured on leaf blades using the method of Martre *et al.* (2001). A single fully expanded leaf (~30 cm long) on each individual was harvested and placed in a water bath. In most cases, this leaf was either the same one that was used for gas exchange, or was an adjacent leaf of similar size and developmental stage. Previous studies in similar-sized leaves (~40–50 cm long) of other grasses found that average vessel length was < 1.5 cm, and maximum lengths did not exceed 2.8 cm (Martre & Durand, 2001). Thus, to ensure that our samples did not contain vessels that were open at both ends, leaves were re-cut under water such that a 4–6-cm segment located at the midpoint of the leaf blade was isolated (e.g. Martre & Durand, 2001). However, we have no direct measurements of vessel length for *A. barbata*, so we cannot rule out the possibility that open vessels artificially increased the hydraulic conductivity of leaf segments.

The leaf segment was wrapped around a 1-cm-diameter aluminium bar that had been covered with plumber's putty (Oatey Inc., Brampton, ON, Canada) to provide a watertight seal. Teflon tape was wrapped around this assemblage to secure

the leaf in place. Leaf segments were inserted into vinyl tubing that was attached to a Xylem meter (Xylem Embolism Meter; Bronkhorst, Montigny les Cormeilles, France; Cochard, 2002) which utilizes a high-resolution liquid mass flow meter to measure the volume of water flow. Axial hydraulic conductivity of leaf segments was measured under laboratory irradiance conditions (< 15 $\mu\text{mol m}^{-2} \text{s}^{-1}$) by perfusing them at a pressure of 80 kPa with filtered (0.2 μm) distilled water. Volume flow rates (Q ; kg s⁻¹) typically reached steady state within 10–15 min, after which data were recorded. Hydraulic conductivity (K_H ; kg m MPa⁻¹ s⁻¹) was expressed as the volume flow rate divided by the pressure gradient (MPa m⁻¹) and corrected to water viscosity at 20°C. Because K_H increases with leaf width in grasses, we report hydraulic conductivity per unit leaf width (K_W ; kg MPa⁻¹ s⁻¹; as in Neufeld *et al.*, 1992). The use of pure water, rather than a KCl solution, can result in underestimation of K_H because of hydrogels in the pit membrane (Zwieniecki *et al.*, 2001). However, we used pure water because the effect of KCl on K_H varies considerably among individuals of a single species (Zwieniecki *et al.*, 2001), which could bias calculations of broad-sense heritability (Arntz & Delph, 2001).

To determine if there were differences in development among RILs that could be correlated with gas exchange and leaf hydraulics (e.g. Geber & Dawson, 1997), we recorded the day on which each individual flowered, relative to the time of seed germination. The experiment was terminated in September 2004, after approx. 6 months of growth. The aboveground portions of each plant were harvested, dried to constant mass in a forced convection oven for 48 h and weighed.

Statistical analyses

We examined genetic variation for photosynthetic rate, stomatal conductance, leaf hydraulic conductivity, flowering time and aboveground biomass by calculating the intra-class correlation ($\tau = (\sigma_g^2)/(\sigma_g^2 + \sigma_e^2)$), where σ_g^2 is the between-line variance and σ_e^2 is the within-line environmental variance. Because individuals within an *A. barbata* inbred line are 96.75% homozygous (Latta *et al.*, 2007), variation from individual to individual within each line is caused almost entirely by random environmental effects. As a result, the intra-class correlation is equivalent to the broad-sense heritability (H^2) (Falconer & McKay, 1996). The broad-sense heritability represents all the possible genetic contributions to phenotypic variation, including additive genetic variation, dominance, epistasis (gene interactions) and maternal effects (Lynch & Walsh, 1998). Because RILs were homozygous at each locus, dominance effects could be discounted. In addition, prior generations were all grown under the same environmental conditions, so maternal effects should be similar for all RILs. Terms for the intra-class correlation were calculated using the mean squares (MS) from one-way analyses of variance (ANOVAs) with RIL as a factor and temporal block as a covariate (SYSTAT 8.0; Systat Software Inc., San Jose, CA, USA). The error mean squares (MSe) term

Table 1 Parental ecotype means, recombinant inbred line (RIL) population means and broad-sense heritabilities (H^2) for physiological, development and biomass traits in *Avena barbata*

Trait	Parental ecotypes		Recombinant inbred lines		H^2	P
	Xeric mean (± 1 SD)	Mesic mean (± 1 SD)	Population mean (± 1 SD)	Range of RIL means		
K_W ($\times 10^{-5}$, kg MPa $^{-1}$ s $^{-1}$)	2.34 \pm 0.89	3.00 \pm 1.70	2.78 \pm 0.86	1.46–4.93	0.33	0.0028
A ($\mu\text{mol m}^{-2}$ s $^{-1}$)	7.31 \pm 2.72	5.10 \pm 1.79	6.86 \pm 1.68	3.56–10.3	0.23	0.026
g_s (mol m^{-2} s $^{-1}$)	0.26 \pm 0.03	0.18 \pm 0.05	0.29 \pm 0.06	0.18–0.39	0	0.638
FT (d)	124 \pm 17.9	115 \pm 18.9	100 \pm 13.2	80.3–133	0.62	< 0.0001
Biomass (g)	59.4 \pm 48.3	35.5 \pm 11.8	51.6 \pm 10.1	37.6–76.3	0.05	0.306

Broad-sense heritabilities were calculated as the intra-class correlation ($\tau = (\sigma_g^2)/(\sigma_g^2 + \sigma_e^2)$, where σ_g^2 is the between-line variance and σ_e^2 is the within-line environmental variance). The statistical significance of H^2 was determined with a one-way ANOVA using genotype (RIL) as a fixed factor. K_W , hydraulic conductivity per unit leaf width; A , photosynthetic rate; g_s , stomatal conductance; FT, flowering time.

from the ANOVA is considered equivalent to the within-line environmental variance, σ_e^2 . The genetic line mean squares (MSg) term in the ANOVA is equivalent to $\sigma_e^2 + n\sigma_g^2$, where n is the number of individuals per genotype (Lynch & Walsh, 1998). Thus, to calculate the broad-sense heritability using the intra-class correlation, $\sigma_e^2 = \text{MSe}$ and $\sigma_g^2 = (\text{MSg} - \text{MSe})/n$.

To estimate the phenotypic correlation between pairs of traits, we calculated Spearman rank correlations among individuals. We estimated the genetic correlation among traits by calculating the rank correlation among family means. Because there was a significant block effect on the expression of phenotypic traits, broad-sense heritability calculations and correlation analysis were performed on the residuals of traits calculated after the block effect was removed. Family mean correlations have been widely used to estimate genetic correlation among traits (e.g. Geber & Dawson, 1997; McKay *et al.*, 2003) because they are amenable to standard significance tests and are straightforward to interpret. However, family mean correlations can often be biased because they include a component of environmental variance (Lynch & Walsh, 1998). Consequently, we consider a significant family mean correlation between two traits to be representative of significant genetic covariation if there was also significant heritable variation for each trait (Lynch & Walsh, 1998; Caruso *et al.*, 2005; Culley *et al.*, 2006). Statistical significance of genetic and phenotypic correlations was assessed using one-tailed critical values of the Spearman rank correlation coefficient (Zar, 1999).

Results

There was considerable genetic variation for physiological and development traits in *A. barbata*. We detected significant broad-sense heritabilities for hydraulic conductivity per unit leaf width (K_W), photosynthetic rate (A) and flowering time (Table 1). Parental ecotypes had similar K_W and A , whereas offspring displayed nearly three-fold or more variation in these traits (Fig. 1a,b, Table 1). There was an almost two-fold

Table 2 Phenotypic correlations (upper off-diagonal) and family mean (genetic) correlations (lower off-diagonal) for physiological, development and aboveground biomass traits of *Avena barbata*

	K_W	A	g_s	FT	Biomass
K_W	–	0.096	0.042	–0.167	0.051
A	0.456**†	–	0.549***†	–0.072	0.179
g_s	0.346*	0.492**	–	–0.074	–0.096
FT	–0.200	–0.043	–0.154	–	–0.108
Biomass	–0.093	0.365*	–0.114	0.163	–

K_W , leaf hydraulic conductivity per unit leaf width; A , photosynthetic rate; g_s , stomatal conductance; FT, flowering time. Statistically significant phenotypic correlations are shown in bold. Statistically significant family mean correlations are in bold only when there was also significant heritable variation for each trait. Correlations that remained significant after a Bonferroni correction for multiple comparisons are marked with a †.

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$.

difference between the highest and lowest ranking lines for flowering time (Table 1). Broad-sense heritabilities for stomatal conductance (g_s) and aboveground biomass were not statistically different from zero (Table 1). Leaf width, which was used to calculate K_W , was also not heritable ($P = 0.58$).

There was only one significant phenotypic correlation among traits in *A. barbata*; A was strongly positively correlated with g_s (Table 2). By contrast, there were four statistically significant correlations among family means, primarily among leaf hydraulic conductivity and gas exchange traits (Table 2). However, evidence for genetic covariation among these traits was strongest when there was also significant genetic variance for each trait (Lynch & Walsh, 1998; Culley *et al.*, 2006). For example, even though there was a significant family mean correlation between A and g_s (Table 2), genetic covariation between these two traits would be weak because there was no genetic variation for g_s (Table 1). By contrast, the significant family mean

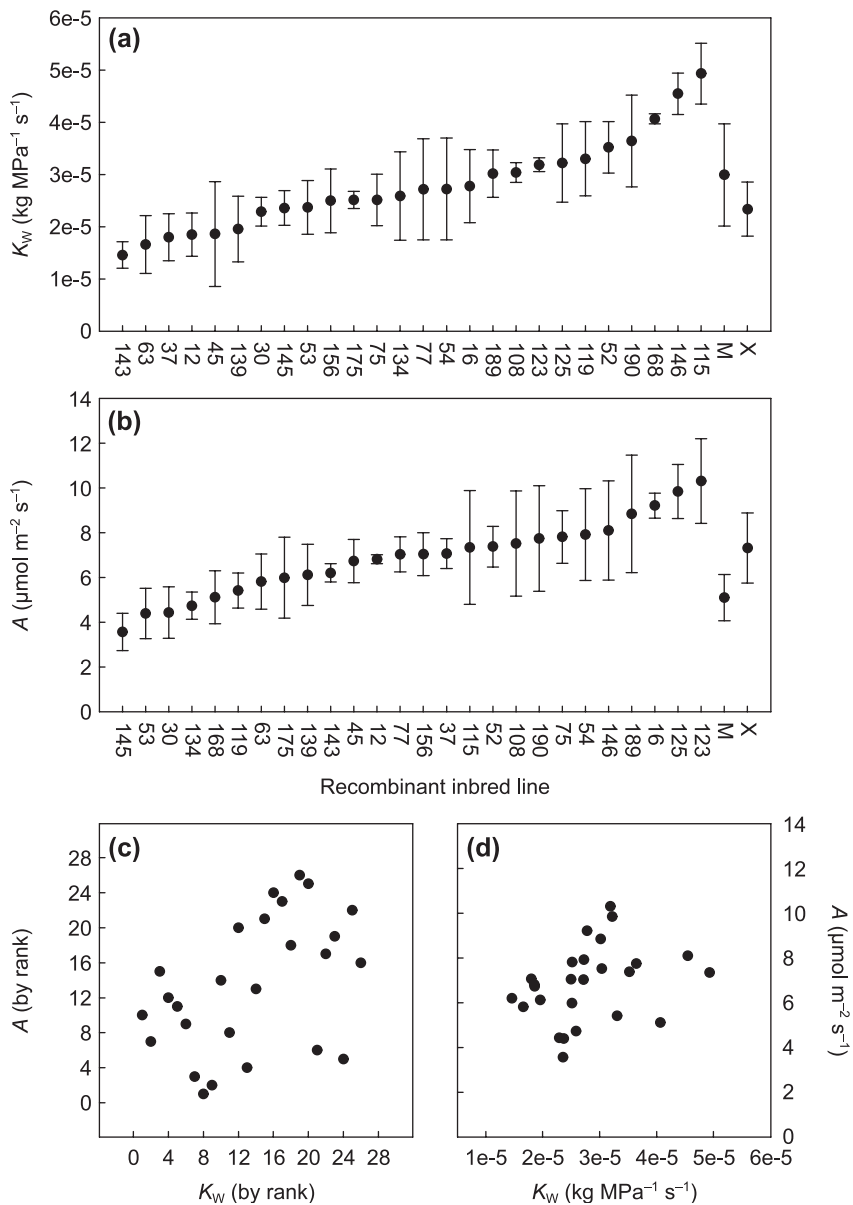


Fig. 1 Mean (± 1 SE) hydraulic conductivity per unit leaf width (K_w) (a) and light-saturated photosynthetic rate (A) (b) for 26 recombinant inbred line (RIL) families and xeric and mesic parental ecotypes of *Avena barbata*. RILs are ranked by magnitude. Several RILs fell outside the range of K_w and A associated with the two parental ecotypes (X and M). The relationship between K_w and A is shown using RIL ranks (c) and RIL means (d).

correlation between K_w and A (Fig. 1c,d) represents genetic covariation because there was heritable variation for each trait (Table 1). There was no genetic covariation between K_w and flowering time, or between A and flowering time; family mean correlations between these pairs of traits were not statistically significant (Table 2).

Discussion

Whereas previous studies have documented heritable genetic variation for leaf gas exchange (e.g. Arntz & Delph, 2001; Caruso *et al.*, 2005) and photosynthetic biochemistry (Geber & Dawson, 1997), we obtained the first evidence of genetic variation for K_w , a trait that underlies variation in leaf gas

exchange (Meinzer & Grantz, 1990; Sack *et al.*, 2003; Sperry, 2004; Brodrigg *et al.*, 2007). Variation among RILs for K_w also exceeded that of the range of phenotypes exhibited by the parental ecotypes (Fig. 1), suggesting that there was transgressive segregation for this trait (Rieseberg *et al.*, 1999; Juenger *et al.*, 2005; Johansen-Morris & Latta, 2006). Moreover, the broad-sense heritability for K_w was moderate to high, compared with that of other physiological traits in plants (Ackerly *et al.*, 2000; Arntz & Delph, 2001; Geber & Griffen, 2003; Caruso *et al.*, 2005; Culley *et al.*, 2006). Although we did not determine whether there was genetic variation for K_w within each ecotype, quantitative genetic variation within populations of each ecotype would be low because *A. barbata* is primarily selfing (Geber & Griffen, 2003). However, the release of genetic

variation following out-crossing in environments where both parental ecotypes coexist (Hamrick & Allard, 1972; Latta *et al.*, 2004) would allow natural selection to shape the evolution of K_{W} .

Our finding of genetic covariation between K_{W} and A is consistent with biophysical expectations because water transport through leaf veins is necessary to hydrate mesophyll cells where photosynthesis takes place (Sack & Holbrook, 2006; Brodribb *et al.*, 2007). More generally, our results suggest that natural selection on one of these traits will cause indirect selection on the other, resulting in the correlated evolution of leaf hydraulic capacity and photosynthesis in natural populations. Genetic covariation between K_{W} and A may be caused by pleiotropy, tight physical linkage or linkage disequilibrium associated with natural selection to increase both K_{W} and A (Lynch & Walsh, 1998; McKay *et al.*, 2003). The rounds of recombination that preceded the creation of the $F_6 A. barbata$ RIL population should have overcome linkage disequilibrium (Lynch & Walsh, 1998), suggesting that pleiotropy or tight physical linkage is the most likely cause of covariation between K_{W} and A . Although we cannot distinguish between pleiotropy and linkage as causes of genetic covariation, recent detailed genetic studies of *Arabidopsis thaliana* suggest that photosynthetic capacity and transpiration can be influenced by the same gene (Masle *et al.*, 2005).

Because leaf hydraulic capacity is correlated with stomatal opening across species (Sack *et al.*, 2003), we also expected positive genetic covariation between K_{W} and g_s . Although there was a significant family mean correlation between K_{W} and g_s (Table 2), the absence of genetic variation for g_s suggests that genetic covariance between K_{W} and g_s was weak (Lynch & Walsh, 1998). One explanation for low genetic variation for g_s compared with A and K_{W} is that within-plant and within-line variation (σ^2) may have been much greater for g_s than for A or K_{W} , resulting in a lower broad-sense heritability (Falconer & McKay, 1996; Arntz & Delph, 2001). Higher within-plant and within-line variation for g_s may have occurred because stomatal opening is more sensitive than photosynthesis to humidity variation during gas exchange measurement (e.g. Maherali *et al.*, 2003). Similarly, K_{W} is less likely to be influenced by environmental variation than g_s because it is measured on detached tissue under steady-state conditions. Using similar methods, previous studies (Geber & Dawson, 1997; Caruso *et al.*, 2005; Culley *et al.*, 2006) have documented genetic variation for g_s in other species, so it is possible that this trait is not heritable in *A. barbata* under the well-watered conditions of our study. It is also possible that strong directional selection eliminated genetic variation for g_s in natural populations of *A. barbata*.

We expected that photosynthetic rate would be positively genetically correlated with stomatal conductance (Wong *et al.*, 1979; Farquhar & Sharkey, 1982) because increased stomatal opening is necessary to increase CO_2 diffusion into leaves. However, the absence of genetic variation for g_s also prevents the conclusion that there was strong genetic covariation between

A and g_s . One implication of this result is that *A. barbata* would not be constrained from evolving increased water use efficiency by maximizing carbon fixation (Farris & Lechowicz, 1990; Dudley, 1996a; Heschel *et al.*, 2002). However, *A. barbata* would be constrained from evolving increased water use efficiency through lower g_s because there was no genetic variation for this trait (e.g. Caruso *et al.*, 2005).

Our observation of high broad-sense heritability for flowering time is consistent with previous studies in *A. barbata* (Latta *et al.*, 2007). Variation in flowering time is also an indicator of whether plants escape or tolerate water stress (Grime, 1977; Ludlow, 1989; McKay *et al.*, 2003). This is because high water use and photosynthesis in early-flowering genotypes facilitates fast growth and the completion of reproduction before the onset of stress. By contrast, late-flowering genotypes may avoid dehydration by having low water use and photosynthesis during periods of resource limitation (Geber & Dawson, 1990, 1997). Although previous studies have detected negative genetic correlations between flowering time and gas exchange (White, 1993; Geber & Dawson, 1997; McKay *et al.*, 2003) we did not observe this pattern in our results. Thus, our results suggest that the trade-off between the rate of development and water use physiology is not universal. Nevertheless, our conclusions are based on steady-state measurements of physiology, which ignore the dynamic responses of liquid and vapour phase conductance to water stress (Meinzer, 2002; Mencuccini, 2003). If there is indeed no trade-off between water use and development time, then we predict that the sensitivity of stomata to water limitation should not differ between *A. barbata* RILs that differ in flowering time.

One potential limitation of our study is that hydraulic measurements were made on cut leaf samples, and not entire leaves. Although we used leaf samples that were longer than previous determinations of maximum vessel length for similarly sized grass leaves (Martre & Durand, 2001), the use of cut leaves could have resulted in an overestimate of K_{W} if there were open vessels in the sample. To examine this possibility, we compared *A. barbata* leaf hydraulic traits with those of other grasses reported in the literature (Table 3). To allow comparisons with values reported for other species, we expressed hydraulic traits as leaf-blade hydraulic conductance (K ; mass flow per unit pressure applied; $\text{kg MPa}^{-1} \text{s}^{-1}$) and hydraulic conductivity (K_{H} ; mass flow per unit pressure gradient applied; $\text{kg m MPa}^{-1} \text{s}^{-1}$) as well as K_{W} . Overall, hydraulic traits in *A. barbata* were within the broad range reported for grasses. *Avena barbata* K_{W} was about half of that reported in two species of sugar cane (*Saccharum officinarum* and *Saccharum spontaneum*; Neufeld *et al.*, 1992). *Avena barbata* K_{H} was an order of magnitude higher than that of *Festuca arundinacea* (Martre & Durand, 2001) but nearly two orders of magnitude lower than that of *Zea mays* (Wei *et al.*, 1999). Finally, *A. barbata* K was about twice that of *Oryza sativa* (Stiller *et al.*, 2005). Leaf hydraulic traits in *A. barbata* and other grasses are not comparable to values of whole-leaf hydraulic conductance (K_{leaf})

Table 3 A comparison of leaf hydraulic traits between *Avena barbata* and other grass species

Species	K (kg MPa ⁻¹ s ⁻¹)	K_H (kg m MPa ⁻¹ s ⁻¹)	K_W (kg MPa ⁻¹ s ⁻¹)	Reference
<i>Avena barbata</i>	5.34×10^{-06}	2.54×10^{-07}	2.78×10^{-05}	This study
<i>Festuca arundinacea</i>	–	2.70×10^{-08}	–	Martre & Durand (2001)
<i>Oryza sativa</i>	2.18×10^{-06}	–	–	Stiller <i>et al.</i> (2005)
<i>Saccharum officinarum</i>	–	–	5.80×10^{-05}	Neufeld <i>et al.</i> (1992)
<i>Saccharum spontaneum</i>	–	–	4.67×10^{-05}	Neufeld <i>et al.</i> (1992)
<i>Zea mays</i>	–	1.67×10^{-05}	–	Wei <i>et al.</i> (1999)

To facilitate comparisons with values reported in the literature, *A. barbata* hydraulic function has been expressed in three different formulations: hydraulic conductance (K), hydraulic conductivity (K_H) and hydraulic conductivity per unit leaf width (K_W).

K , hydraulic conductance, calculated as volume flow rate (kg s⁻¹) per unit pressure applied (MPa).

K_H , hydraulic conductivity, calculated as K /length of leaf segment (m).

K_W , hydraulic conductivity per unit leaf width, calculated as K_H /leaf width (m).

for dicots reported in the literature (Sack & Holbrook, 2006) because hydraulic measurements in grasses have been made on cut leaf blades, rather than entire leaves. As a result, values for grasses probably overestimate whole-leaf conductance because much of the hydraulic resistance associated with the entire leaf lamina has been removed.

Although we observed significant genetic variation and covariation among some traits, it is possible that we failed to detect significant broad-sense heritabilities and genetic covariances in some cases because of low statistical power. For the same reason, however, the statistically significant broad-sense heritabilities and genetic covariances we observed are underestimates of stronger patterns. We also note that genetic variation and covariation vary with growth environment because of genotype by environment interactions (Lynch & Walsh, 1998). Thus the broad-sense heritabilities and genetic covariances we report here are specific to the growth environment of our study, and may change if plants were grown under different conditions.

We have shown significant heritable variation for leaf hydraulic conductivity, photosynthesis, and flowering time in an annual grass species. The genetic covariances among these traits could influence the evolution of mechanisms of drought adaptation in *A. barbata*. Positive genetic covariation between A and K_W suggests that natural selection for increased photosynthetic capacity in arid environments would also cause indirect natural selection to increase xylem water transport capacity. This type of coordinated evolutionary change could reduce hydraulic limitations on gas exchange (Meinzer, 2002; Mencuccini, 2003; Brodribb *et al.*, 2007) as well as increase nitrogen delivery to leaves for photosynthetic enzymes (Donovan *et al.*, 2007). By contrast, positive genetic covariation between A and K_W could also prevent the evolution of increased water conservation in arid environments by preventing the simultaneous evolution of increased photosynthesis and reduced transpiration (Caruso *et al.*, 2005). Overall, our results indicate that the evolution of a positive correlation between xylem and leaf function among species would be facilitated by positive genetic covariation for these traits within species.

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