Original papers

Genetic variation in and covariation between leaf gas exchange, morphology, and development in *Polygonum arenastrum*, an annual plant

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Summary. We present evidence of genetic variation in and covariation between leaf-level gas exchange properties and leaf size among family lines of *Polygonum arenastrum*. This self-fertilizing annual had previously been shown to vary genetically in developmental phenology and in morphology (size of leaves, internodes, flowers and seeds) (Geber 1990). Significant family differences were found in photosynthetic carbon assimilation rate (A), leaf conductance to water vapor (g), instantaneous water-use efficiency (WUE), and leaf carbon isotope discrimination (Δ). A strong positive genetic correlation between A and g suggested that there was stomatal limitation on A. In addition, higher g led to relatively greater increases in transpiration, E, than in assimilation, A, so that families with high rates of gas exchange had lower instantaneous WUE and/or higher carbon isotope discrimination values. Leaf size and gas exchange were genetically correlated. In earlier studies leaf size was found to be genetically correlated with developmental phenology (Geber 1990). The pattern that emerges is one in which small-leaved families (which also have small internodes, flowers, and seeds) tend to have high gas exchange rates, low WUE, rapid development to flowering and high early fecundity, but reduced life span and maximum (vegetative and reproductive) yield compared to large-leaved families. We suggest that this pattern may have arisen from selection for contrasting suites of characters adapted to environments differing in season length.

Key words: *Polygonum arenastrum* – Photosynthesis – Water-use efficiency – Development – Morphology

Little is known about the genetics of physiological variation in wild plant populations, and how physiology combines with morphology and development to affect plant performance (McGraw and Wulff 1983, Scheiner et al. 1984; Garbutt 1986; Lechowicz and Blais 1988). Although plant size is often a good measure of success (Samson and Werk 1986), and growth to a large size depends on photosynthetic carbon assimilation, genetic correlations between leaf-level photosynthetic assimilation rate (A), plant growth rate, and yield (vegetative or seed biomass) are not consistently positive in domesticated plants or wild species (McGraw and Wulff 1983; Scheiner et al. 1984; Garbutt 1986; Nelson 1988; Dijkstra and Lambers 1989). Greater A may not translate into greater yield if there is a trade-off between A and water loss. Because plants inevitably lose water through stomata in the process of CO_2 uptake, high A may come at the expense of high transpiration rates (E) and reduced water-use efficiency (WUE) when it is achieved through increased stomatal conductance (g) (Wong et al. 1979) rather than through biochemical adjustment of photosynthetic capacity (Sharkey 1985; Woodrow and Berry 1988).

The relative importance of A versus WUE to performance will likely depend on the phenological development and life expectancy of plants and on the environmental conditions during growth. In crops, lower WUE is often associated with shorter life span and more rapid development (Ehleringer et al. 1990; Hall et al. 1990). In a study of *Xanthium strumarium* along an environmental gradient, Lechowicz and Blais (1988) found that at the water-limited and resource-poor end of the gradient, high gas exchange rates, low WUE, and rapid early growth increased reproductive yield, while the converse set of traits were favored at the opposite end of the gradient. Ehleringer et al. (1990) suggested that lower WUE would contribute positively to yield in ephemeral genotypes (and species) if correspondingly higher A allowed for greater and more rapid biomass accumulation. Conversely, predictable drought might favor higher WUE in longer-lived annual and perennial genotypes (or species) (Ehleringer et al. 1990). Conserva-

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tive water use (high WUE) might also be advantageous in a deteriorating environment, especially in slow-growing plants (Cowan 1982). The advantage of high WUE and low A to longer-lived genotypes in conditions where water is not limiting is unclear (Lechowicz and Blais 1988). Nonetheless, consideration of the developmental phenology of a plant may contribute to our understanding of the link between A, WUE, and performance.

We examined genetic variation in leaf physiology in *Polygonum arenastrum*, a self-fertilizing annual, that exhibits considerable phenotypic variation in developmental phenology and morphology. A greenhouse study of inbred family lines from a single population in Salt Lake Co., UT, USA, revealed extensive genetic variation in the developmental timing of axillary bud commitment to branching (vegetative growth) versus flowering (reproduction) and the morphology (size) of leaves, internodes, flowers, and seeds (Geber 1990). Developmental variation had profound effects on life span and age-specific life history characteristics, and there were strong genetic correlations between development and morphology (Geber 1990). Genotypes which committed axillary buds to reproduction early in development achieved high fecundity early in life, but they did so at the expense of a shorter life span, reduced growth and fecundity later in life, and a smaller maximum yield (biomass and reproductive output). Rapid development was also correlated with small leaves, internodes, flowers, and seeds. The opposite suite of life history and morphological characters were associated with delayed development to flowering (Geber 1990).

Using field-grown plants, we asked whether there was evidence 1) of genetic variation in leaf-level gas exchange properties and WUE, and 2) of a trade-off between A and WUE due to stomatal limitation on A. In the case of stomatal limitation, we expected a positive genetic correlation between A and g (Wong et al. 1979). Two measures of WUE were used. The ratio of A to E provides an instantaneous measure of WUE. A long-term indicator of WUE integrated over the lifetime of the leaf is the leaf carbon isotope discrimination (Δ) (Farquhar et al. 1989). For plants with the C_3 photosynthetic pathway, Δ has been shown to correlate negatively with long-term WUE (Farquhar et al. 1989). We also considered genetic correlations between leaf-level physiology, morphology, and development. In particular, we asked whether plants with rapid development to flowering had high A and low WUE compared with plants exhibiting delayed development?

Methods

Breeding and planting design

Polygonum arenastrum is of Eurasian origin and is now widely naturalized in western North America. It grows in disturbed habitats, ranging from poorly vegetated road beds with highly compacted soils to more heavily vegetated, mesic ditches and lawns (Geber 1989). *Polygonum arenastrum* appears to be entirely self-fertilizing: flowers are very small (2–3 mm), and anthers dehisce

in close proximity to the stigma; all flowers set seed in the greenhouse in the absence of pollinators; and, we have never seen insect visitors to flowers in the field. We assume that all seeds from a single parent are inbred full-siblings. Seeds collected at a roadside population in the Red Butte Canyon Natural Research Area, Salt Lake Co., UT, USA were used to establish inbred family lines in 1986 (Geber 1990). Information on developmental variation comes from greenhouse studies of 26 randomly chosen lines in 1987 (Geber 1990). Thirteen of these lines were chosen at random for field studies of physiology and morphology. Plants were grown at the Biology Department Experimental Garden Facility, located approximately 2 km from the population's site of origin. Seedlings were transplanted to the tilled garden site on June 27–28, 1988, in a randomized block design consisting of 5 blocks containing 10 seedlings from each line. Seedlings were watered daily until establishement and then once every four days with an overhead sprinkler system. Plants in three blocks were destroyed by herbivores so that measurements were restricted to the remaining two blocks.

Physiological and morphological measurements

The daily course of leaf photosynthetic carbon assimilation (A) and leaf conductance to water vapor (g) was measured in four family lines known to differ in leaf size and developmental pattern. The four family lines (nos. 2, 12, 19, 26) were chosen on the basis of a Principal Component Analysis in which all 26 families from the developmental studies were ordered according to branching frequency and leaf size. In the analysis, four distinct groups of families emerged. One family line among the 13 included in the physiological studies were selected from each group: 1) the largest group, consisting of 16 families, had relatively low branching frequency (i.e., rapid development to flowering) and small leaf size (=line 2); 2) the next largest group of 6 families had relatively frequent branching (i.e., delayed development to flowering) and large leaf size (=line 26); the third group of 3 families had limited branching and large leaves (=line 19); and the final group, consisting of a single family (=line 12) had small leaves and frequent branching. Lines 2, 26, and 19 were typical of their family groupings.

On August 9, midway between two watering cycles, and prior to flowering, the daily course of gas exchange was measured on three individuals from each of the four lines. Measurements were made every $1\frac{1}{2}$ hours between 8:00 and 21:00 hr local time (=9 sampling times), with a LI-6200 portable gas exchange system (Li-Cor Instruments, Lincoln, NE). The order in which individuals were measured was randomized at each sampling time. Instantaneous water-use efficiency (WUE) was calculated as the ratio of A to E; E is the leaf transpiration rate and is equal to $g \cdot \Delta w$, where Δw is the leaf-to-air vapor pressure gradient. The vapor pressure gradient is a function of leaf temperature (measured inside the chamber) and relative humidity (measured outside the chamber). Leaf temperature ranged between 28.2 and 36.6° C, and did not differ among families over the course of the day ($F=0.49$, $df=3,8$, $P>0.69$). Family differences in transpiration would therefore be attributable primarily to differences in g.

Measurement of the daily course of gas exchange indicated that maximal rates of assimilation and conductance were achieved between 9:30 and 11:00 hr local time (Fig. 1). On August 10, maximum assimilation rates (A_{max}) and corresponding conductances (g_{max}) were measured on 3 individuals from all 13 lines between 9:15 and 11:00 hr local time (ambient air temperature: 26–31° C; photosynthetic photon flux density: 1.1–1.6 $mol \cdot m^{-2} \cdot s^{-1}$; w: 44.3–62.6 $mmol \cdot mol^{-1}$). Leaf temperature did not differ among families ($F=0.40$, $df=12,26$, $P>0.95$). Instantaneous WUE was again calculated as $A \div E$. Leaf size was measured with a LI-3000 (LiCor Instruments, Lincoln, NE) leaf area meter. Carbon isotope discrimination of leaves (Δ) was obtained from dried leaf material following the methods of Ehleringer and Osmond (1989).
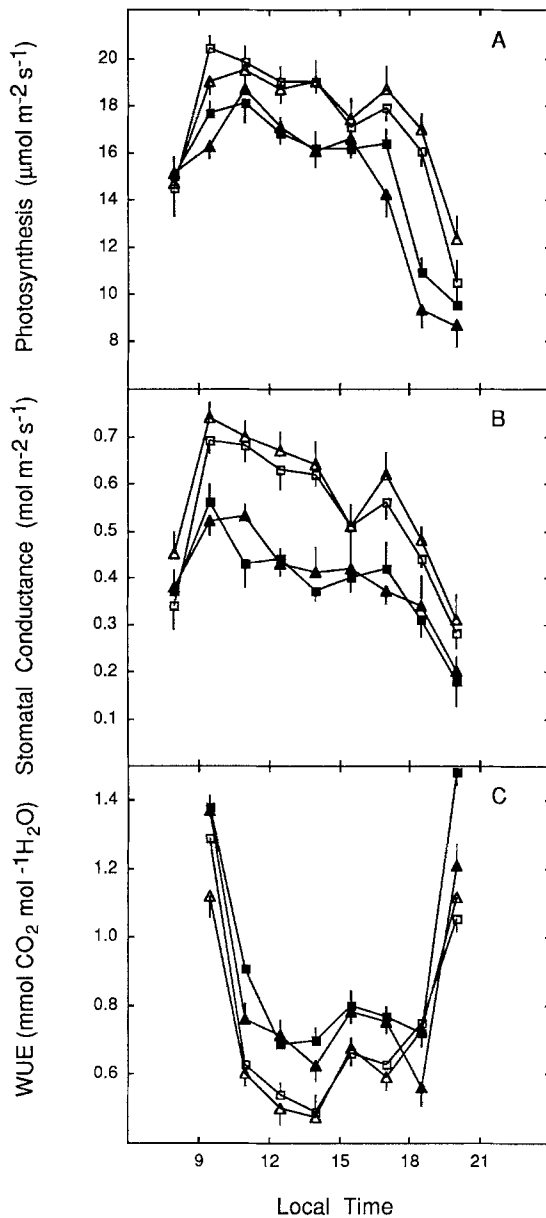
Fig. 1A–C. Daily course of family mean (**A**) leaf photosynthetic carbon assimilation, (**B**) stomatal conductance to water vapor, and (**C**) instantaneous water-use efficiency (WUE) in four families of *Polygonum arenastrum*. Families 2 (□) and 12 (△) have small leaves, and families 19 (■) and 26 (▲) have large leaves. In greenhouse studies, families 2 and 19 had rapid development to flowering, while families 12 and 26 had delayed development to flowering

Analysis

Family differences in the daily course of A, g, and instantaneous WUE were analyzed in a Repeated Measures Analyses of Variance (ANOVAR in proecdure GLM, SAS Institute, 1985), where the multiple measurements of A, g, and WUE on a given plant over the course of the day constituted the repeated variables. Values of WUE were log_e-transformed to conform to normality assumptions ANOVAR. In each ANOVAR, the main effect of family was tested as a between-subjects effect. Diurnal variation in gas exchange, and differences among families in diurnal variation (Time × Family interaction) were tested as within-subjects effects (see Potvin et al. 1990 for the application of ANOVAR to ecophysiological data). Exact univariate tests of within-subject effects in

ANOVAR make an assumption of compound symmetry of the variance covariance matrix (Winer 1971). Mauchly's criterion test of compound symmetry could not be performed because of the insufficient error degrees of freedom. In the absence of compound symmetry, the ANOVAR may still be used if the Huynh-Feldt conditions are met (Huynh and Feldt 1970). The Huynh-Feldt epsilon measures the strength of the violation of compound symmetry, the violation being strong for small values of ε ($\ll 1$). In the analysis of A, g, and WUE, the Huynh-Feldt epsilon values were 0.82, 2.17, and 0.60 respectively, and indicate mild violations of the compound symmetry assumption. Our analyses of within-subject effects are based on Huynh-Feldt corrected significance levels. Contrasts comparing large versus small leaved lines (2 and 12 versus 19 and 26) and lines with rapid development versus delayed development to flowering (2 and 19 versus 12 and 26) were tested.

To examine the pattern of correlation between A, g, and WUE across families, we first calculated Pearson's correlations among the family means of characters at each of the 9 sampling times. The 9 correlations between each pair of characters were then averaged (after first applying the z-transformation; Sokal and Rohlf 1981) to obtain an estimate of the average correlation.

Genetic differences in A_{max}, g_{max}, instantaneous WUE, Δ, and leaf size were tested in a Model II ANOVA of family effects (SAS Institute, 1985). Values of WUE were log_e-transformed to conform to normality assumptions of the ANOVA model. Phenotypic correlations among pairs of characters were calculated as Pearson's product-moment correlation between character values. Genetic correlations among pairs of characters were estimated as Pearson's correlation among the family means of characters (Via 1984).

Results

Significant family differences were found in the daily course of A, g, and WUE (see Family and Time × Family effects in Table 1, Fig. 1). These differences were princi-

Table 1. Repeated Measures Analyses of Variance of the daily course of leaf photosynthetic carbon assimilation (A), conductance to water vapor (g) and water-use efficiency (WUE) in four family lines of *Polygonum arenastrum*. Significance level of within subject effects represent Huynh-Feldt corrected P values. ns: non-significant; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$

Sources of Variation	numerator df denominator df	F values and significance levels		
		A	g	WUE
Between subjects				
Family	3,8	31.9 ****	128.1 ****	25.7 ***
Contrasts				
rapid vs slow development	1,8	0.4 ns	12.4 **	12.1 **
small vs large leaf size	1,8	93.2 ****	369.4 ****	65.5 ****
Within subjects				
Time	8,64	66.0 ****	72.2 ****	336.0 ****
Time × Family	24,64	31.6 ***	2.9 ***	1.9 *
Contrasts				
Time × rapid vs slow development	8,64	0.7 ns	0.6 ns	0.4 ns
Time × small vs large leaf size	8,64	7.6 ***	6.6 ***	3.8 ***

pally due to differences in leaf size among families. Small-leaved families achieved higher rates of assimilation and conductance earlier, and maintained higher rates throughout the day compared to large-leaved families (see Contrasts comparing large versus small leaved lines in Table 1, Fig. 1). Conversely, instantaneous WUE was lower in small compared with large-leaved families. There were significant main effects of development on conductance and WUE (see Contrasts comparing rapid versus slow development to flowering in Table 1). This is attributable to the fact that, within each leaf size group, the families with delayed development to flowering (12 and 26) tended to have higher rates of conductance and lower WUE throughout the day compared to families with rapid development (Fig. 1). The effects of development on physiology were small relative to the effects of leaf size (see F values, Table 1, and Fig. 1).

Daily A and g were positively correlated (average correlation: $r = 0.93$), and both were negatively correlated with WUE among the four families. Correlations of WUE with g were stronger (average correlation: $r = -0.97$) than the correlations of WUE with A (average family mean correlation: $r = -0.79$).

In the set of 13 families, significant genetic variation was found in all characters except maximum g (g_{max}) and WUE (Table 2). Genetic correlations indicated, nevertheless, that families with high average g_{max} also had

high average A_{max} (Table 3). Instantaneous WUE was not significantly correlated with either g_{max} or A_{max}. The lack of a significant association between instantaneous WUE and either g_{max} or A_{max} may result from the fact that WUE was changing rapidly during the portion of the day when measurements were made (see Fig. 1 between 9:00 and 11:00 am local time). However, $\varDelta$ values were positively correlated with g_{max} and A_{max} (Table 3), indicating that long-term WUE was negatively correlated with g_{max} and A_{max}.

There were strong genetic correlations between leaf size and gas exchange physiology. Small-leaved families had high gas exchange rates and low long-term WUE, and vice versa for large-leaved families (Table 3). In general, genetic correlations among characters were larger than phenotypic correlations (Table 3). The exception to this pattern was the highly negative and significant phenotypic correlation between g_{max} and WUE compared to the less negative and non-significant genetic correlation (Table 3). This appears to have been due to a single family (21) whose members were quite variable and spanned the range of g_{max} and WUE values. Within family 21 there was a strong negative relationship between g_{max} and WUE, and this contributed to the strong negative phenotypic correlation. At the family mean level, however, family 21 had a high WUE for its g_{max} relative to other families; this lowered the genetic correlation. Exclusion of family 21 changed the genetic correlation from -0.40 to -0.56, which was then significant. Other genetic correlations were not greatly altered by the exclusion.

Table 2. Overall mean ($\pm$sd), range of family means, F values and significance level associated with family differences, for characters measured on 13 family lines in *Polygonum arenastrum*. ns: non-significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$

Character (units) effect	Mean $\pm$ sd	Range of family means	F value (and P level) of family
Leaf Size (cm^2)	1.24 $\pm$ 0.48	0.54– 1.85	13.74 ****
A_{max} (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	16.99 $\pm$ 2.49	13.92–19.52	2.79 *
g_{max} (mol $H_2O \cdot m^{-2} \cdot s^{-1}$)	0.43 $\pm$ 0.11	0.32– 0.54	0.85 ns
WUE (mmol $CO_2 \cdot mol_{-1} H_2O$)	0.80 $\pm$ 0.19	0.70– 0.98	0.34 ns
$\varDelta$ (‰)	20.70 $\pm$ 0.44	20.07–21.17	2.97 **

Table 3. Phenotypic (above diagonal) and genetic (below diagonal) character correlations in *Polygonum arenastrum*

	Leaf Size	A_{max}	g_{max}	WUE	$\varDelta$
Leaf Size		-0.54**	-0.28	-0.02	-0.51**
A_{max}	-0.71**		0.47*	0.24	-0.46*
g_{max}	-0.64**	0.68**		-0.58***	-0.62***
WUE	0.18	0.26	-0.40		-0.23
$\varDelta$	-0.79**	0.70**	-0.79**	-0.21	

A correlation is significantly different from 0 at *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$

Discussion

We found evidence of genetic variation in leaf-level gas exchange properties and leaf size among field-grown family lines of *Polygonum arenastrum*, a weedy annual species. These same family lines, which were derived from seed collected at a single population, had previously been shown to vary genetically in developmental phenology and in morphology (Geber 1990). Thus, there is considerable intra-populational genetic variation in this species for many characters that are potentially important to plant performance.

The strong positive genetic correlation between A and g suggest that family differences in stomatal conductance account at least in part for differences in photosynthetic carbon assimilation rate. We do not have evidence, as yet, that differences in biochemical capacity for photosynthesis account for variation in A_{max}. Leaf Kjeldahl nitrogen concentration – a measure of organic nitrogen, and an indicator of photosynthetic protein concentration – was obtained for all plants and was found to vary among families ($F = 3.11$ $df = 12,36$, $P < 0.01$). Kjeldahl N was not correlated, however, with A_{max} nor with other characters. Nonetheless, because determinations of Kjeldahl N are on the basis of leaf mass (mg $N \cdot g^{-1}$ leaf dry mass), while A is expressed on the basis of leaf area (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), and because it is not known whether families differ in specific leaf weight ($g \cdot m^{-2}$),

it is premature to conclude that leaf nitrogen content and photosynthetic carbon assimilation are not related (Field and Mooney 1986; Evans 1989).

Among families, the correlation between g and instantaneous WUE was strongly negative when measured on plants over the course of an entire day. The correlation was not particularly strong, however, when calculated from a single measurement of conductance and assimilation in plants (Table 3). The reason may be that instantaneous WUE is very sensitive to small variation in either A or g. However, the single measure of conductance was strongly correlated with Δ among families. Thus, this long-term measure of WUE discriminated better among families than instantaneous measures.

While assimilation increased with conductance, transpiration increased even more. This resulted in a trade-off between A and WUE. Stomatal limitation on instantaneous or long-term WUE has been found in ecotypes of ponderosa pine (Monson and Grant 1988) and has been suggested to occur in cultivars of bean (Nerkar et al. 1981; Ehleringer et al. 1990) and wheat (Condon et al. 1987).

In *P. arenastrum*, smaller-leaved families had higher leaf gas exchange rates and lower WUE. Negative correlations between leaf size and gas exchange rates are commonly reported in crops and other plants (Chabot and Hicks 1982; Bhagsari and Brown 1986). In some instances, the correlation has been attributed to lower stomatal densities in larger-leaved varieties (see Bhagsari and Brown 1986; also in *Capsicum annuum*, Dawson unpubl.), or to differences in cell size and specific leaf weight.

From greenhouse studies we know leaf size to be genetically correlated with developmental phenology, age-specific life history characteristics, and maximum yield and life span. To the extent that character expression is similar in the field and greenhouse, it follows, then, that gas exchange physiology and development are also genetically correlated, with rapid developers having high gas exchange rates and low WUE. We believe that the assumption of similar character expression is justified because family leaf size was strongly correlated across environments ($r = 0.85$, $n = 13$, $P < 0.001$), and plant growth and development were similar in the field and in the greenhouse.

An association between delayed development, low gas exchange rates, and high WUE has been reported in several crops, and the correlation may apply to broader comparisons among species differing in life span (Bazzaz 1979; Ehleringer et al. 1990; Hall et al. 1990). We suggest that in *P. arenastrum* the combination of rapid development, high gas exchange rates, and low WUE is favored in sites or in years where the growing season is short and disturbance is high because it allows a plant to reach maturity quickly and produce some seed. This is a bet hedging strategy that guards against total reproductive failure in poor environments at the expense of a lower reproductive potential in good environments (Philippi and Seger 1989). The greater yield potential of delayed development, on the other hand, is best realized when growing conditions are favorable

(e.g., long growing season, low amounts of disturbance or damage). Such environments are characterized by higher vegetation cover where competitive interactions become important. The large seed and seedling sizes, dense shoot canopy, and large leaf size of these genotypes (Geber 1990) would all contribute to greater competitive ability (McGinley et al. 1987). Preliminary evidence from competition trials indicates that genotypes with delayed development and large leaves tend to be dominant (Geber unpubl.).

In this population of *P. arenastrum*, rapid development to flowering is typically associated with small leaf size (and vice versa for delayed development) (Geber 1990). However, families with atypical character combinations exist (i.e., early flowering and large leaf size, or delayed flowering and small leaf size), and were among the four families used in the daily gas exchange measurements (see Methods). In these families, leaf physiological characteristics were found to follow leaf size more closely than plant development (Fig. 1). *Polygonum arenastrum* offers the possibility, therefore, of testing the contributions of developmental versus morphological and physiological characters to performance in contrasting environments.

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