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Genetic variation in stomatal and biochemical limitations to photosynthesis in the annual plant, Polygonum arenastrum

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Abstract Terrestrial plant photosynthesis may be limited both by stomatal behavior and leaf biochemical capacity. While inferences have been made about the importance of stomatal and biochemical limitations to photosynthesis in a variety of species in a range of environments, genetic variation in these limitations has never been documented in wild plant populations. Genetic variation provides the raw material for adaptive evolution in rates of carbon assimilation. We examined genetic variation in gas exchange physiology and in stomatal and biochemical traits in 16 genetic lines of the annual plant, *Polygonum arenastrum*. The photosynthesis against leaf internal CO_2 (*A–ci*) response curve was measured on three greenhouse-grown individuals per line. We measured the photosynthetic rate (A) and stomatal conductance (g), and calculated the internal CO_2 concentration (ci) at ambient CO_2 levels. In addition, the following stomatal and biochemical characteristics were obtained from the *A–ci* curve on each individual: the degree of stomatal limitation to photosynthesis (L_s), the maximum ribulose 1,5-biphosphate carboxylase-oxygenase (Rubisco) activity (Vc_{max}) and electron transport capacity (J_{max}). All physiological traits were genetically variable, with broad sense heritabilities ranging from 0.66 for L_s to 0.94 for J_{max}. Strong positive genetic correlations were found between Vc_{max} and J_{max}, and between g and biochemical capacity. Path analyses revealed strong causal influences of stomatal conductance and leaf biochemistry on A and ci. Path analysis also indicated that L_s confounds both stomatal and biochemical effects, and is an appropriate measure of stomatal influences on photosynthesis, only when biochemical variation is accounted for. In total, our results indicate that differences among lines in photosynthesis and ci result from simultaneous changes in biochemical and stomatal characteristics and are consistent with theoretical predictions that there should be co-limitation of photosynthesis by ribulose-1,5-biphosphate (RuBP) utilization and regeneration, and by stomatal conductance and leaf biochemistry. Gas exchange characteristics of genetic lines in the present study were generally consistent with measurements of the same lines in a previous field study. Our new results indicate that the mechanisms underlying variation in gas exchange include variation in both stomatal conductance and biochemical capacity. In addition, A, g, and ci in the present study tended also to be positively correlated with carbon isotope discrimination (Δ), and negatively correlated with time to flowering, life span, and leaf size based on earlier work. The pattern of correlation between physiology and life span among genetic lines of *P. arenastrum* parallels interspecific patterns of character correlations. We suggest that the range of trait constellations among lines in *P. arenastrum* represents a continuum between stress avoidance (rapid development, high gas exchange metabolism) and stress tolerance (slow development, low gas exchange metabolism), and that genetic variation in these character combinations may be maintained by environmental variation in stress levels in the species' ruderal habitat.

Key words Stomatal conductance · Leaf biochemistry · Genetic variation · *Polygonum arenastrum* · Morphology

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Introduction

Terrestrial plants face an important trade-off between carbon fixation and water loss, because of the shared pathway of CO_2 and H_2O diffusion into and out of the leaf through stomata. Plants that limit water loss by closing their stomata also limit carbon gain. Physiologists have established that stomatal limitation of photosynthesis is a common feature in plants (Farquhar and Sharkey 1982; Schulze 1986) in the sense that actual photosynthetic rates are typically lower than the maximal rates possible, if stomata were wide open. These observations suggest that the trade-off between water loss and CO_2

uptake has been important in shaping stomatal behavior. The influence of this trade-off on physiological and morphological adaptations has been the subject of many past and present studies in plant ecology and evolution (Ehleringer and Werk 1986; Givnish 1986; Lechowicz and Blais 1988; Farris and Lechowicz 1990; Dawson and Ehleringer 1993; Donovan and Ehleringer 1994; Dudley 1996a, b).

In addition to stomatal limitation, however, plants may experience biochemical limitations to photosynthesis (Sharkey 1985; Woodrow and Berry 1988). Two important sources of biochemical limitations derive from: (1) the limits on ribulose 1,5-bisphosphate (RuBP) carboxylase-oxygenase (Rubisco) activity, and (2) the limits on the regeneration of RuBP during electron transport. Rubisco activity is the chief biochemical limitation to carbon fixation when the concentration of CO_2 inside the leaf (*ci*) is low. Conversely, at high *ci*, photosynthesis is limited principally by the leaf's ability to regenerate RuBP. Thus, the primary source of biochemical limitation to photosynthesis depends in part on *ci*. Theory predicts that C_3 plants should operate at a *ci* at which photosynthesis is co-limited within the chloroplast by RuBP consumption and regeneration (Farquhar and Sharkey 1982).

Inferences have been made about the importance of stomatal and biochemical limitations to photosynthesis in a variety of species under a wide range of environmental conditions (Flanagan and Jefferies 1988; Sharkey and Seemann 1989; Day et al. 1991; Ni and Pallardy 1992). To our knowledge, however, genetic variation in these limitations has never been documented in wild plant populations, even though data from several studies suggest that such variation should be present (Comstock and Ehleringer 1992; Isebrands et al. 1988; Pereira et al. 1987). Genetic variation in stomatal and biochemical characteristics provides the raw material for adaptive evolution in rates of carbon assimilation.

The first goal of our study was to examine genetic variation in stomatal and biochemical influences on photosynthesis in the annual plant, *Polygonum arenastrum*. We examined reductions in photosynthesis resulting from stomatal closure. We also examined two potential sources of biochemical limitation: (1) the maximum Rubisco activity, and (2) the maximum electron-transport capacity (Kirschbaum and Farquhar 1984).

We focus on *P. arenastrum* because natural populations are genetically variable for leaf photosynthetic rates and for leaf carbon isotope discrimination (Δ), an integrated measure of physiology (Geber and Dawson 1990). Δ provides a long-term measure of *ci* in leaves, which is affected by the balance between CO_2 drawdown through photosynthesis and CO_2 entry into (and water loss from) the leaf through stomata. Thus, Δ can serve as an measure of long-term water-use efficiency (WUE; Farquhar et al. 1989); it may also be a good indicator of gas exchange metabolism (Ehleringer 1993a, b; Dawson and Ehleringer 1993). In *P. arenastrum*, photosynthetic rates are positively correlated with stomatal conductance and Δ, and hence negatively correlated with long-term WUE (Geber and Dawson 1990). These patterns suggest that photosynthetic rates may be under strong stomatal control in *P. arenastrum*. However, up till now, we have not investigated biochemical sources of variation in photosynthesis. Thus, our study is aimed at understanding the mechanisms underlying genetic variation in gas exchange physiology in *P. arenastrum*.

Our interest in mechanism is motivated by the fact that leaf gas exchange and Δ are not only genetically variable in *P. arenastrum* but are also genetically correlated with life history and morphology (Geber 1990; Geber and Dawson 1990). *P. arenastrum* genotypes vary widely in life history because of developmental differences in the node of first flowering (Geber 1990). Indeed, genotypes that flower at an early node have an early onset of flowering, attain a small final size, and complete their life cycle rapidly. Furthermore, early-flowering genotypes have small leaf sizes, high leaf photosynthetic rates, high stomatal conductance, and high Δ reflecting low integrated WUE. The opposite suite of morphological, physiological and life history traits characterize genotypes that flower at an advanced node (Geber and Dawson 1990). A second goal of our study was to examine the relationship between stomatal and biochemical characteristics measured in the present study and physiological (gas exchange and Δ), developmental (node of first flowering), and morphological (leaf size) characteristics established in earlier studies (Geber 1990; Geber and Dawson 1990). Are genotypes consistent in their physiology across studies? Do genotypes with contrasting leaf gas exchange characteristics, development, and morphology differ in degree of stomatal versus biochemical limitation? And if so, how do they differ? Answers to these question may help elucidate the functional significance of the associations between development, morphology, and physiology.

Materials and methods

Plants and treatments

P. arenastrum is a cosmopolitan weed of Eurasian origin. It is commonly found in disturbed habitats (Geber 1989). Because the species is strictly self-pollinating (pollen is shed onto stigmas before flowers open), all seeds from a single plant are inbred full-siblings. In 1986, seeds were collected from a large number of randomly selected plants in a roadside population in the Red Butte Canyon Research Natural Area, east of Salt Lake City, Utah, United States) (Geber 1990). From 26 of these plants, inbred lines were established by single seed descent (Geber 1990). A subset of 16 lines were chosen for the present investigation. All experiments were conducted on greenhouse-grown plants between May and October 1994. Seeds from each line were germinated on moist peat moss and then transplanted into tube-shaped pots (25.4 cm^3) filled with fritted clay. Fritted clay was chosen as the growing medium because it is chemically inert, has low bulk density and high porosity, and has excellent desorption properties (van Bavel et al. 1978); it therefore drains quickly and dries uniformly and repeatably (Emerman and Dawson 1996). After transplanting, plants watered daily to saturation for the first three weeks, and then every other day. Plants were fertilized every 16 days (Peters 20-20-20).

Physiological measurements were made five weeks after transplanting, before any plants had begun to flower. In these tubes, plants attained sizes typical of plants in the field.

Physiological measurements

CO_2 and H_2O vapor exchange measurements were made with an open gas exchange system (Campbell Scientific Inc., model MPH-1000). The system used mass flow controllers (Edwards High Vacuum Int., Datametrics model 825) to precisely mix 1% CO_2 in air with CO_2-free air. Part of the dry, mixed, gas passed through degassed water to humidify the air, which was remixed with the dry air to a known dew point and monitored with a chilled-mirror dew point hygrometer (General Eastern Corp., model dew-10). The CO_2 concentration ($[CO_2]$) of this same air was measured with an infrared gas analyzer (LiCor Inc., model 6252). Leaf and cuvette temperatures were measured with fine-wire lead-constant thermocouples. Light was supplied by a 1000-W sodium-vapor lamp (General Electric, Inc.) mounted above a water-filter to remove infrared radiation. Measurements of the dew point and $[CO_2]$ of the air stream returning from the leaf cuvette were also made (as above), and used in calculations of gas exchange rates and *ci*, following Harley and Sharkey (1991).

Branch tips with five to nine newly expanded leaves (total leaf area of 12–19 cm^2) were enclosed in a temperature-controlled cuvette (10.16×7.62×7.62 cm) in which air was pulled across the leaf surfaces to form a very small and uniform boundary layer. Genotypes have similar rates of leaf (=node) production (Geber 1990), and thus had equivalent numbers of newly expanded leaves on branch tips. A model shoot constructed from moistened filter paper was used to determine the boundary layer conductance for use in the final gas exchange calculations. Response curves of photosynthesis versus leaf internal $[CO_2]$ (the *A–ci* curve) were measured by holding photon flux density (PFD; 1200±150 μmol m^{-2} s^{-1}), leaf temperature (28±1.1° C), and the leaf-to-air vapor pressure difference (Δw; 10±1.2 mmol H_2O mol^{-1} air) constant and varying $[CO_2]$ in the leaf cuvette in steps of 50 μl l^{-1} from 50 to 750 μl l^{-1}. Measurements were first taken at a cuvette $[CO_2]$ of 360 μl l^{-1}. The $[CO_2]$ in the cuvette was then increased, decreased, and finally returned to 360 μl l^{-1} to generate the *A–ci* curves for plants from the two treatments (Fig. 1).

Physiological characters

Gas exchange

For each plant, we estimated the photosynthetic (A, μmol m^{-2} s^{-1}) and the stomatal conductance to water vapor (g, mmol m^{-2} s^{-1}) that would be expected under ambient field $[CO_2]$ from gas exchange measurements at a cuvette $[CO_2]$ of 360 μl l^{-1} (Fig. 1). The leaf internal $[CO_2]$ at a cuvette $[CO_2]$ of 360 μl l^{-1} was calculated from A and g according to Farquhar and von Caemmerer (1982) (Fig. 1).

In the paper, we use plain text font (A, g, ci) to designate the values of photosynthesis, stomatal conductance and the internal $[CO_2]$ at a cuvette $[CO_2]$ of 360 μl l^{-1}. We use italicized font (*A*, *g*, *ci*) when referring to photosynthesis, stomatal conductance and the internal $[CO_2]$ as variables (e.g., *A–ci* curve).

An estimate of the rate of photosynthesis (A_0, μmol m^{-2} s^{-1}; Fig. 1) in the absence of stomatal limitation was obtained from the *A–ci* curve when the internal *ci* was equal to 360 μl l^{-1}. A_0 estimates the rate of photosynthesis when stomatal resistance to CO_2 diffusion is essentially zero.

Stomatal limitation

The percent stomatal limitation to photosynthesis (L_s, %) was calculated from the *A–ci* curve as,

$$L_s=100\cdot(A_0-A)/A_0 \quad (1)$$

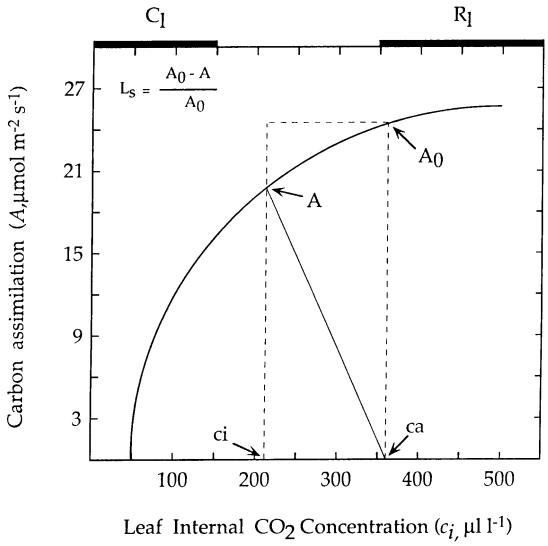

Fig. 1 Hypothetical *A–ci* curve. Parameters used in the analysis of stomatal and biochemical limitations to photosynthesis are illustrated. The ambient $[CO_2]$ is indicated by ca. For each curve, the *diagonal line* between the x-axis and the *A–ci* curve is the CO_2 supply function and is proportional to stomatal conductance (*g*). The intersection of the line with the curve corresponds to photosynthesis at ambient $[CO_2]$, A; the leaf internal $[CO_2]$ corresponding to this point on the *A–ci* curve is ci. A_0 is the photosynthetic rate when the leaf internal $[CO_2]$ is equal to ca, i.e., the photosynthetic rate in the absence of stomatal limitation. Stomatal limitation, L_s reflects the percentage reduction in A_0 attributable to stomatal closure (see formula). The *bar* at the top of the figure labelled C_l is the region of the *A–ci* response that is most limited by low ci and by Rubisco activity (Vc_{max}). The *bar* labelled R_l is the region of the *A–ci* response that is most limited by electron transport capacity (J_{max}) and the regeneration of RuBP

(Farquhar and Sharkey 1982; Fig. 1). L_s estimates the percentage decrease in *A* imposed by stomatal closure. Although L_s is generally referred to as stomatal limitation, its value depends on the shape of the *A–ci* curve between ci and an internal $[CO_2]$ of 360 μl l^{-1}, and so is likely to be influenced by biochemistry. Through data analysis and in the Discussion, we address the value of using L_s as a measure of stomatal limitation (see also Flanagan and Jefferies 1988).

Biochemical model, and curve fitting procedures

Estimates of a plant's maximum Rubisco activity (Vc_{max}, μmol CO_2 m^{-2} s^{-1}) and electron-transport capacity (J_{max}, μmol CO_2 m^{-2} s^{-1}) were determined from an analysis of the *A–ci* curve, using the biochemical model of von Caemmerer and Farquhar (1981).

The biochemical model. CO_2 assimilation (*A*) can be related to *ci* as

$$A=-R_{day}+(1-\Gamma_*/ci)\cdot Vc \quad (2)$$

where R_{day} represents day respiration resulting from CO_2 release in the light by processes other than photorespiration; Γ_* is the CO_2 compensation point in the absence of R_{day}; and Vc is the rate of carboxylation.

In the absence of any limitations due to inorganic phosphate regeneration,

$$Vc=min\ \{Wc, Wj\} \quad (3)$$

where *min* designates the minimum of Wc and Wj. Wc represents the Rubisco-limited (i.e., RuBP-saturated) rate of carboxylation,

and Wj represents the electron-transport-limited (i.e., RuBP-regeneration-limited) rate of carboxylation. Wc and Wj, in turn, are functions of Vc_{max} and J_{max}. In particular,

$$WC=(Vc_{max}\cdot ci)/[(ci+K_c\cdot(ci+O/K_o)] \quad (4)$$

where Vc_{max} is the maximum rate of carboxylation with non-limiting RuBP, CO_2, and with full activation of Rubisco. K_c and K_o are the Michaelis-Menten constants for carboxylation and oxygenation, respectively, and O is the partial pressure of O_2.

$$Wj=J\cdot ci/[4(ci+2\Gamma_*)] \quad (5)$$

where J is the potential rate of electron transport at a given irradiance, and the factor 4 accounts for the fact that the transport of four electrons generates sufficient ATP and NADPH to regenerate RuBP in the Calvin cycle (Farquhar and von Caemmerer 1982). For a given irradiance, I, J can be expressed as:

$$J=(J_{max}\cdot I)/(I+2.1\ J_{max}) \quad (6)$$

where J_{max} is the maximum electron transport capacity.

Following Kirschbaum and Farquhar (1984), we used the following values for parameters: R_{day}=1.0 μmol CO_2 m^{-2} s^{-1}; Γ_*=42 μmol CO_2 mol^{-1}; K_c=310 μmol mol^{-1} CO_2; K_o=155 mmol mol^{-1} O_2; and O=210 mmol mol^{-1}.

Estimation of Vc_{max} and J_{max} from A–ci *curves.* A second-order polynomial regression was fit to the *A–ci* curve of each plant, to obtain predicted values of *A* for *ci* ranging between 20 and 580 μl l^{-1}, in 20 μl l^{-1} increments. This range encompassed the measured range of *ci* values for all plants. For each plant, the second-order polynomial explained 95–99% of the variation in *A–ci* data.

We used non-linear regression techniques (SAS Institute 1994) to estimate Vc_{max} and J_{max} by fitting the biochemical model (Eqs. 2–6) to the predicted *A–ci* values. Following Wullschleger (1993), we estimated Vc_{max} by assuming that *A* was entirely limited by the Rubisco-limited rate of carboxylation, Wc, at *ci* below 200 μl l^{-1} (i.e., from Eq. 2, with Vc=Wc in Eq. 4). We then substituted the estimated value of Vc_{max} into the model equations, and estimated J_{max} by fitting the model to the entire range of *A–ci* values, at an irradiance, I, of 1200 μmol m^{-2} s^{-1}.

The limited number of data points for each *A–ci* curve did not allow us to estimate Vc_{max} and J_{max} simultaneously. Whether Vc_{max} and J_{max} are estimated jointly or sequentially from the same *A–ci* curve, a statistical correlation may arise between the two estimates. We tried to address the issue of statistical correlation by examining how Vc_{max} and J_{max} were correlated *within* each genetic line. To the extent that individuals from the same genetic lines have nearly identical *A–ci* responses (see Results), covariation of Vc_{max} and J_{max} estimates within lines would provide evidence of a statistical association between parameter estimates.

In summary, the following characteristics were determined on all plants: A, g, and ci, A_0, L_s, Vc_{max}, and J_{max}. Data were analyzed for all traits, except A_0, because A_0 entered directly into the calculation of L_s.

Analysis

Genetic variation in physiological traits

We tested for genetic variation in each of the six traits measured by one-way analyses of variance (ANOVA), with genetic lines as a random factor. The ANOVA on the six physiological traits were not independent of each other because all traits were measured on the same plants. The significance levels for tests of genetic effects were therefore inflated. To correct for multiple tests on non-independent data sets, we applied a sequential Bonferroni procedure to evaluate the significance of genetic effects on trait variation (Rice 1989).

For each trait, we estimated the within- (σ_e^2) and between- (σ_g^2) line components of variance in trait expression from the error (MS_e) and genetic line (MS_g) mean squares in the ANOVA (Falconer 1989). The variance component estimates were then used to calculate the intraclass correlation, τ, for each trait as $\tau=\sigma_g^2/(\sigma_g^2+\sigma_e^2)$. The inctraclass correlation is both a measure of the degree of resemblance between related individuals and of genetic differences between unrelated individuals. Thus, when the resemblance between individuals from the same genetic line is high, there is little within-line variation in trait expression (σ_e^2~0), and τ approaches 1 because most of the variation in a trait is due to variation among lines (σ_g^2). Conversely, τ is near 0 when there is little resemblance between relatives (σ_e^2 large), or differences among lines in trait expression are small (σ_g^2~0). In the case of comparisons among fully inbred lines, τ is equal to the ratio of the total genetic variance to the total phenotypic variance in a trait, and thus provides an estimate of the trait's broad sense heritability, h_g^2 (Falconer 1989). Standard errors for τ were calculated as in Falconer (1989, p. 182).

Genetic correlations among traits

Genetic character correlations were calculated between all pairs of traits as product-moment correlations between the means of traits from each genetic line. Correlations calculated from family (=line) means tend to overestimate the true (full-sib) genetic correlations, because they include maternally-inherited environmental covariance in addition to genetic covariance (Via 1984). Past work with *P. arenastrum* has indicated very close agreement between family-mean correlations and correlations based on genetic covariance components (Geber 1990).

The statistical significance of genetic correlations was tested by converting the correlation coefficients to their z-transforms and then constructing confidence intervals around the z-transforms (Snedecor and Cochran 1989). To adjust for the fact that confidence intervals were being computed on a set of non-independent correlations, we again applied the sequential Bonferroni procedure in estimating the width of the confidence intervals (Rice 1989).

The patterns of pairwise trait correlations can be useful in determining whether stomatal or biochemical characteristics influence variation in photosynthesis and ci among genetic lines. However, because stomatal and biochemical characteristics may themselves be correlated, their separate effects on A and ci may be difficult to detect from simple pairwise correlations. We used path analysis to partition the genetic correlations of stomatal and biochemical traits with A and ci into components based on a hypothesis of causation (Pedhazur 1982; Kingsolver and Schemske 1991). Our path analysis was based on family-means of stomatal, biochemical and photosynthetic characters, rather than on individual plant values. In addition, all characters were standardized to mean zero and variance 1, by expressing each family mean as a deviation from the grand mean and dividing by the standard deviation of family means. We are justified in using path analysis, which assumes linear relationships between dependent and independent variables, because pairwise plots (not shown) between stomatal, biochemical and/or photosynthetic family means showed linear relationships among variables, in spite of the non-linearity of the biochemical model.

The path diagram in Fig. 2a represents our *a priori* hypothesis of stomatal and biochemical effects on A or ci. A single-headed arrow between two variables connotes and hypothesis of direct causation, whereas a double-headed arrow reflects correlation without necessarily a direct causal relationship. Thus, a double-headed arrow between variables X and Y ($r_{X,Y}$) may result from a direct causal effect of X on Y or of Y on X, or it may reflect an indirect relationship caused by mutual dependence of a third unmeasured variable. In our path diagram, a double-headed arrow between X and Y is equal to the simple genetic correlation between the two variables. The single-headed arrows ($p_{X,Y}$) are equivalent to partial genetic correlation coefficients, when a dependent variable (e.g., A) is regressed against more than one independent variable (e.g., stomatal and biochemical characteristics).

We hypothesized that g has a direct causal effect ($p_{g,L}$) on L_s, because, all else being equal, increased stomatal conductance should reduce stomatal limitation; biochemistry may also directly affect L_s ($p_{B,L}$), because, for a fixed g, L_s will depend on the shape

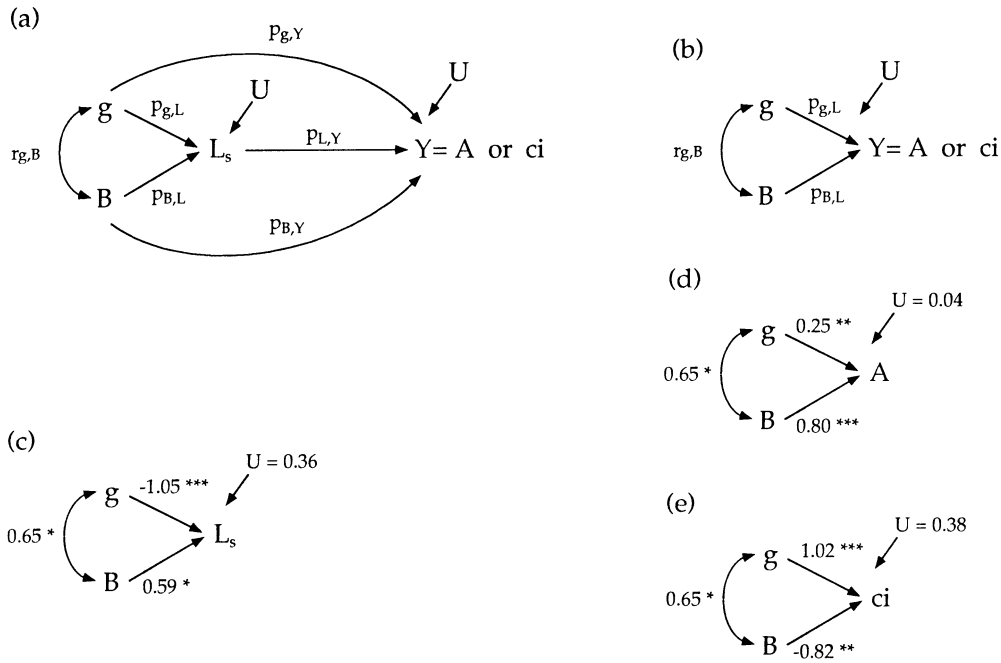

Fig. 2 **a** Hypothesis of causation between stomatal and biochemical characteristics and A or ci. **b** Simplified hypothesis of causation, eliminating L_s. **c** Path diagram of the effects of stomatal conductance and biochemistry on L_s. **d** Path diagram of the effects of stomatal conductance and biochemistry on A. **e** Path diagram of the effects of stomatal conductance and biochemistry on ci. The statistical significance of a path coefficient is based on a test of its significance in the regression analysis, or as a correlation coefficient. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

of the *A–ci* curve between ci and an internal [CO_2] of 360 µl l^{-1}. Stomatal conductance was hypothesized to be correlated with biochemical characteristics, but not necessarily in a direct causal fashion ($r_{g,B}$). For example, stomatal behavior may not directly affect biochemical capacity or vice versa; and the two traits are not likely to be determined by the very same genes. On the other hand, stomatal behavior and biochemistry may co-vary because the genes affecting these traits are linked in this set of inbred lines, or because the two sets of traits are similarly affected by other genes. Stomatal (g, L_s) and biochemistry (B) traits were also hypothesized to have direct causal effects ($p_{g,Y}$, $p_{L,Y}$, $p_{B,Y}$) on A and ci (Fig. 2b).

In our path analysis, we did not attempt to partition individual causal effects of Vc_{max} and J_{max} on other variables because the two biochemical traits were very highly correlated among lines and some of this correlation may be statistical rather than biological in origin (see Discussion). Instead, we created a composite variable, B, consisting of the first principal component of Vc_{max} and J_{max}. The first principal component explains 98% of the variation in biochemical characteristics.

Relatioship between physiology, development, and morphology

To examine the relationship between physiology, development and morphology, we calculated Spearman's rank correlations between the family means of physiological traits in the present study and the family means of the following characteristics measured in earlier studies on 13 of the same lines (from Geber 1990; Geber and Dawson 1990): A, g, ci, Δ, node of first flowering (FN) and the average leaf size (LA).

Results

Genetic variation in physiology

Table 1 shows the mean values of the six physiological traits for the entire set of 48 plants, as well as the range of trait means among the 16 genetic lines. For all traits, average values ranged widely among lines. Indeed, for all traits except ci, there was a two-fold difference in the mean trait value of the lowest and highest ranked lines (Table 1).

Table 1 Means±phenotypic standard deviations of physiological traits in *Polygonum arenastrum*. Means are based on 48 plants (3 individuals from each of 16 genetic lines). The range of genetic line means for each trait is also shown in *parentheses*

A	18.1±3.0 (12.4–22.2)
A_0	21.8±3.3 (14.6–26.9)
ci	271±15 (248–295)
g	0.49±0.13 (0.25–0.72)
L_s	21.0±6.0 (14.1–34.6)
Vc_{max}	89.6±15.1 (63.6–113.7)
J_{max}	175.8±29.6 (109.0–216.6)

In contrast to the between-line variance in physiological traits, there was a remarkable consistency within lines in the physiology of individuals. This is best seen in Fig. 3 where the *A–ci* responses of three individuals from eight genetic lines are plotted. These eight genetic lines were chosen because they represent the full range of measured *A–ci* responses, including the more extreme responses (highest and lowest A, g, L_s, Vc_{max} and J_{max}). In all cases, individuals from the same line were nearly identical in *A–ci* response.

The low within-line variance relative to the high-between line variance is indicative of high levels of genetic variation for all traits. Intraclass correlations (=heritabilities) ranged from 0.66 for L_s to 0.94 for J_{max} (Table 2).

Figure 3 also illustrates the nature of the variation in biochemical and stomatal characteristics among *P. are-*

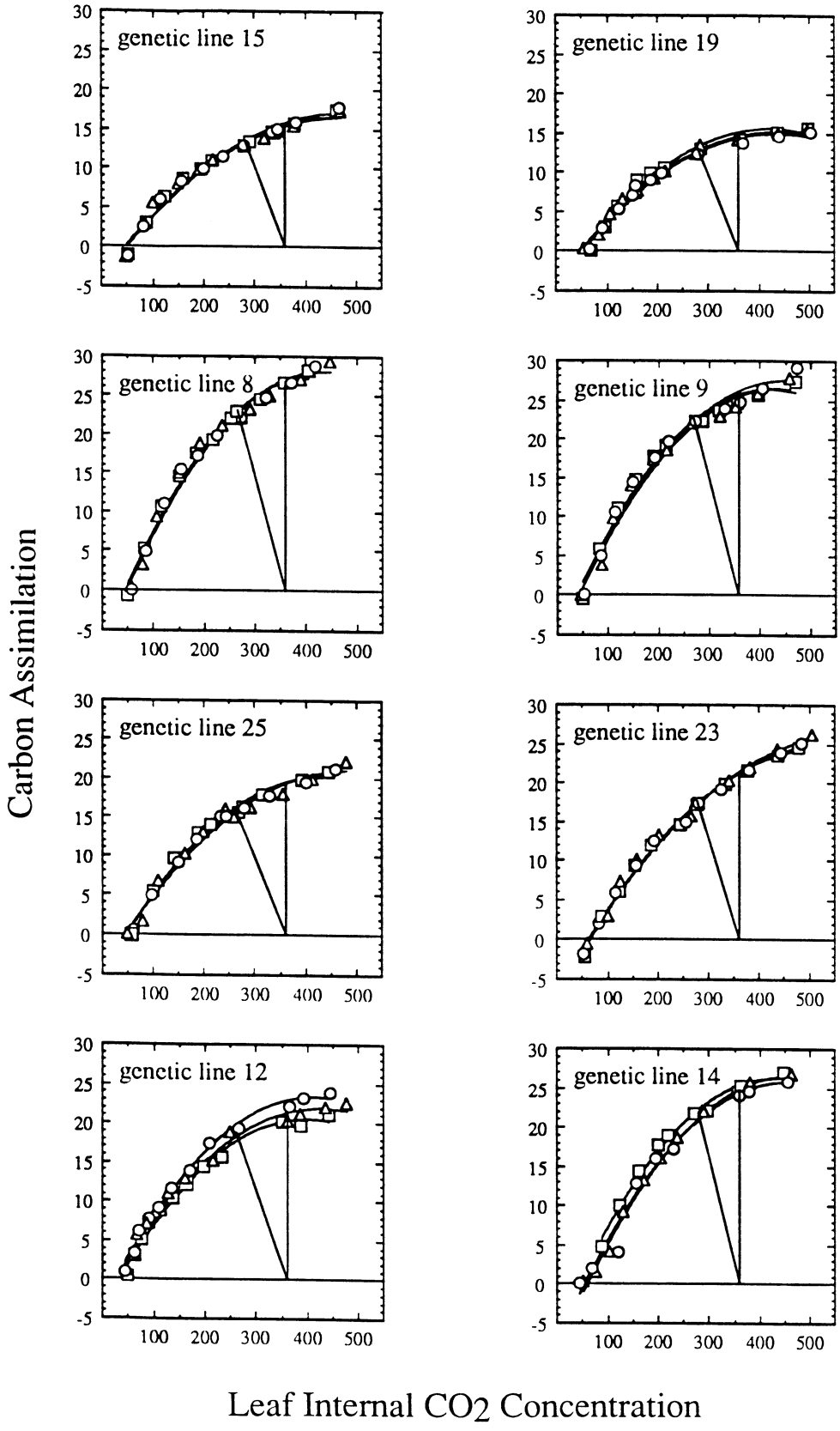

Fig. 3 *A–ci* responses of eight genetic lines of *Polygonum arenastrum*. Each plot shows the *A–ci* data for three individuals from one genetic line. The three *curves* in each plot are the second-degree polynomials that were fit to the *A–ci* data of the three individuals. The *diagonal line* in each plot corresponds to the average supply-function for the genetic line. The *vertical line* intersects the *A–ci* curve at A_0

Table 2 Intraclass correlations, τ±SD, of physiological traits for 16 genetic lines in *P. arenastrum*. In *P. arenastrum*, where genetic lines are fully inbred, the intraclass correlation is equal to the broad-sense heritibality of a trait. All intraclass correlations are significantly different from zero, based on sequential Bonferroni tests with overall α=0.05

A	0.87±0.05
ci	0.81±0.07
g	0.71±0.10
L_s	0.66±0.12
Vc_{max}	0.82±0.07
J_{max}	0.94±0.03

nastrum lines. Genetic lines 15 and 19 have low Vc_{max}, which is reflected in the shallow slopes of the *A–ci* curves at low *ci*; these lines also have low J_{max}, which is reflected in the low asymptote of the *A–ci* curve at high *ci*. Genetic lines 8 and 9, by contrast, have high Vc_{max} and J_{max}.

Genetic lines 23 and 25 have high L_s, because A_0 is considerably greater than A. In genetic lines 12 and 14, on the other hand, the *A–ci* response quickly asymptotes between ci and 360 µl l^{-1}; in these lines, A_0 and A are not very different, and L_s is low.

Lines 8 and 23 were the only two lines whose *A–ci* responses showed little evidence of reaching an asymptote at high *ci*. When the *A–ci* response does not asymptote, J_{max} is likely to be underestimated. Other measured (A, g) or calculated parameters (ci, L_s) are not affected by the lack of an asymptote at high *ci*. Elimination of lines 8 and 23 from subsequent analyses of character correlations only strengthened the patterns of correlations.

Stomatal and biochemical influences on photosynthesis

Stomatal conductance and biochemical characteristics were strongly positively correlated (Table 3), indicating that genetic lines with high transpiration rates also had high biochemical capacity. In addition, Vc_{max} was positively correlated with J_{max}, indicating that lines with high Rubisco activity also had high electron-transport capacity.

Because the estimates of Vc_{max} and J_{max} for each plant were obtained from the same *A–ci* curve, it is possible that part of the positive genetic (among-family) correlation between Vc_{max} and J_{max} is due to a statistical correlation between the estimates of these two parameters obtained for each plant. We therefore examined the within-line correlations between Vc_{max} and J_{max} (see above). Within-line correlations between Vc_{max} and J_{max} ranged from –0.8 to +0.9, with an average of correlation of 0.19. The average within-line correlation is considerably lower than the between-line family mean correlation of 0.88 (Table 3), suggesting that co-variation between Vc_{max} and J_{max} among lines is not simply a statistical artifact.

Based on pairwise correlations among genetic line means, A was strongly and positively correlated with g, Vc_{max} and J_{max} (Table 3). A was negatively correlated with L_s, but the correlation was non-significant (Table 3). There was a marginally significant positive correlation between ci and g, and a strong and significant negative correlation between ci and L_s; ci was not significantly correlated with A, Vc_{max} or J_{max} (Table 3).

In view of the positive correlations between stomatal and biochemical characteristics, and in light of their joint influence of photosynthesis and ci, we used path analysis to partition the genetic correlations of stomatal and biochemical traits with A and ci into direct causal and indirect causal or unanalyzed components (Fig. 2a). As indicated earlier, we did not attempt to partition individual causal effects of Vc_{max} and J_{max} on other variables in our path analysis, but created instead a composite variable, B (for biochemistry), consisting of the first principal component (Vc_{max} and J_{max}. The genetic (family-mean) correlations between B and other traits are shown in Table 3).

In analysing the direct effects of g and B on L_s, the path coefficient $p_{g,L}$ was estimated to be equal to –1 (Fig. 2c), indicating that g and L_s are measures of essentially the same trait, *once* biochemical variation is accounted for. We therefore simplified the path analysis of stomatal and biochemical effects on A and ci by dropping L_s from the analysis, producing the simpler path diagram illustrated in Fig. 2b. g and B had significant direct causal effects on both A and ci (Fig. 2d, e); as expected the effects on A were both positive whereas the effects on ci were of opposite sign. B had a stronger direct effect on A than did g, whereas g had a somewhat stronger effect on ci than did B.

Table 3 Matrix of genetic (family-mean) character correlations for 16 genetic lines of *P. arenastrum*. Correlations that are *underlined* and in *bold type* differ significantly from zero, based on significance tests from parametric methods, adjusted by the sequential Bonferroni procedure. Character B is the first principal component of Vc_{max} and J_{max}; correlations with B are shown for comparison with path analysis

	ci	g	L_x	Vc_{max}	J_{max}	B
A	0.08	**0.77**	–0.32	**0.92**	**0.94**	0.96
ci		0.49	**–0.75**	–0.16	0.13	–0.15
g			**–0.66**	**0.66**	0.61	0.65
L_s				–0.28	0.06	–0.09
Vc_{max}					**0.88**	0.97
J_{max}						0.97

Table 4 Spearman's rank correlations between family means of physiological characters measured on 13 lines in the current study and physiological (A, ci, g, Δ), developmental (FN) and leaf size (LA) characters measured on the same lines in past studies. Because of the small number of lines, only correlations greater than 0.56 in absolute value (*bold and underlined*) are significantly different form zero at α=0.05; correlations greater than 0.47 in absolute value (*bold*) are significantly different from zero at α=0.10

		Current Study					
		A	ci	g	L_s	Vc_{max}	J_{max}
Past	A	0.39	0.30	0.40	**–0.49**	0.35	0.14
Studies	ci	**0.58**	**0.49**	**0.57**	**–0.48**	**0.56**	**0.47**
	g	0.08	0.18	0.19	–0.24	0.23	–0.05
	Δ	**0.57**	0.27	**0.77**	**–0.53**	**0.57**	0.28
	FN	0.03	**–0.71**	–0.12	**0.50**	0.25	0.19
	LA	–0.39	**–0.59**	**–0.49**	**0.48**	–0.26	–0.29

Relationship between physiology, development, and morphology

The gas exchange characteristics of lines measured in this study were generally consistent with results from earlier field and studies (Geber 1990, Geber and Dawson 1990). Thus, lines with high A, g and/or ci in the present study were the same lines with high A and ci in our earlier work (Geber and Dawson 1990). There was no strong correlation between gas exchange rates in the current study and g in the field study; however, g in the field was not genetically variable (Geber and Dawson 1990).

Gas exchange characteristics were also correlated with integrated physiology, development, and morphology (Table 4). As expected, lines with high gas exchange rates in this study were the same lines that had high carbon isotope discrimination (Δ) (Geber and Dawson 1990), early flowering (low FN) and small leaves (LA) (Geber 1990) in our previous work. High L_s in this study was linked to low A, ci, and Δ, late flowering and large leaf size in the same lines in earlier studies. Lines with high biochemical capacity (Vc_{max} and J_{max}) tended to be the ones with high gas exchange rates, high Δ and small leaf size in earlier work (Table 4).

Due to the small number of lines being compared across studies, only eight of the Spearman's rank correlations between characteristics measured in the present study and those measured in earlier work were significantly different from zero at α=0.05. Sixteen correlations were significantly different at α=0.1. In addition, all correlations greater than 0.2 in absolute value were of the expected sign.

Discussion

Although there is widespread evidence of interspecific variation in gas exchange physiology (Evans 1989), in integrated physiology based on Δ (Ehleringer and Cooper 1988; Smedley et al. 1991, Schuster et al. 1992a, Ehleringer 1993a, b), and in biochemical characteristics related to the regulation of photosynthesis (Wullschleger 1993), there have been few investigations of intra-specific variation in physiology, and especially of biochemical variation.

Biochemical variation has been documented at the intra-specific level, but only among cultivars of crop species. For example, significant genetic differences in biochemistry have been observed in sunflower hybrids (Gimenez et al. 1992), and in cultivars of wheat (Pyke and Leech 1985; Gummuluru et al. 1989), corn (Rocher et al. 1989), fescue (Nelson and Sleper 1983; Nelson 1988), and pea (Hobbs and Mahon 1985). Genetic variation in photosynthesis has been attributed to variation in Rubisco content or activity (von Caemmerer and Farquhar 1981; Hobbs and Mahon 1985; Pyke and Leech 1985; Woodrow and Berry 1988), to variation in chlorophyll content (Hobbs and Mahon 1985; Gummuluru et al. 1989), or to differences in other key enzymes involved in carbon fixation and sucrose synthesis (Cameron and Bassett 1988; Rocher et al. 1989). The focus of interspecific comparisons and in cultivar studies has typically been on evaluating stomatal and biochemical sources of variation in photosynthetic responses to stress; in other words, the aim has been to document variation in stomatal and biochemical *adjustments* to a deteriorating environment; relatively few studies have examined variation among species/cultivars in stomatal and biochemical traits within environments.

In wild plant species, investigations of intra-specific physiological variation have focused on inter-population (*viz* ecotypic) comparisons (Gurevitch et al. 1986; Kalisz and Teeri 1986; Dawson and Bliss 1989; Comstock and Ehleringer 1992). In these studies, the emphasis has been on documenting variation in gas exchange physiology, in instantaneous or integrated measures of water-use efficiency, to the exclusion of biochemical variation. The same can be said of the few studies of genetic variation in physiology *within* populations of wild species (Geber and Dawson 1990; Schuster et al. 1992b; Ehleringer 1993c; Dawson and Ehleringer 1993; Donovan and Ehleringer 1994; Dudley 1996b). The tendency, therefore, has been to focus on the efficiency of carbon acquisition relative to water loss, with the perception that stomata exert a dominant control over carbon fixation. This bias is reinforced by the oft reported correlation between photosynthetic rate and stomatal conductance within and

among species (e.g. Wong et al. 1979, 1985). Theory predicts, however, that photosynthesis in C_3 plants should not only be co-limited by ribulose 1,5-bisphosphate consumption (Vc_{max}) and regeneration (J_{max}) (Farquhar and Sharkey 1982), but also by stomatal relative to biochemical factors (Cowan 1986). Thus, if photosynthesis is found to covary with stomatal characteristics among plants or genotypes, we might also expect to find corresponding variation in mesophyll biochemistry.

Genetic variation in physiology

In this study, we not only confirmed results from an earlier field investigation that gas exchange physiology is genetically variable in populations of *P. arenastrum* (Geber and Dawson 1990), we also showed that *P. arenastrum* is genetically variable for stomatal (g, L_s) and biochemical traits (Vc_{max}, J_{max}) (Table 2). Indeed, the heritabilities (=intraclass correlations) for biochemical traits were as high as those for stomatal characteristics (Table 2).

The high heritabilities estimated for all traits (Table 2) reflect the marked differences in *A–ci* responses among lines, as well as the remarkable similarity in the *A–ci* responses of individuals within lines (Fig. 3). This striking similarity is attributable, no doubt, to the highly inbred nature of genetic lines in this species, and to the controlled environmental settings of this greenhouse study. Field estimates of trait heritabilities are considerably lower than greenhouse estimates (compare field estimates of intraclass correlations for A: 0.25±0.19, g: 0.0±0.17; ci: 0.22±0.19 to Table 2). Indeed, in the field, there was no detectable genetic variation in g, based on a single measure of stomatal conductance at midday in 13 lines, although a more detailed examination of the daily course of stomatal conductance in four lines did reveal significant genetic variation (Geber and Dawson 1990). Thus, while the expression of genetic differences in the field will not be as pronounced as in the greenhouse, the general consistency in the gas exchange characteristics of genetic lines between the two studies suggests that physiological differences among lines are real and biologically meaningful (Table 4).

The range of variation in gas exchange characteristics and in stomatal and biochemical traits in 16 genetic lines is quite impressive, with most characters varying twofold among lines (Table 1). The range of variation in A, on the order of 10 μmol m^{-2} s^{-1}, is as large as variation in photosynthetic rates observed among several species of Death Valley annuals (Mooney et al. 1981), even though the absolute rates are lower in *Polygonum*. Similarly, the range of variation in Vc_{max} and in J_{max} in a single population of *P. arenastrum* is fully 1/3 as large as the range of variation reported from 40 species of dicot crops (Wullschleger 1993). Average Rubisco activity and electron-transport capacity across all lines of *Polygonum* are similar to values reported for dicot crops (Wullschleger 1993). This similarity may reflect *P. arenastrum*'s association with agricultural crops and weedy environments (Geber 1989).

The intriguing question is why would there be such a broad range of gas exchange physiology in populations of *P. arenastrum*? How is genetic variation in physiology maintained? What is the functional significance of variation in physiology? Definitive answers to these questions, and especially to the last question, can only come from evolutionary studies of the fitness consequences of variation in physiology. These studies are currently underway (M.A. Geber and T.E. Dawson, unpublished work). In the interim, the pattern of correlation among physiological traits, and between physiology and other important phenotypic characters, such as life history and morphology, may help inform hypotheses about the functional significance of variation in physiology.

Relationships among physiological characters

We find support for several theoretical predictions in the pattern of genetic correlations among physiological traits in *P. arenastrum*. First, the positive correlation between Vc_{max} and J_{max} (Table 3) suggests that biochemical differences among lines result from simultaneous changes in both Rubisco activity and regeneration (see also Fig. 3, top 4 panels), and supports the prediction of co-limitation by Rubisco consumption and regeneration in C_3 plants (Farquhar and Sharkey 1982). Co-limitation does not simply mean that Vc_{max} and J_{max} both have an influence on photosynthetic rates within a plant; clearly they do. Rather co-limitations means that across environments or among genotypes and species, as Vc_{max} changes so to should J_{max}. A positive correlation between Vc_{max} and J_{max} has been reported in interspecific comparisons (see Wullschleger 1993). Our analysis of within-line correlations of Vc_{max} and J_{max} suggests that the between-line covariation is real and not due entirely to a statistical artifact.

We also found a strong positive correlation between g and biochemical traits among lines (Table 3), indicating that lines differ with respect to both stomatal behavior and leaf biochemistry. Furthermore, the joint influence of stomata and biochemistry on variation in photosynthesis and ci among lines (Table 3; Fig. 3) is consistent with the theoretical prediction that C_3 plants should be co-limited by stomatal and biochemical factors (Cowan 1986). In *P. arenastrum*, lines with high photosynthetic rates, not only have high stomatal conductance but also high biochemical capacity, and vice versa for lines with low photosynthetic rates.

The path analysis indicated that biochemistry has a somewhat greater direct influence on variation in photosynthesis than does stomatal conductance in *P. arenastrum*. Thus, a strong positive correlation between stomatal conductance and photosynthesis should not be taken as evidence that stomata exert the primary control over carbon assimilation.

The path analysis also indicated that variation in stomatal limitation (L_s) was affected both by variation in g and in biochemistry (Table 3; Fig. 2b). Furthermore, L_s is only a true measure of stomatal effects on photosynthesis once variation in biochemistry has been accounted for (Fig. 2b).

Relationship between physiology, development and morphology

A common pattern that emerges from interspecific comparison is that shorter-lived taxa often have higher rates of gas exchange (Bazzaz 1979; Evans 1989; but see Nelson 1984), higher carbon isotope discrimination (Ehleringer and Cooper 1988; Smedley et al. 1991; Schuster et al. 1992a; Ehleringer 1993a, b), and higher biochemical capacity (Wullschleger 1993).

We report evidence of an identical pattern of character association among genetic lines of a single species (Table 4). In *P. arenastrum*, life span is greatly affected by developmental variation in the node of first flowering. Genetic lines that begin flowering at an early node become limited in their ability to continue vegetative growth because they have committed meristems to the determinate flowering fate. As a result, they complete their life cycle early, well in advance of genotypes that delay flowering until an advanced node (Geber 1990). Early flowering genetic lines are also characterized by high rates of gas exchange, higher carbon isotope discrimination (Geber and Dawson 1990), and, according to this latest study, show a tendency for high biochemical capacity (Table 4).

One hypothesis for the association between life-span, gas exchange rates and carbon isotope discrimination at the inter-specific level is based on the idea that the continuum from short to long-lived species also represents a continuum from stress avoidance to stress tolerance (Grime 1979; Chapin 1980; Hoffman and Parsons 1991). One theme that emerges from both plant and animal studies is that low metabolic rates may improve stress tolerance by reducing the demand for resources. At the same time, low metabolic rates may be incompatible with stress avoidance which often requires that organisms cease activity or complete their life cycle in advance of an impending stress. This hypothesis may explain the observed negative correlation between metabolic rate and lifespan among taxa (Calder 1984; Hoffman and Parsons 1991).

In plants, low metabolic rates are reflected in low leaf photosynthetic rates, stomatal conductances to water vapor, and respiration rates. Ehleringer (1993a) has argued that the intercellular CO_2 concentration in plant leaves (ci) may be viewed as a "set point" for gas exchange metabolism, reflecting a complex balance between rates of photosynthesis (i.e, biochemical capacity) and water loss and use; high set points for gas exchange metabolism are reflected in high ci and, over the long-term, in high carbon isotope discrimination. The pattern of correlation between physiological and developmental (*viz* life history) traits across genetic lines of *P. arenastrum* is consistent with this metabolic hypothesis. The relative tolerance of genetic lines to drought stress, a common feature of this population's environment (Ehleringer et al. 1991) is currently being investigated. Preliminary evidence suggests that lines with slow development, longer life span, and lower gas exchange metabolism are indeed more tolerant of drought stress (M.A. Geber, unpublished work).

The significance of the correlation between morphology (*viz* leaf size) and physiology or development is less clear. Negative correlations between leaf size and gas exchange rates have been reported in crops and other plants (Chabot and Hicks 1982; Bhagsari and Brown 1986; Dudley 1996b). In some crops, negative correlations have been attributed to lower stomatal densities in larger-leaved varieties (Bhagsari and Brown 1986) or to differences in cell size and specific leaf weight. Data on *P. arenastrum* suggest, however, that larger-leaved genetic lines tend to have higher stomatal densities, higher specific leaf weights, and higher nitrogen contents per unit leaf area (M.A. Geber, unpublished work). Thus, the patterns for *P. arenastrum*, though weak, would lead to the prediction of higher gas exchange rates in larger-leaved lines, which is just the opposite of what is observed. It is possible that the greater leaf thickness (higher specific leaf weight) of larger leaved lines results in increased mesophyll resistance (r_{mes}) which then limits CO_2 influx to the sites of carboxylation and reduces A_{max}, in spite of the higher stomatal densities (Nobel 1991).

From a functional standpoint, it has been argued that smaller leaves may be favored in drier environments, because small leaves provide less surface area for transpirational water loss or because they have lower temperature (Givnish 1979; Nobel 1991; Dudley 1996a). We do not know, at present, whether the range of leaf sizes among lines of *P. arenastrum* is of sufficient magnitude (0.53–1.16 cm^2) to be of great functional significance. Furthermore, preliminary evidence suggests that lines with small leaves (and high gas exchange rates) are less tolerant of drought than large-leaved lines (see above).

Small leaf size in lines with rapid development may also reflect a developmental constraint. Guerrant (1988) has argued that rapid development may be achieved through a reduction in development time or in the size of primorida, and that this leads to smaller organ sizes (e.g., leaves) and smaller plant size.

Conclusions

This is the first study to demonstrate both genetic variation in gas exchange physiology and in the stomatal and biochemical mechanisms underlying photosynthetic variation in a population of a wild plant species. Genetic lines of *P. arenastrum* varied two-fold in average photosynthetic rates (A), stomatal conductance (g), stomatal limitation (L_s), and in Rubisco activity (Vc_{max}) and elec-

tron transport capacity (J_{max}). The average internal CO_2 concentration (ci) varied by 50 μl l^{-1} among lines.

Theoretical predictions that photosynthesis in C_3 plants should be co-limited by RuBP (Vc_{max}) and regeneration (J_{max}) (Farquhar and Sharkey 1982), and by stomatal relative to biochemical factors (Cowan 1986) were also supported by our data. Not only were there strong positive genetic correlations between Vc_{max} and J_{max}, and between g and biochemical traits, but a path analysis showed that genetic variation in both stomatal and biochemical traits accounted for large portions of the genetic variation in A and ci.

By comparing the gas exchange characteristics of genetic lines in the present study to their physiology in a previous field investigation, we were able to show that physiology remained consistent across studies and growth environments. In addition, we confirmed that lines with high (low) photosynthetic, stomatal conductance and ci are the same lines that had high (low) carbon isotope discrimination (Δ), rapid (slow) development to flowering and short (long) life span, as well as small (large) leaf size. The pattern of correlation between physiology and life span among genotypes of *P. arenastrum* parallels interspecific patterns of character correlations. We suggest that the range of trait constellations among lines in *P. arenastrum* represents a continuum between stress avoidance (rapid development, high gas exchange metabolism) to stress tolerance (slow development, low gas exchange metabolism), and that genetic variation in these character combinations may be maintained by environmental variation in stress levels in the species' ruderal habitat.

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References

Bavel CHM van, Lascano R, Wilson DR (1978) Water relations of fritted clay. J Soil Sci Soc Am 42:675–659

Bazzaz FA (1979) The physiological ecology of plant succession. Annu Rev Ecol Syst 10:351–271

Bhagsari AA, Brown RH (1986) Leaf photosynthesis and its correlation with leaf area. Crop Sci 26:127–132

Caemmerer S von, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376–387

Calder WA (1984) Size, function and life history. Harvard University Press, Cambridge

Cameron RG, Bassett CL (1988) Inheritance of C_4 enzymes associated with carbon fixation in *Flaveria* species. Plant Physiol 88:532–536

Chabot BF, Hicks DJA (1982) The ecology of leaf life spans. Annu Rev Ecol Syst 13:229–259

Chapin FS (1980) The mineral nutrition of wild plants. Annu Rev Ecol Syst 11:233–260

Comstock JP, Ehleringer JR (1992) Correlating genetic variation in carbon isotopic composition with complex climatic gradients. Proc Natl Acad Sci 89:7747–7751

Cowan IR (1986) Economics of carbon fixation in higher plants. In: Givnish TJ (ed) On the economy of plant form and function. Cambridge University Press, Cambridge, pp 133–177

Dawson TE, Bliss LC (1989) Intraspecific variation in the water relations of *Salix arctica*, an arctic-alpine dwarf willow. Oecologia 79:322–331

Dawson TE, Ehleringer JR (1993) Gender-specific physiology, carbon isotope discrimination, and habitat distribution in boxelder, *Acer negundo*. Ecology 74:798–815

Day TA, Heckathorn SA, DeLucia EH (1991) Limitations of photosynthesis in *Pinus taeda* L. (Loblolly pine) at low solid temperatures. Plant Physiol 96:1246–1254

Donovan LA, Ehleringer JR (1994) Potential for selection on plants for water-use efficiency as estimated by carbon isotope discrimination. Am J Bot 81:927–935

Dudley SA (1996a) Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. Evolution 50:92–102

Dudley SA (1996b) The response to differing selection on plant physiological traits: evidence for local adaptation. Evolution 50:103–110

Ehleringer JR (1993a) Gas-exchange implications of isotopic variation in arid-land plants. In: Smith JAC, Griffiths H (eds) Water deficits: plant responses from cell to community. Bios Scientific, pp 265–284

Ehleringer JR (1993b) Carbon and water relations in desert plants: an isotopic perspective. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon-water relations. Academic Press, San Diego, pp 155–172

Ehleringer JR (1993c) Variation in carbon isotope discrimination in *Encelia farinosa*: implications for growth, competition, and drought survival. Oecologia 95:340–346

Ehleringer JR, Cooper TA (1988) Correlations between carbon isotope and microhabitat in desert plants. Oecologia 76:562–566

Ehleringer JR, Werk KS (1986) Modifications of solar-radiation absorption patterns and implications for carbon gain at the leaf level. In: Givnish TJ (ed) On the economy of plant form and function. Cambridge University Press, Cambridge, pp 57–82

Ehleringer JR, Arnow LA, Arnow T, McNulty IB, Negus NC (1991) Red Butte Canyon research natural area: history, flora, geology, climate and ecology. Great Basin Nat 52:95–121

Emerman SH, Dawson TE (1996) The role of macropores in the cultivation of bell pepper in salinized soil. Plant and Soil 181:241–249

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78:9–19

Falconer DS (1989) Introduction to quantitative genetics. Longman, Essex

Farquhar GD, Caemmerer S von (1982) Modelling of photosynthetic response to environmental conditions. In: Lange O, Nobel PS, Osmond CV, Ziegler H (eds) Physiological plant ecology II. Water relations and carbon assimilation (Encyclopedia of plant physiology, new series, vol 12B). Springer, Berlin Heidelberg New York, pp 549–588

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Annu Rev Plant Physiol 33:317–345

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Mol Biol 40:503–537

Farris MA, Lechowicz MJ (1990) Functional interactions among traits that determine reproductive success in a native annual plant. Ecology 71:548–557

Flanagan LB, Jefferies RL (1988) Stomatal limitation of photosynthesis and reduced growth of the halophyte, *Plantago maritima* L., at high salinity. Plant Cell Environ 11:239–245

Geber MA (1989) The interplay of plant morphology and development on size inequality: a *Polygonum* greenhouse study. Ecol Monogr 59:267–288

Geber MA (1990) The cost of meristem limitation in *Polygonum arenastrum*: negative genetic correlations between fecundity and growth. Evolution 44:799–819

Geber MA, Dawson TE (1990) Genetic variation in and covariation between leaf gas exchange, morphology, and development in *Polygonum arenastrum*, an annual plant. Oecologia 85:153–158

Gimenez C, Mitchell VJ, Lawlow DW (1992) Regulation of photosynthetic rate of two sunflower hybrids under water stress. Plant Physiol 98:516–524

Givnish TJ (1979) On the adaptive significance of leaf form. In: Solbrig OT, Jain S, Johnson GB, Raven PH (eds) Topics in plant population biology. Cambridge University Press, New York, pp 171–214

Givnish TJ (1986) On the economy of plant form and function. Cambridge University Press, Cambridge

Grime JP (1979) Plant strategies and vegetation processes. Wiley, New York

Guerrant EO Jr (1988) Heterochrony in plants the intersection of evolution ecology and ontogeny. In: McKinney ML (ed), Heterochrony in evolution. Plenum, New York, pp 111–133

Gummuluru S, Hobbs SLA, Jana S (1989) Genotypic variability in physiological characters and its relationship to drought tolerance in durum wheat. Can J Plant Sci 69:703–712

Gurevitch J, Teeri JA, Wood AM (1986) Differentiation among populations of *Sedum wrightii* (Crassulaceae) in response to limited water availability: water relations, CO_2 assimilation, growth, and survivorship. Oecologia 70:198–204

Harley PC, Sharkey TD (1991) An improved model of C_3 photosynthesis at high CO_2: reversed O_2 sensitivity explained by lack of glycerate reentry into the chloroplast. Photosynthesis Res 27:169–178

Hobbs SLA, Mahon JD (1985) Inheritance of chlorophyll content, ribulose 1,5-bisphosphate carboxylase activity and stomatal resistance in peas. Crop Sci 25:1031–1034

Hoffmann AA, Parsons PA (1991) Evolutionary genetics and environmental stress. Oxford University Press, Oxford

Isebrands JG, Ceulemans R, Wiard B (1988) Genetic variation in photosynthetic traits among *Populus* clones in relation to yield. Plant Physiol Biochem 26:427–437

Kalisz S, Teeri J (1986) Population-level variation in photosynthetic metabolism and growth in *Sedum wrightii*. Ecology 67:20–26

Kingsolver JG, Schemske DW (1991) Path analyses of selection. Trends Ecol Evol 6:276–280

Kirschbaum MUF, Farquhar GD (1984) Temperature dependence of whole-leaf photosynthesis in *Eucalyptus pauciflora* Sieb. ex Spreng. Aust J Plant Physiol 11:519–538

Lechowicz MJ, Blais PA (1988) Assessing the contributions of multiple interacting traits to plant reproductive success: environmental dependence. Evol Biol 1:255–273

Mooney HA, Field C, Gulmon SL, Bazzaz FA (1981) Photosynthetic capacity in relation to leaf position in desert versus old-field annuals. Oecologia 50:109–112

Nelson CJ (1988) Genetic associations between photosynthetic characteristics and yield: review of the evidence. Plant Physiol Biochem 26:543–554

Nelson CJ, Sleper DA (1983) Using leaf-area expansion rate to improve yield of tall fescue. In: Smith JA, Hays VW (eds) Proceedings of the XIV International Grassland Congress. Westview, Boulder, pp 413–416

Nelson ND (1984) Woody plants are not inherently low in photosynthetic capacity. Photosynthetica 18:600–605

Ni B-R, Pallardy SG (1992) Stomatal and non-stomatal limitations to net photosynthesis in seedlings of woody angiosperms. Plant Physiol 99:1502–1508

Nobel PS (1991) Physiochemical and environmental plant physiology. Academic Press, San Diego

Pedhazur EJ (1982) Multiple regression in behavioral research. Holt, Rinehart and Winston, New York

Pereira JS, Tenhunen JD, Lange OL (1987) Stomatal control of photosynthesis of *Eucalyptus globulus* Labill. trees under field conditions in Portugal. J Exp Bot 38:1678–1688

Pyke KA, Leech RM (1985) Variation in ribulose 1,5-bisphosphate carboxylase content in a range of winter wheat genotypes. J Exp Bot 36:1523–1529

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Rocher JP, Prioul JL, Lecharny A, Reyss A, Joussaume M (1989) Genetic variability in carbon fixation, sucrose-p-synthesis and ADP glucose pyrophosphorylase in maize plants of differing growth rate. Plant Physiol 89:416–420

SAS Institute (1994) JMP Statistics and graphics guide. SAS Institute, Cary

Schulze E-D (1986) Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. Annu Rev Plant Physiol 37:247–274

Schuster WSF, Sandquist DR, Phillips SL, Ehleringer JR (1992a) Comparisons of carbon isotope discrimination in populations of aridland plants differing in lifespan. Oecologia 91:332–337

Schuster WSF, Phillips SL, Sandquist DR, Ehleringer JR (1992b) Heritability of carbon isotope discrimination in *Gutierrezia microcephala* (Asteraceae). Am J Bot 79:216–221

Sharkey TD (1985) Photosynthesis in intact leaves of C_3 plants: physics, physiology and rate limitations. Bot Rev 51:53–105

Sharkey TD, Seemann JR (1989) Mild water stress effects on carbon-reduction-cycle intermediates, ribulose bisphosphate carboxylase activity, and spatial homogeneity of photosynthesis in intact leaves. Plant Physiol 89:1060–1065

Smedley MP, Dawson TE, Comstock JP, Donovan LA, Sherill DE, Cook CS, Ehleringer JR (1991) Seasonal carbon isotope discrimination in a grassland community. Oecologia 85:314–320

Snedecor GW, Cochran WG (1989) Statistical methods. Iowa State University Press, Ames

Via S (1984) The quantitative genetics of polyphagy in an insect herbivore. II. Genetic correlation in larval performance within and among host plants. Evolution 38:896–905

Wong S-C, Cowan IR, Farquhar GD (1985) Leaf conductance in relation to rate of CO_2 assimilation. III. Influences of water stress and photoinhibition. Plant Physiol 78:830–834

Wong S-C, Cowan IR, Farquhar GD (1979) Stomatal conductance correlates with photosynthetic capacity. Nature 282:424–426

Woodrow IE, Berry JA (1988) Enzymatic regulation of photosynthetic CO_2 fixation in C_3 plants. Annu Rev Plant Physiol Mol Biol 39:533–594

Wullschleger SD (1993) Biochemical limitations to carbon assimilation in C_3 plants – a retrospective analysis of the A/ci curves from 109 species. J Exp Bot 44:907–920