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POTENTIAL FOR SELECTION ON PLANTS FOR WATER-USE EFFICIENCY AS ESTIMATED BY CARBON ISOTOPE DISCRIMINATION¹

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Water-use efficiency is thought to be related to plant performance and natural selection for plants in arid habitats, based on a general expectation that increased water-use efficiency is associated with decreased carbon gain and biomass accumulation. Using leaf carbon isotope discrimination (Δ) to determine integrated water-use efficiency, we estimated genetic variance for, and examined the relationships among Δ , biomass, and gas exchange characters for full-sibling families of the woody shrub, *Chrysothamnus nauseosus*, grown from seed collected at Tintic, Utah. In both well-watered greenhouse and common garden experiments, and water-limited common garden experiments, there were significant family differences for Δ , biomass, and morphological characters, indicating a potential for genetic change in response to selection. However, estimates of broad-sense heritabilities for Δ were low, indicating that the rate of change in response to selection would be relatively slow. This was consistent with the large amount of phenotypic plasticity observed for Δ as it differed with water treatment and year in the garden experiment. Phenotypically, aboveground biomass and Δ were negatively correlated within the well-watered treatments (i.e., more water-use efficient plants were larger), not correlated within the water-limited treatment, and positively correlated for combined well-watered and water-limited garden treatments, suggesting that variation in both photosynthetic capacity and stomatal limitation contribute to the variation in Δ . In contrast to the phenotypic correlations, genetic correlations for biomass and Δ were consistently negative within each treatment, and selection for higher water-use efficiency through low Δ for *C. nauseosus* plants in this population would tend to shift populations toward larger plants. For *C. nauseosus*, increased water-use efficiency is not necessarily associated with decreased carbon gain.

Instantaneous water-use efficiency has been defined at the leaf level as ratio of photosynthetic carbon gain to transpirational water loss (A/E), and at the whole plant level, transpiration efficiency is the biomass accumulated per total water consumed (W). Hereafter, both terms are referred to as water-use efficiency. At both the leaf and whole plant levels, plants are thought to be under selective pressure for water-use efficiency in water-limited environments (Cohen, 1970; Cowan, 1982; Passioura, 1982). There is a potential for lower water-use efficiency to be associated with greater carbon gain, and we have suggested that a low water-use efficiency may be advantageous for young or small establishing plants if it is associated with greater biomass accumulation (Donovan and Ehleringer, 1992; Sandquist et al., 1993). Larger plant size or biomass may reduce the probability of seedling and juvenile mortality resulting from water stress (Cook, 1979; Donovan, Mausberg, and Ehleringer, 1993; Donovan and Ehleringer, in press). In addition, reproductive output or fitness is often positively correlated with plant size or biomass (Solbrig and Solbrig, 1984; Farris and Lechowicz, 1990). In order to understand the potential for natural selection (genetic or microevolutionary change) with respect to water-use efficiency (A/E or W), it is useful to determine its relationship to biomass, in addition to determining if there is genetic variance for the trait.

Estimation of W is a labor-intensive process that involves measurement of total biomass accumulated and water consumed. A/E is a more easily determined ratio of the leaf level processes that predominately determine W , but respiratory carbon losses and nonstomatal water losses must also be considered when scaling up from the leaf A/E to whole plant W , and instantaneous measurements of A/E are not generally integrated. Since E is a function of g (stomatal conductance to water vapor) and ν (leaf-to-air vapor pressure deficit), A/E can be estimated by A/g under conditions where ν does not vary substantially, and by c_i/c_a (ratio of internal leaf to ambient CO_2 concentration, determined by A and g). Thus, the theoretical and experimentally confirmed relationships between carbon isotope discrimination (Δ) and c_i/c_a suggest that Δ can be used to provide estimates c_i/c_a , A/g , A/E , and then W (Farquhar, O'Leary, and Berry, 1982; Farquhar and Richards, 1984; O'Leary, 1988; Farquhar, Ehleringer, and Hubick, 1989). Hence, with consideration of factors such as leaf temperature, respiration, and nonstomatal water losses, Δ can be used to investigate genetic variation for water-use efficiency.

Genetic variation for Δ has been documented for non-agricultural and agricultural plants (e.g., Hubick, Farquhar, and Shorter, 1986; Condon, Richards, and Farquhar, 1987; Hubick and Farquhar, 1989; Martin et al., 1989; Geber and Dawson, 1990; Hall et al., 1990; Meinzer, Goldstein, and Grantz, 1990; Virgona et al., 1990; Johnson and Bassett, 1991; Schuster et al., 1992). A number of these studies have shown positive correlations between Δ and total or aboveground biomass for plants grown in a similar water treatment (Condon, Richards, and Farquhar, 1987; Farris and Lechowicz, 1990; Johnson et al., 1990; White, Castillo, and Ehleringer, 1990), where-

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TABLE 1. Statistics [overall means and ranges of family means, F values, and intraclass correlation coefficients (τ)] for characters measured on *Chrysothamnus nauseosus* grown in a well-watered greenhouse study; 45 families, average 23 replicates per family for morphological measures and six replicates per family for carbon isotope discrimination (Δ).

	Mean (\pm SD)	Range	F (sig.) ^a	τ (\pm SD)
Height (cm)	6.47 \pm 1.27	3.90–8.87	11.00***	0.303 \pm 0.051
Leaf length (cm)	3.47 \pm 0.35	2.55–4.13	6.27***	0.187 \pm 0.039
Shoot biomass (g)	0.13 \pm 0.04	0.05–0.23	11.55***	0.322 \pm 0.052
Leaf/shoot biomass (g)	0.70 \pm 0.05	0.57–0.78	5.42***	0.167 \pm 0.037
Total biomass (g)	0.33 \pm 0.08	0.15–0.50	10.71***	0.304 \pm 0.051
Shoot/total biomass	0.39 \pm 0.04	0.27–0.46	6.55***	0.203 \pm 0.041
Δ (‰)	22.54 \pm 0.41	21.71–23.48	2.31***	0.174 \pm 0.060

^a Significance levels for family effects: ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

as others have observed negative correlations (Hubick, Farquhar, and Shorter, 1986; Condon, Farquhar, and Richards, 1990; Virgona, 1992) or no significant correlations (Martin and Thorstenson, 1988; Read et al., 1992; Schuster et al., 1992). The relationship between Δ (and W) and biomass varies as a function of whether Δ is changing as a result of differences in photosynthetic capacity and/or stomatal limitation (Hubick, Farquhar, and Shorter, 1986; Virgona et al., 1990; Virgona, 1992). Investigations of genetic variation for Δ , and the relationship of Δ to biomass, for additional nonagricultural plants will contribute to the understanding of water-use efficiency in relation to growth and survival in natural populations.

This study investigated relationships between Δ , biomass and morphology, and gas exchange characters for the aridland habitat shrub *Chrysothamnus nauseosus* (Pallas) Britt. The specific objectives were to 1) examine the genetic and environmental components of variance for Δ and biomass, and 2) determine the relationships among Δ , biomass, and gas exchange characters.

MATERIALS AND METHODS

Seed source and parent plant leaf collections—The source population for *C. nauseosus* seed was the Tintic Range Experiment Station, Tintic, Utah (39°55'N, 122°03'W, elevation 1,775 m). *C. nauseosus* is reported to be predominately self-fertilized with occasional out-crossing (Anderson, 1966). Seeds were collected in the fall of 1989 from 45 reproductive plants that were greater than 1 m in height. The reproductive shrubs were located within a 1,750-m² area of sagebrush steppe habitat encompassing relatively uniform topography and soil type (Donovan and Ehleringer, 1992). Each parent plant was sampled (ten to 15 leaves combined) for determination of leaf carbon isotope composition in June and August of 1989, and again in June of 1990 and 1991.

Carbon isotope measurements—Carbon isotopic composition was measured on dried, ground leaf samples (Ehleringer and Osmond, 1989), using an isotope ratio mass spectrometer (delta S, Finnigan MAT, San Jose, CA). Carbon isotope ratios ($\delta^{13}\text{C}$) were calculated from isotope compositions, relative to the PeeDee Belemnite standard, and converted to carbon isotope discrimination (Δ , ‰) values, using atmospheric carbon dioxide values of -10‰ and -8‰ as measured for the greenhouse and garden environments, respectively.

Greenhouse experiment—The greenhouse study was conducted at the University of Utah, Salt Lake City campus (elevation 1,450 m), starting in November 1989. On 14 November, air-dried seeds from parent plants were placed in a mist bench on a mixture of one-third silica sand, one-third perlite, and one-third vermiculite. Seeds germinated within 7 d of planting, and seedlings were transplanted to 5-cm peat pots of greenhouse soil on 27 November. Greenhouse soil consisted of 4/8 commercial topsoil, 4/8 bark, 4/8 perlite, 3/8 vermiculite, 2/8 peat moss, and 1/8 silica sand. The design consisted of 45 families, with family defined as seeds collected from one parent plant in the field population, and a range of 12 to 40 replicate seedlings per family (Table 1). High intensity metal halide lamps were used to supplement sunlight, and light intensities (400–700 nm) were greater than 1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 12 hr per day. Potted seedlings were arranged haphazardly on greenhouse benches, and trays were rearranged on a weekly basis to minimize the effects of environmental patchiness. Although it was not a complete randomized design, we feel that the weekly rearrangements minimized the effects of localized environment in the greenhouse. Seedlings were well-watered (twice daily), and fans were used to circulate air in the greenhouse. CO_2 concentrations in the greenhouse averaged approximately 370 ppm, and daytime temperatures averaged approximately 26 C. All seedlings were assessed for height and length of longest leaf on 7 February, after 77 d of growth, and harvested for biomass of roots, stems, and leaves. Leaves from six replicate plants per family were analyzed for carbon isotope composition.

Garden experiment—The garden experiment was conducted outside at the University of Utah. Seedlings for the garden experiment were started from seed on 5 March 1990, using 25 families (randomly selected from the 45 used in the greenhouse study) and the same seed collections and germination conditions as for the greenhouse study. Seedlings were transplanted to individual growing containers on 29 March, and maintained in the greenhouse until the end of April. Seedlings were then acclimated in a cold frame until 23 May, when they were transplanted into the garden. The experimental design in the garden consisted of nine blocks that were randomly assigned to either well-watered (four blocks) or water-limited (five blocks) treatments. The unbalanced design for water treatments was chosen on the basis of greater expected mortality in the water-limited treatments. Each block contained four subplots, and one replicate of each

of the 25 families was randomly located in each subplot. Seedlings were planted 25 cm apart, and all blocks were well-watered for several weeks to alleviate transplant shock. On 19 June, the differential water treatments were started: well-watered blocks were irrigated twice a week, and water-limited blocks received no further irrigation. At this point in time, families were already significantly different for plant height, designated as initial height. The effects of differences in initial height on subsequent measurements were assessed as described in the data analysis and Results sections.

On 12 September, at the end of the first growing season, all plants were measured for height and number of stems greater than 5 cm, and leaves were collected for determination of carbon isotope composition. Just prior to morphological measurements, ecophysiological measurements (water potentials and gas exchange) were made on nine randomly selected families. Predawn xylem pressure potentials (Ψ_{pd}) were measured on one branch per plant for nine replicates per family with a pressure chamber (PMS Instruments, Corvallis, OR). Stomatal conductance (g) and net photosynthesis (A) were measured for leaves on intact branches with a portable gas exchange system (LI-6200, Li-Cor, Inc., Lincoln, NE). Measurements of daily course of gas exchange, on a subset of five plants per treatment, determined that maximum rates occurred between 8:30 and 10:00 a.m. local time. Measurements of maximum rates were then made on two consecutive days, from 8:30 to 10:00 a.m. local time, at light intensities greater than $1,700 \mu\text{mol m}^{-2} \text{s}^{-1}$, for six replicates per family in each water treatment. Leaf areas were determined with a Li-Cor 3100 area meter (Li-Cor, Inc., Lincoln, NE). The leaf-to-air vapor pressure gradient divided by total atmospheric pressure (ν) was calculated using leaf and air temperature inside the chamber and absolute humidity of air outside of the chamber, minimizing the potential error for humidity if it was not exactly balanced by the flow rate. Leaf temperatures during the gas exchange measurements differed by less than 2 C for plants in the two water treatments. Transpiration rate (E) was then calculated as the product of g and ν . After morphological measurements were made in September, three-fourths of the replicates for each family were harvested for determination of leaf and stem biomass.

To determine the stability of the characters and their relationships through time, plants not harvested after the first growing season were followed through the 1991 growing season. The well-watered treatments were irrigated every other week for June–August, and the water-limited treatments received no irrigation. On 9–13 September 1991, the end of the second season, plants were assessed for height, leaf length, leaf absorptance of photosynthetically active radiation or PAR (Ehleringer, 1981), above-ground biomass, and Δ .

Data analysis—Family effects for each character were analyzed with analysis of variance procedures using the JMP statistical software package (SAS Institute Inc., 1989). Variation among families are reported as intraclass correlation coefficients, τ , which express the among-family variance as a proportion of the total variance (Falconer, 1981; Sokal and Rohlf, 1981). Standard deviations for τ were calculated by the method of Falconer (1981). As-

suming that the progeny from each family were full-siblings (Anderson, 1966), broad-sense heritabilities were calculated as 2τ (Falconer, 1981).

Phenotypic and genetic correlations among characters were determined with Pearson's product moment correlation coefficients. Phenotypic correlations were calculated using data for individual plants in the analyses. Genetic correlations were calculated from family means (Geber, 1990; Schuster et al., 1992). Although genetic correlations can alternatively be calculated from analyses of variance and covariance (Via, 1984), several studies have found the results from these alternative methods for calculating genetic correlations to be similar (Geber, 1990; Schuster et al., 1992). Genetic correlations were not calculated with characters for which no significant family effects were detected. Many variables required transformations (log, square root, etc.) in order to approach the normality assumptions for the analysis of variance and correlation procedures. Normality was tested using Shapiro-Wilk W test in the JMP statistical package (SAS Institute, Inc., 1989). Variables were changed back to original scale for presentation of means.

For the garden experiments, families were significantly different for plant height, designated as initial height, at the initiation of the water treatments. These family differences in initial height could have been due to genetic family effects, which would appropriately be included in the estimates of genetic variation, and/or differences of environment prior to transplants, which would result in an overestimate of genetic variation. To assess the magnitude of this potential error, tests of family effects, and phenotypic and genotypic correlation matrices were calculated for both the actual character values, and character values with the effects of initial height removed (residual character values). The effects of initial height differences were removed through an analysis of covariance where actual character values were regressed against initial height, and the resulting residuals used as characters in subsequent analyses (Geber, 1990). Because leaf Δ and morphological characters were correlated with initial height in the first year of the garden study, family effects are presented for both actual character values and residual character values. A comparison of correlation matrices constructed using actual character values and residual character values indicated that the relationships were predominately of the same sign, magnitude, and significance, and only the matrices using the actual data are presented.

For the garden experiment, the water treatment effects were determined with a two-way analysis of variance procedure with water treatment and family as independent variables. The effect of year was determined with analysis of variance procedures that included year, water treatment, and family in the model.

RESULTS

Family effects—Families differed significantly for morphological characters, biomass, and Δ in the well-watered greenhouse study (Table 1) and in the well-watered and water-limited garden treatments in year one (Tables 2, 3). Intraclass correlation coefficients, τ , estimate among-family variance (Tables 1–3). For the greenhouse, 30% of the variation in biomass and 17% of the variation in Δ

TABLE 2. Statistics, [overall means and ranges of family means, F values, and intraclass correlation coefficients (τ)] for morphological and physiological characters measured on *Chrysothamnus nauseosus* grown in the well-watered garden treatment. F and τ values in parentheses represent analyses with residuals after effects of initial size removed (see Materials and Methods for distinction); carbon isotope discrimination (Δ), photosynthetic carbon assimilation (A), stomatal conductance to water vapor (g), ratio of internal leaf to ambient CO_2 concentration (c_i/c_a), predawn water potential (Ψ_{pd}), and photosynthetically active radiation (PAR).

	# Families, # Replicates	Means (\pm SD)	Range	F (sig.) ^a	τ (\pm SD)
1990					
Height, initial (cm)	25, 16	2.89 \pm 0.74	1.86–4.71	11.21***	0.408 \pm 0.079
Height, Sept. (cm)	25, 16	35.75 \pm 3.64	30.64–44.86	3.07*** (1.91**)	0.122 \pm 0.046 0.058 \pm 0.033
# Stems, Sept.	25, 16	28.80 \pm 8.40	15.73–46.84	2.37*** (1.86**)	0.089 \pm 0.040 0.056 \pm 0.032
Shoot biomass (g)	25, 12	10.87 \pm 4.12	5.58–22.12	2.34*** (1.99**)	0.108 \pm 0.044 0.083 \pm 0.038
Leaf/shoot biomass	25, 12	0.48 \pm 0.03	0.43–0.54	3.13*** (3.16***)	0.189 \pm 0.058 0.165 \pm 0.054
Δ (‰)	16, 12	22.10 \pm 0.27	21.58–22.58	2.06* (1.93*)	0.099 \pm 0.060 0.087 \pm 0.057
A ($\mu\text{mol m}^{-2} \text{sec}^{-1}$)	9, 6	26.18 \pm 1.69	23.74–28.99	0.55 ns	—
g ($\text{mol m}^{-2} \text{sec}^{-1}$)	9, 6	0.71 \pm 0.07	0.61–0.79	1.66 ns	—
c_i/c_a	9, 6	0.75 \pm 0.02	0.72–0.77	1.61 ns	—
Ψ_{pd} (MPa)	9, 9	-1.07 \pm 0.10	-0.95–-1.27	6.52***	0.390 \pm 0.148
1991					
Height, Sept. (cm)	25, 4	67.0 \pm 6.1	54.8–79.6	1.41 ns	—
Shoot biomass (g)	25, 4	122.1 \pm 66.3	44.1–355.3	1.57 ns	—
Leaf length (cm)	25, 4	5.50 \pm 0.50	4.5–6.5	1.18 ns	—
Leaf absorptance of PAR (%)	25, 4	0.65 \pm 0.03	0.58–0.69	1.80*	0.181 \pm 0.105
Δ (‰)	12, 4	20.43 \pm 0.75	19.17–21.5	2.03 ns	($P = 0.06$)

^a Significance levels for family effects: ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

could be attributed to family effects (Table 1). For the garden experiment, family effects are presented for each water treatment, even though there were no significant interactions between family and water treatment, because

correlations among biomass, Δ , and gas exchange characters differed in the treatments. For the garden experiments the F and τ values are presented both for the actual character values and for residual character values that

TABLE 3. Statistics [overall means and ranges of family means, F values, and intraclass correlation coefficients (τ)] for morphological and physiological characters measured on *Chrysothamnus nauseosus* grown in the water-limited garden treatment. F and τ values in parentheses represent analyses with residuals after effects of initial size removed (see Materials and Methods for distinction); carbon isotope discrimination (Δ), photosynthetic carbon assimilation (A), stomatal conductance to water vapor (g), ratio of internal to ambient leaf CO_2 concentration (c_i/c_a), predawn water potential (Ψ_{pd}), and photosynthetically active radiation (PAR).

	# Families, # Replicates	Means (\pm SD)	Range	F (sig.) ^a	τ (\pm SD)
1990					
Height, initial (cm)	25, 20	2.93 \pm 0.73	1.97–4.59	19.34***	0.489 \pm 0.078
Height, Sept. (cm)	25, 20	31.22 \pm 2.58	26.70–35.61	2.10** (2.07**)	0.054 \pm 0.028 0.053 \pm 0.028
# Stems, Sept.	25, 20	13.78 \pm 4.37	6.87–24.20	3.20*** (2.77***)	0.103 \pm 0.039 0.085 \pm 0.035
Shoot biomass (g)	25, 15	3.98 \pm 1.85	1.77–10.77	3.72*** (3.40***)	0.158 \pm 0.054 0.148 \pm 0.052
Leaf/shoot biomass	25, 15	0.36 \pm 0.03	0.30–0.44	2.04*** (2.08**)	0.071 \pm 0.037 0.070 \pm 0.037
Δ (‰)	16, 12	20.46 \pm 0.53	19.47–21.51	2.40*** (2.62*)	0.117 \pm 0.064 0.133 \pm 0.068
A ($\mu\text{mol m}^{-2} \text{sec}^{-1}$)	9, 6	26.61 \pm 2.22	22.43–29.35	0.69 ns	—
g ($\text{mol m}^{-2} \text{sec}^{-1}$)	9, 6	0.48 \pm 0.03	0.45–0.53	0.37 ns	—
c_i/c_a	9, 6	0.68 \pm 0.02	0.66–0.71	0.44 ns	—
Ψ_{pd} (MPa)	9, 9	-1.37 \pm 0.13	-1.55–-1.21	4.07***	0.254 \pm 0.133
1991					
Height, Sept. (cm)	25, 5	50.5 \pm 5.2	38.8–60.0	1.42 ns	—
Shoot biomass (g)	25, 5	50.2 \pm 5.2	38.7–59.9	1.42 ns	—
Leaf length (cm)	25, 5	5.06 \pm 0.45	4.12–6.06	0.95 ns	—
Leaf absorptance of PAR (%)	25, 5	0.59 \pm 0.03	0.50–0.66	1.39 ns	—
Δ (‰)	12, 4	18.53 \pm 0.34	17.89–18.89	0.64 ns	—

^a Significance levels for family effects: ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

TABLE 4. Phenotypic (above the diagonal) and genetic (below the diagonal) correlation matrix, and significance levels^a for morphological characters, biomass, and carbon isotope discrimination (Δ) for *Chrysothamnus nauseosus* in well-watered and water-limited treatments in year one of the garden study (see Table 2 for units and abbreviations [$N = 25$ families for morphological and biomass characters, $N = 16$ families for Δ]).

	Initial height	September height	September # stems	Shoot biomass	Leaf/shoot biomass	Δ
Well-watered						
Initial height	—	0.359***	0.265***	0.264**	-0.228***	-0.184*
September height	0.689***	—	0.487***	0.597***	-0.338***	-0.224**
September # stems	0.458*	0.497*	—	0.875***	-0.155*	-0.122 ns
Shoot biomass	0.369 ns	0.495*	0.869***	—	-0.152*	-0.272**
Leaf/shoot biomass	-0.381 ns	-0.569**	-0.104 ns	0.022 ns	—	-0.077 ns
Δ	-0.081 ns	-0.087 ns	-0.081 ns	-0.616*	-0.124 ns	—
Water-limited						
Initial height	—	0.203***	0.138***	0.223***	-0.167**	0.035 ns
September height	0.371 ns	—	0.463***	0.607***	-0.209**	0.059 ns
September # stems	0.442*	0.709***	—	0.833***	0.056 ns	0.142 ns
Shoot biomass	0.380 ns	0.712***	0.802***	—	0.016 ns	0.008 ns
Leaf/shoot biomass	-0.183 ns	-0.398*	-0.115 ns	0.049 ns	—	-0.219*
Δ	0.211 ns	-0.152 ns	-0.081 ns	-0.540*	-0.611*	—

^a Significance levels: ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

have the effects of initial height removed (see Materials and Methods for distinction). The analysis of the actual character values would tend to overestimate family effects because environmental effects on initial height would be included, and the analysis of residual character values would tend to underestimate family effects to the extent that genetic differences expressed prior to the initiation of the water treatment would be removed. Although a comparison of F and τ values for actual and residual characters did suggest that some environmental and/or family effects had been expressed prior to initiation of the water treatments, the differences were not substantial. For the garden, within each water treatment, less than 15% of the variation of both biomass and Δ could be attributed to family effects, regardless of whether τ values for actual or residual character values were used. Since progeny from a single family (parent shrub) were full-

siblings, broad-sense heritabilities (h^2) for Δ in the well-watered greenhouse, well-watered garden, and water-limited garden experiments were 0.35, 0.20, and 0.24, respectively (Falconer, 1981). Although h^2 for Δ can potentially be estimated from the regression of offspring (family mean) on parent character values, there were no significant correlations between family means (for either greenhouse or garden experiments) and parent plants in the field (on any date).

Significant family differences were also found for pre-dawn water potentials, but not for gas exchange characters in year one of the garden study (Tables 2, 3). Family effects were also not significant for morphological characters in year two of the garden study, with the exception of leaf absorptance of PAR in the well-watered treatment.

Correlations within water treatments—In the garden experiment, phenotypic and genotypic correlations among the estimates of size (canopy height, number of stems, and aboveground biomass) were positive within both the well-watered and the water-limited treatments (Table 4). The phenotypic correlation for shoot (leaf and stem) biomass and Δ was negative for the well-watered treatment (i.e., more water-use efficient plants were larger) and not significant for the water-limited treatment (Table 4; Fig. 1). Genetic correlations for shoot biomass and Δ were negative for both water treatments in the garden (Table 4). For the greenhouse treatment, the correlations were consistent with those for the garden well-watered treatment; negative phenotypic correlations of Δ with both shoot biomass ($P < 0.001$, $r = -0.32$, $N = 286$) and total (root and shoot) biomass ($P = 0.001$, $r = -0.19$, $N = 286$), and negative genetic correlations of Δ with both shoot biomass ($P < 0.001$, $r = -0.54$, $N = 45$ families) and total biomass ($P = 0.002$, $r = -0.45$, $N = 45$ families).

For the garden study, phenotypic correlations for the predawn water potentials, gas exchange characters, aboveground biomass, and Δ were calculated for the subset of families and individuals for which all characters had been evaluated (Table 5). Even with fewer numbers of families and numbers of individuals in this analysis, phenotypic correlations between biomass and Δ were consistent with

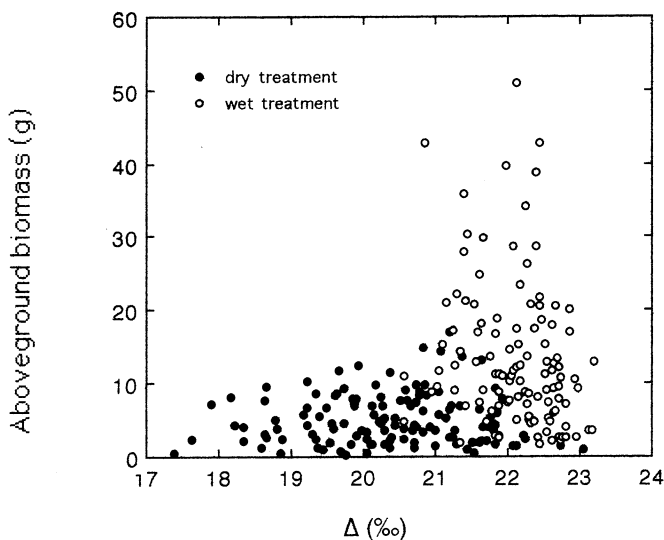


Fig. 1. The relationship between biomass and carbon isotope discrimination (Δ) for *Chrysothamnus nauseosus* in well-watered and water-limited treatments in the garden study. Actual data are plotted here, and statistics in the text are reported for these data transformed to meet normality assumptions (inverse hyperbolic sine).

those in Table 2 and 3; negatively correlated in the well-watered treatment and not correlated in the water-limited treatment. In both the well-watered and water-limited treatments, there was a significant positive correlation between c_i/c_a and Δ . As expected, E and g were positively correlated for both the well-watered ($P < 0.001$, $r = 0.77$, $N = 52$) and the water-limited treatments ($P < 0.001$, $r = 0.78$, $N = 51$), and A/E and A/g were positively correlated for both the well-watered ($P < 0.001$, $r = 0.72$, $N = 52$) and the water-limited treatments ($P < 0.001$, $r = 0.64$, $N = 51$). Thus, E and A/E are excluded from the correlation matrices for the sake of brevity.

In both the well-watered and water-limited treatments, Δ was positively correlated with c_i/c_a and g, but not with A (Table 5). In addition, c_i/c_a was positively correlated with g, but negatively correlated with A. This was somewhat surprising since A was positively correlated with g. The gas exchange data are plotted with A as a function of c_i (Fig. 2), and a model is proposed in the discussion. Ψ_{pd} was positively correlated with Δ only in the water-limited treatment.

Response to water treatments—*C. nauseosus* plants in the well-watered garden treatment were significantly different ($P < 0.01$) from plants in the water-limited treatment for all variables listed in Tables 2 and 3, with the exceptions of initial height and A, which were not significantly different. In 1990, families in the well-watered treatment had an aboveground biomass of 10.87 g and a Δ value of 22.10‰ as compared to the water-limited treatment with a biomass of 3.98 g and a Δ value of 20.46‰. Although the water-limited treatment Ψ_{pd} was only 0.3 MPa more negative at the time of the gas exchange measurements, the water-limited treatment g and c_i/c_a were 65% and 87% lower, respectively, than those of the well-watered treatment (Tables 2, 3). There were no significant interactions between water treatment and family for any characters.

Comparison of year one and year two in the garden—There were no significant interactions between year and family or water treatment for height, biomass, and Δ char-

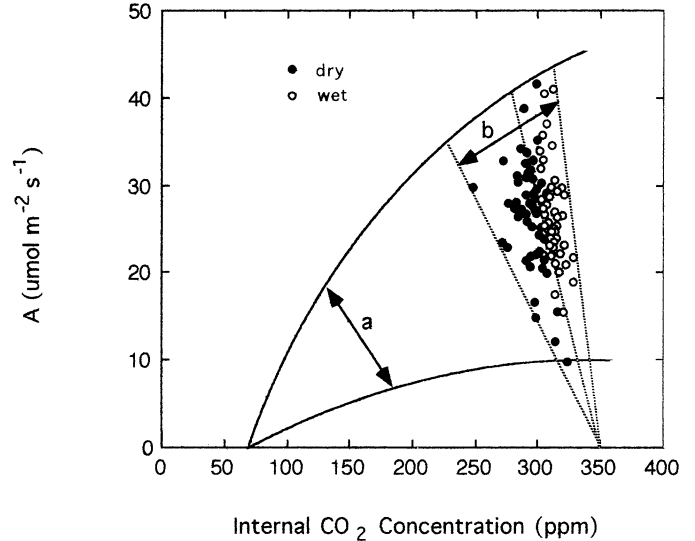


Fig. 2. A model representing the relationship of photosynthetic rate (A) to internal carbon dioxide concentration which includes the gas exchange data for *Chrysothamnus nauseosus* in the well-watered and water-limited treatments in the garden. The potential variation in photosynthetic rate is represented by the range of initial slopes (a). The range in stippled lines (b) represents the range in stomatal conductances, grouped by well-watered and water-limited treatments.

acters. Thus, family rankings for each character were maintained even though family effects were not significant in year two. This is supported by a significant correlation between Δ in 1990 and in 1991 ($r = 0.61$, $P < 0.001$, $N = 63$). The phenotypic relationships between biomass and Δ were also the same for year two, negatively correlated in the well-watered treatment ($r = -0.41$, $P = 0.006$, $N = 44$) and not correlated in the water-limited treatment ($r = 0.10$, $P = 0.514$, $N = 48$). For the individual plants measured for Δ in both years of the study, Δ was significantly more negative the second year. Plants in the well-watered treatment shifted from a mean of 21.96 to 20.49‰ ($F = 47.61$, $P < 0.001$), and plants in the water-limited treatment shifted from a mean of 20.30 to 18.49‰ ($F = 87.50$, $P < 0.001$).

TABLE 5. Phenotypic correlations and significance levels^a for xylem pressure potentials, gas exchange characters, biomass, and carbon isotope discrimination (Δ) for *Chrysothamnus nauseosus* in well-watered and water-limited treatments in year one of the garden study. See Table 2 for character abbreviations and units ($N = 8$ families with six to 12 replicates per family).

	Shoot biomass	Ψ_{pd}	A	g	c_i/c_a	Δ
Well-watered						
Initial height	0.351*	-0.035 ns	-0.105 ns	-0.189 ns	-0.179 ns	-0.095 ns
Shoot biomass	—	0.245 ns	-0.033 ns	-0.241 ns	-0.257 ns	-0.436**
Ψ_{pd}		—	0.016 ns	-0.021 ns	-0.064 ns	-0.016 ns
A			—	0.568***	-0.352**	0.085 ns
g				—	0.484***	0.433**
c_i/c_a					—	0.403**
Water-limited						
Initial height	0.119 ns	0.049 ns	-0.043 ns	-0.038 ns	-0.052 ns	0.172 ns
Shoot biomass	—	0.069 ns	0.222 ns	0.169 ns	-0.198 ns	0.040 ns
Ψ_{pd}		—	0.040 ns	0.265 ns	0.261 ns	0.384***
A			—	0.698***	-0.457***	-0.046 ns
g				—	0.273*	0.417**
c_i/c_a					—	0.549***

^a Significance levels: ns = not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

DISCUSSION

In this experiment, leaf Δ was positively correlated with c_i/c_a , as expected from theory (Farquhar, Ehleringer, and Hubick, 1989) and shown for a number of species. Hence we used Δ as an estimate of c_i/c_a and A/g . Also, leaf temperature did not vary substantially within and across water treatments, so that Δ provided a reasonable integrated estimate of A/E (Ehleringer, Phillips, and Comstock, 1992). For this study, discussions of differences in Δ or c_i/c_a or A/g or A/E refer to the same basic parameter or physiological set-point that is determined by the interaction of photosynthetic capacity and the stomatal limitation to photosynthesis.

Genetic variation in Δ —Genetic variation for Δ has previously been documented for agricultural and nonagricultural plants (see introduction for references). For *C. nauseosus*, we found significant family effects, and hence genetic variation, for Δ , biomass, and other morphological characters, assuming no significant maternal environment effects. Based on a full-siblings relationship among each family (progeny from one parent plant), broad-sense heritabilities (h^2) for Δ are estimated at 0.35, 0.20, and 0.24, for the well-watered greenhouse, well-watered garden, and water-limited garden experiments, respectively. Broad-sense heritabilities include the additive effects of genes, as well as dominance and epistatic effects. All of these factors (i.e., total genetic variation between families) determine the potential for a population of an inbred species (such as would result from continual self-fertilization) to respond to selection, rather than just additive genetic variation (Falconer, 1981; Geber, 1990). Thus, broad-sense h^2 provides a reasonable upper limit estimate for potential response of *C. nauseosus* to selection. Maternal environment effects on Δ would decrease estimates of h^2 , but they have been found to be relatively small for other species (Hubick, Shorter, and Farquhar, 1988; M. Brick, Colorado State University, Fort Collins, CO, personal communication).

For *C. nauseosus*, the broad-sense h^2 estimates range from 0.20 to 0.35 for Δ , and appear to be relatively low when compared to the broad-sense h^2 estimates of 0.81 and 0.92 for *Gutierrezia microcephala* (Asteraceae), an out-crossing shrub that co-occurs with *C. nauseosus* (Schuster et al., 1992). Our estimates of h^2 for Δ were higher for the greenhouse as compared to the garden, but were similar for the two water treatments in the garden. For plants such as crested wheatgrass (Johnson et al., 1990) estimates of h^2 have also been found to differ with environmental conditions. On the other hand, similar h^2 estimates for well-watered and water-limited treatments have been found for cowpea plants (h^2 of 0.76, Hall et al., 1990), and for wheat (h^2 of approximately 0.72, Ehdaie et al., 1991). In all, we did find significant family differences for Δ in each environment, suggesting that there was genetic variation upon which the process of natural selection could act. However, the low estimates of τ and h^2 suggest that the potential rate of change in response to selection would be relatively slow.

Biomass and Δ —Biomass is the character in this study that most closely approximates fitness. We have suggested

that a higher Δ (lower water-use efficiency) might be advantageous for young or small, establishing plants in natural populations, if it were associated with increased biomass accumulation (i.e., positive correlation between Δ and biomass) and greater access to soil moisture (Donovan and Ehleringer, 1991, 1992; Sandquist et al., 1993). In this study, phenotypic correlations between biomass and Δ depended on the environment; negative in the well-watered greenhouse and garden treatments (less water-use efficient plants were smaller), not significant in the water-limited garden treatment, and positive for combined well-watered and water-limited treatments (an environmentally induced relationship). However, genetic correlations between biomass and Δ were negative within all of the treatments. The potential for a change in Δ in response to selection on biomass would be dependent on the genetic correlations between Δ and biomass, which were consistently negative in these experiments. For *C. nauseosus*, selection for greater biomass or size would be associated with lower Δ (greater water-use efficiency), contrary to our previous selective arguments.

The relationships between biomass and Δ have been reported to vary in strength and sign, even when data were restricted to those comparing individuals of one species and in one environment (see introduction). Mechanistically, the different relationships are generated by variation in photosynthetic capacity and/or stomatal limitation (Hubick, Farquhar, and Shorter, 1986; Virgona et al., 1990). A negative relationship between Δ and biomass occurs when variation in photosynthetic capacity is more prevalent than stomatal limitation (Virgona, 1992). Although we do not have direct measures of either photosynthetic capacity or stomatal limitation for *C. nauseosus*, the expected variations in photosynthetic capacity (based on the relationships between Δ and biomass) are consistent with the instantaneous gas exchange measurements from the garden (Table 5; Fig. 2). Figure 2 is a plot of the photosynthetic rate plotted as a function of internal CO_2 concentration (c_i) where ambient CO_2 (c_a) is 350 ppm, and a hypothetical model superimposed over the data. The model assumes a CO_2 compensation point of approximately 70 ppm and assumes that each gas exchange point lies either at or below the point where A is limited by RuBP regeneration (sensu Farquhar and Sharkey, 1982). Photosynthetic capacities can be visualized at the slopes of the model, i.e., from the CO_2 compensation point to each data point (Fig. 2). Within each treatment, variation in photosynthetic capacity is suggested by negative correlations between A and c_i/c_a (Table 5; Fig. 2) and the range of initial slopes in the model (Fig. 2). Also within each treatment, positive correlations between A and g suggest that stomatal limitation also contributes to variation in c_i/c_a and Δ (Ehleringer, 1990; Ehleringer et al., 1990).

Well-watered treatment plants had the same A but greater aboveground biomass than water-limited treatment plants. This apparent lack of a relationship between carbon uptake (A) and biomass accumulation could result from two sources. First, instantaneous A measured at one point during the growing season may not reflect the long-term pattern. However, the instantaneous c_i/c_a values (calculated from A and g for the well-watered and water-limited treatments) match the independent estimates of

c_i/c_a from Δ and hence support our relative measurements of Δ . Second, plants in the water treatments may have had similar photosynthetic rates but different allocation patterns. If plants in the well-watered treatment partitioned more photosynthate to leaves early in the study, then the greater leaf area would have resulted in a compound interest effect of overall carbon gain even if rates per unit leaf area were not different (Hubick and Farquhar, 1989).

Plasticity for Δ —The differences in Δ values for the water treatments and the differences in Δ values for year one and two demonstrate that there is substantial phenotypic plasticity for this character. Plants in the water-limited garden treatment had Δ values that were approximately 1.6‰ more negative, on average, than those in the well-watered treatment. This difference in Δ is predominately due to lower rates of g in the water-limited environment (Tables 2, 3, 5; Fig. 2), suggesting increased predominance of stomatal limitation, and is consistent with the positive relationship between Δ and biomass when data for both treatments are combined (Fig. 1). Values of Δ values both in the well-watered and water-limited treatments were approximately 1.5‰ more negative, on average, in the second year of the study as compared to the first year. This shift in Δ as plants become taller and accumulate more biomass corroborates the negative relationship between Δ and size found for the population of *C. nauseosus* that included all of the parent plants for this study (Donovan and Ehleringer, 1992). Although these relationships suggest the potential for a developmental or ontogenetic shift in Δ (Donovan and Ehleringer, 1992), a more likely explanation is variation in environmental factors, such as nitrogen, that would affect photosynthetic capacity. An understanding of the role of Δ and water-use efficiency in aridland plant populations will require further investigation of the relationship between growth and the functional control of Δ (photosynthetic capacity and stomatal limitation) under various environmental regimes.

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