

Phenotypic constraints and community structure: Linking trade-offs within and among species

Amy L. Angert,^{1,2} Sarah Kimball,³ Megan Peterson,⁴ Travis E. Huxman,^{3,5} and David L. Venable⁶

¹Departments of Botany and Zoology, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

²E-mail: amy.angert@botany.ubc.ca

³Center for Environmental Biology, University of California, Irvine, California 92697

⁴Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, California 95064

⁵Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697

⁶Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721

Received August 19, 2013

Accepted July 29, 2014

Trade-offs are central to many topics in biology, from the evolution of life histories to ecological mechanisms of species coexistence. Trade-offs observed among species may reflect pervasive constraints on phenotypes that are achievable given biophysical and resource limitations. If so, then among-species trade-offs should be consistent with trade-offs within species. Alternatively, trait variation among co-occurring species may reflect historical contingencies during community assembly rather than within-species constraints. Here, we test whether a key trade-off between relative growth rate (RGR) and water-use efficiency (WUE) among Sonoran Desert winter annual plants is apparent within four species representing different strategies in the system. We grew progeny of maternal families from multiple populations in a greenhouse common garden. One species, *Pectocarya recurvata*, displayed the expected RGR–WUE trade-off among families within populations. For other species, although RGR and WUE often varied clinally among populations, among-family variation within populations was lacking, implicating a role for past selection on these traits. Our results suggest that a combination of limited genetic variation in single traits and negative trait correlations could pose constraints on the evolution of a high-RGR and high-WUE phenotype within species, providing a microevolutionary explanation for phenotypes that influence community-level patterns of abundance and coexistence.

KEY WORDS: Community assembly, G-matrix, genetic correlation, growth rate, stress tolerance, water-use efficiency, winter annual plants.

The concept of trade-offs is an organizing principle that integrates many areas of ecology and evolution. Trade-offs are invoked to explain everything from resource partitioning and predator–prey dynamics (Tilman 1994; Rees et al. 2001; Amarasekare 2003) to the evolution of behaviors and life histories (Stearns 1992; Werner and Anholt 1993; Wolf et al. 2007). Ecologists have hypothesized that many of the same trade-offs that shape life histories, behaviors, and growth strategies also affect species interactions, coexistence, and community structure (Grubb 1977; Chesson and Huntly 1988; Tilman and Pacala 1993; Geritz et al. 1999; Turnbull

et al. 1999; Rees et al. 2001; Suding et al. 2003; Vasseur et al. 2011). Similarly, trade-offs are described at many different levels of biological organization, from genetic trade-offs within species (Herms and Mattson 1992; Stearns 1992; Robinson et al. 1996; Lankford et al. 2001) to broad comparisons across species and habitats (Reich et al. 1999; Fine et al. 2004; Schiesari et al. 2006; Mooney et al. 2010).

Missing trait combinations in interspecific comparisons are often interpreted as evidence of deeper constraints operating within species that are set by biophysical and allocational



Table 1. Summary of ANCOVA for each trait (water-use efficiency, WUE and relative growth rate, RGR).

Trait	Effect	<i>F</i> /cov	df	<i>P</i>
WUE	Species	19.48	3, 8	0.0005
	Germination date	1.79	1, 131	0.1827
	Transplant date	0.70	1, 131	0.4052
	Population	0.07	1	0.0001
	Seed family	0	1	1.0000
RGR	Species	37.54	3, 8	<0.0001
	Germination date	1.24	1, 262	0.2660
	Transplant date	40.66	1, 262	<0.0001
	Population	0.01	1	0.3360
	Seed family	0.05	1	0.2681

Species was treated as a fixed effect. Date of germination and date of transplant were included as covariates. Population (nested within species) and seed family (nested within population) were included as random effects. Analysis is restricted to three sites from which we collected all four species and thus includes a subset of the populations that are used in single-species analyses. Significance of random effects was tested using likelihood ratio tests with 1 degree of freedom (df). We report *F*-values for fixed effects and covariance parameter estimates (cov) for random effects. Statistically significant values ($P < 0.05$) are indicated in bold.

constraints on phenotypes (Grime 1979; Tilman 1988; Vincent et al. 1996; Rees et al. 2001; Suding et al. 2003; Bonsall et al. 2004; Kneitel and Chase 2004). If this view is correct, then trade-offs should appear fractal, remaining evident as one zooms in on ever-finer levels of biological organization. However, mechanisms other than phenotypic constraints can give rise to apparent trade-offs at higher levels. For example, phenotypes may be missing from a community not because of phenotypic constraints but because species with those trait combinations are unavailable in the regional species pool or cannot stably persist in the local community (Belyea and Lancaster 1999; Weiher et al. 2011; Ricklefs 2012). Alternatively, phenotypes may be missing because particular trait combinations are not favored by natural selection (“selective constraints” sensu Arnold 1992). Few studies have explicitly linked constraints within species to the diversity of strategies among coexisting species (Tessier et al. 2000; Dudley et al. 2007; Rees and Venable 2007)

In a literature largely isolated from community ecology, evolutionary biologists have discussed how to define and quantify phenotypic constraints and their underlying sources at genetic, developmental, or physiological levels (Antonovics and Van Tienderen 1991; Houle 1991; Arnold 1992; Zera and Harshman 2001). We use the hierarchical conceptual framework proposed by Arnold (1992), in which developmental and functional constraints affect patterns of mutational input (i.e., the spectrum of mutations that are possible) and hence the pattern of genetic (co)variation for a set of traits. Phenotypic evolution could be limited

simply by a lack of additive genetic variation in a trait or set of traits. Although standing genetic variation is usually plentiful (Lewontin 1974; Gomulkiewicz and Houle 2009), Blows and Hoffman (2005) reviewed a number of cases where additive genetic variation in ecologically important traits is surprisingly low. When considering multiple traits, constraints can arise from the pattern of genetic covariation for a set of traits, which is the product of mutational input, linkage disequilibrium, and pleiotropy from shared developmental pathways or coupled physiological processes (Arnold 1992; Conner 2002). Although phenotypic correlations determine the pattern of trait variation that is exposed to selection, the underlying genetic variances and covariances determine the direction and rate of response to selection. Because recombination can reduce all but the strongest linkage disequilibrium within several generations, genetic covariation due primarily to linkage disequilibrium may not present a long-term phenotypic constraint (Falconer and Mackay 1996). An integration of ecology and evolutionary biology would benefit from evaluations of trade-offs where knowledge about the genetic architecture of traits is robust, or at minimum, functional hypotheses for pleiotropy as the cause of a negative association between two traits are justifiable (Zera and Harshman 2001).

Here, we use quantitative genetics to test whether a key trade-off among species is apparent within species using an experimentally tractable and well-studied group of winter annual plants native to the Sonoran Desert. By focusing on distantly related species (members of four genera from three families) that span the range of phenotypes observed in this community, we can examine whether within-species constraints limit the distribution of phenotypic variation upon which selection and community assembly can act. Sonoran Desert winter annuals are highly variable in abundance from year to year (Venable 2007). Although years with high precipitation during the growing season tend to favor all winter annuals, species also exhibit reversals in relative performance from year to year (Kimball et al. 2012). Temporally decoupled population dynamics promote species coexistence via the storage effect, and appear to be related to differences among species for a few key functional traits (Angert et al. 2009).

To understand the functional trait differences that foster coexistence, our previous work has examined traits that affect resource uptake and allocation in the nine most common species (representing seven genera from five families) and that predict variation in environmental responses and population dynamics (Angert et al. 2007, 2010; Huxman et al. 2008, 2013; Kimball et al. 2010, 2011, 2012; Gremer et al. 2012; Barron-Gafford et al. 2013). Species differ greatly in relative growth rate (RGR; Angert et al. 2007) and in leaf carbon isotope discrimination (Huxman et al. 2008), which is inversely related to integrated water-use efficiency (WUE; Dawson et al. 2002). Across species, RGR trades-off with WUE: the most water-use efficient species display the lowest

Table 2. Summary of univariate ANOVA for trait variation within each of the four study species.

Position	Species	Trait	Parameter	Population	Germdate	Transdate	Family
High RGR/low WUE	<i>S. micropoides</i>	WUE	<i>F/cov</i>	1.08	–	–	0
			df	5, 131	–	–	1
			<i>P</i>	0.3719	–	–	1.0000
		RGR	<i>F/cov</i>	1.71	0.39	–	0.13
			df	5, 139	1, 324	–	1
			<i>P</i>	0.1372	0.5333	–	0.0674
<i>E. lanosum</i>	WUE	<i>F/cov</i>	0.24	–	–	0.06	
		df	4, 69.9	–	–	1	
		<i>P</i>	0.9142	–	–	0.8072	
	RGR	<i>F/cov</i>	1.63	–	–	0.15	
		df	4, 103	–	–	1	
		<i>P</i>	0.1714	–	–	0.1209	
Low RGR/high WUE	<i>P. recurvata</i>	WUE	<i>F/cov</i>	9.57	–	0.29	0.27
			df	4, 87.8	–	1, 154	1
			<i>P</i>	<0.0001	–	0.5898	0.0001
		RGR	<i>F/cov</i>	5.33	16.51	–	0.12
			df	4, 139	1, 287	–	1
			<i>P</i>	0.0005	<0.0001	–	0.0395
<i>E. texanum</i>	WUE	<i>F/cov</i>	4.41	–	–	0.16	
		df	4, 62.1	–	–	1	
		<i>P</i>	0.0034	–	–	0.1674	
	RGR	<i>F/cov</i>	0.58	0.57	–	0	
		df	4, 226	1, 226	–	1	
		<i>P</i>	0.6787	0.4517	–	1.0000	

Species are ordered according to their previously described position along a trade-off between relative growth rate (RGR) and water-use efficiency (WUE) (Angert et al. 2007; Huxman et al. 2008). Each model included a fixed effect of population and a random effect of seed family nested within population. If supported by the Akaike information criterion, models also included date of germination (Germdate) or date of transplant (Transdate) as a covariate. Significance of fixed effects was tested using Satterthwaite's approximation for denominator degrees of freedom. Significance of random effects was tested using likelihood ratio tests. We report *F*-values for fixed effects and covariance parameter estimates (cov) for random effects. Statistically significant values ($P < 0.05$) are indicated in bold.

growth rates, especially when compared to high-RGR species under conditions of abundant resource availabilities. Studies of phenotypic selection in the field indicate that natural selection favors phenotypes with high values of both RGR and WUE, suggesting that this among-species trade-off exists due to some type of genetic constraint (Kimball et al. 2013). The trade-off appears to manifest in part because species with high RGR can rapidly increase leaf area following periods of high rainfall, when soil moisture persists long enough to allow sufficient carbon gain to offset water loss from large, thin leaves (Angert et al. 2007, 2010). Because periods of high rainfall are rare, species with high RGR suffer relatively lower fitness in dry years that have predominantly small rainfall events (Kimball et al. 2012). Although plasticity in RGR is quite high, species-level differences in WUE are more stable across years (S. Kimball, unpubl. data). Species with high WUE have high leaf nitrogen and photosynthetic characteristics that promote carbon gain at low temperatures (Huxman et al. 2008; Gremer et al. 2012; Barron-Gafford et al. 2013). This allows them to capitalize on small, but relatively frequent, rainfall

events, when it is substantially cooler than during dry prestorm periods (Huxman et al. 2008). But, this incurs performance costs, particularly at high temperatures, because high leaf nitrogen is associated with high respiration rates and thus lower conversion efficiency of photosynthetic assimilates to biomass (Ryan 1995; Amthor 2000; Turnbull et al. 2003; Barron-Gafford et al. 2013).

The overarching hypothesis of this study is that genetic constraints, driven by the functional constraints described above, hinder the construction of a phenotype with both high RGR and high WUE. Thus, we predicted a negative genetic correlation between RGR and WUE within species. We examined genetic correlations at two nested levels within species: among populations and among families within populations. Because genetically based differences in traits within populations are the raw material for evolutionary change, trait correlations among families are an important level of investigation. However, it is also possible that genetic variation in fitness-related traits within populations will be low, hindering statistical detection of a trade-off among families. In this case, evolutionary divergence among populations

Table 3. Summary of linear regressions relating variation in water-use efficiency (WUE) or relative growth rate (RGR) among populations within each species to either latitude or longitude of origin.

Effect	Response	Species	<i>b</i>	ddf	<i>F</i>	<i>P</i>
Latitude	WUE	<i>S. micropoides</i>	0.1708	50	4.33	0.0426
		<i>E. lanosum</i>	-0.0540	54	0.36	0.5536
		<i>P. recurvata</i>	0.1496	78	2.13	0.1489
		<i>E. texanum</i>	0.0716	58	0.54	0.4645
	RGR	<i>S. micropoides</i>	-0.0402	161	0.47	0.4955
		<i>E. lanosum</i>	0.0397	81	0.27	0.6052
		<i>P. recurvata</i>	0.0107	142	0.03	0.8724
		<i>E. texanum</i>	-0.0849	95	1.55	0.2163
Longitude	WUE	<i>S. micropoides</i>	-0.1855	50	5.11	0.0281
		<i>E. lanosum</i>	0.0360	54	0.15	0.6971
		<i>P. recurvata</i>	0.2406	78	5.83	0.0181
		<i>E. texanum</i>	0.0640	58	0.43	0.5161
	RGR	<i>S. micropoides</i>	0.0164	160	0.08	0.7833
		<i>E. lanosum</i>	-0.0652	81	0.73	0.3953
		<i>P. recurvata</i>	0.1603	143	6.37	0.0127
		<i>E. texanum</i>	0.0766	95	1.12	0.2927

We report the linear regression coefficient (*b*), denominator degrees of freedom (ddf), *F*-ratio (*F*), and *P*-value (*P*). Statistically significant values (*P* < 0.05) are indicated in bold.

might provide a more accurate representation of long-term genetic constraints. For example, if populations from cooler, drier environments have evolved higher WUE, we would expect to see correlated decreases in RGR. Trait correlations among families and among populations should be congruent if evolutionary divergence is constrained by genetic covariances that remain consistent over time. To increase our power for detecting trade-offs within species, we sampled populations across gradients with approximately threefold variation in winter precipitation and approximately 5°C variation in mean winter temperature. We conducted the study with plentiful soil moisture and nutrients so that individuals could reach their maximum growth potential, allowing us to detect the trade-off between growth potential and tolerance of low resources. Our specific objectives, questions, and hypotheses are as follows:

1. Examine the relative rankings of species' trait values when grown in a common environment: Do phenotypes in a simplified environment mirror phenotypes in the field? We hypothesized that the previously observed trade-off between RGR and WUE among species would be evident under the uniformly high-resource conditions of the greenhouse.
2. Determine how genetic variation is partitioned among families and populations: Do populations and families differ in RGR and WUE, and are population differences related to climate of origin? We hypothesized that populations from cooler, drier environments would have higher WUE and lower RGR than populations from warmer, wetter environments.
3. Estimate intraspecific covariation in RGR and WUE: Is there evidence for a genetically based trade-off within species (within or among populations)? We hypothesized that RGR and WUE would negatively covary within species, posing a genetic constraint on the construction of a phenotype with both high RGR and high WUE.

We are not aware of any previous studies that have examined genetic constraints within multiple, co-occurring species. The results of this study shed light on the microevolutionary processes that shape functional trait diversity within a community and influence patterns of abundance and coexistence.

Methods

STUDY SPECIES

We selected four species for this study based on their positions along the previously documented trade-off between RGR and WUE and their abundance during the year of collection: *Eriophyllum lanosum* (Asteraceae; high RGR, low WUE), *Stylocline micropoides* (Asteraceae; high RGR, low WUE), *Pectocarya recurvata* (Boraginaceae; low RGR, high WUE), and *Erodium texanum*

Table 4. Summary of multivariate regressions relating variation in water-use efficiency (WUE) or relative growth rate (RGR) among populations of each species to three climatic variables (for the winter months January–March, the average maximum temperature (Tmax_wt), average minimum temperature (Tmin_wt), and average cumulative precipitation (PPT_wt)).

Species	Response	Effect	b	ddf	F	P
<i>S. micropoides</i>	WUE	PPT_wt	-0.1637	50	1.97	0.1669
		Tmax_wt	-0.1299	50	2.27	0.1382
		Tmin_wt	-0.0189	50	0.02	0.8752
	RGR	PPT_wt	-0.1169	161	2.08	0.1509
		Tmax_wt	0.0219	161	0.13	0.7197
		Tmin_wt	-0.1192	161	2.25	0.1360
<i>E. lanosum</i>	WUE	PPT_wt	0.0151	54	0.01	0.9160
		Tmax_wt	0.0381	54	0.10	0.7550
		Tmin_wt	-0.0142	54	0.02	0.9026
	RGR	PPT_wt	-0.2882	81	5.89	0.0175
		Tmax_wt	0.2356	81	5.10	0.0266
		Tmin_wt	-0.1067	81	1.34	0.2496
<i>P. recurvata</i>	WUE	PPT_wt	0.2445	77	3.97	0.0499
		Tmax_wt	-0.1956	77	4.43	0.0386
		Tmin_wt	-0.1020	77	0.87	0.3551
	RGR	PPT_wt	0.2590	142	10.67	0.0014
		Tmax_wt	-0.0774	142	1.53	0.2188
		Tmin_wt	0.0623	142	0.65	0.4209
<i>E. texanum</i>	WUE	PPT_wt	0.0623	58	0.23	0.6312
		Tmax_wt	-0.1890	58	2.61	0.1115
		Tmin_wt	-0.1987	58	3.58	0.0634
	RGR	PPT_wt	0.1307	95	1.83	0.1796
		Tmax_wt	-0.0552	95	0.45	0.5047
		Tmin_wt	0.0268	95	0.12	0.7272

Statistically significant values ($P < 0.05$) are indicated in bold.

(Geraniaceae; low RGR, high WUE). This nomenclature is consistent with previous work on this system; synonymy and currently accepted nomenclature are given in Kimball et al. (2011). *Eriophyllum lanosum* makes relatively large, showy inflorescences and is self-incompatible (Mooring 2002). *Erodium texanum* exhibits variable floral morphology, ranging from showy, magenta flowers to flowers that lack petals and self-pollinate in the bud (A. Angert, unpubl. data). *Pectocarya recurvata* has diminutive, white, nonshowy flowers and is highly self-fertilizing (S. Kimball, unpubl. data). *Stylocline micropoides* is fully autogamous and does not produce open flowers (S. Kimball, unpubl. data).

SOURCE MATERIAL

Five or six populations of each species were sampled from southern Arizona and southeastern California (Fig. 1, Table S1). We extracted climatic variables, averaged over the period 1961–1990, for each site based on its latitude and longitude using the Climate WNA database (Wang et al. 2012). From each population, we collected seeds from up to 30 maternal plants (i.e., families; exact sample sizes in Table S2). For a subset of two to four populations from all species except self-incompatible *E. lanosum*, we also collected living seedlings in the field and transported them back to the University of Arizona greenhouse, where the plants were allowed to grow to reproductive maturity, self-fertilize, and produce seed. In a separate experiment conducted in parallel with the main experiment, we examined the potential for maternal environmental effects by comparing growth rates between plants grown from field-collected versus greenhouse-generated seeds (Appendix S1).

PLANT PROPAGATION

In December 2008, three field-collected seeds per family were sown on 1.5% agar in Petri dishes. Upon germination, seedlings were transplanted to 2.5 cm diameter × 16 cm deep conical pots (Ray Leach “Cone-tainers,” Steuwe & Sons, Inc., Tangent, OR) containing a mixture of 55% potting soil, 30% sand, and 15% vermiculite and transferred to the Colorado State University Greenhouse. The greenhouse ranged between 18°C and 22°C with a 16/8 h photoperiod. Because the species germinate after different lengths of time following seed sowing, species were sown in reverse order of germination speed to minimize variation among individuals in age (SD of germination date = 1.9 days, SD of transplant date = 1.3 days). Nonetheless, to preserve sample sizes and balance in the experimental design, late-germinating individuals were included. This caused germination to span 14 days and transplanting to take place over 10 days. We recorded date of germination and date of transplant as potential covariates for the analyses (see below). One week after initial transplant, when the bulk of individuals had been transplanted, we randomized

individuals among 33 racks, each holding a maximum of 96 pots. Pots were watered to field capacity daily and fertilized weekly to sustain the high-resource conditions in which the trade-off between RGR and WUE is likely to be observed (Angert et al. 2007, 2010; Huxman et al. 2008; Kimball et al. 2012).

TRAIT MEASUREMENT

Growth analysis using harvests of individuals over time showed that differences among species in whole-plant RGR are largely driven by differences in leaf area allocation (Angert et al. 2007, 2010) and the photosynthetic gains derived from this strategy. It is impossible to measure a single individual’s RGR from biomass directly because it requires destructive sampling. Thus, here we estimated individual RGR by measuring total leaf area at three time points: 7 January, 21 January, and 4 February 2009. Because of the large number of plants and the need to make measurements within a short period of time, we took digital images including a size standard to estimate total plant leaf area using the software ImageJ 1.43u (Abràmoff et al. 2004).

To describe relative change in leaf area over time, we compared linear models to Ricker models, which allow for a decrease in growth rate through time. Leaf area was log-transformed. Models were fit in R 2.9.2 using maximum-likelihood estimation in the *bbmle* package (Bolker and Team 2012) for Ricker models and ordinary least squares using the *lm* function for linear models. The Akaike information criterion was lower for the linear model than the Ricker model, so we used slope of ln-transformed size versus time as the estimate of RGR for each individual.

Leaf carbon isotopes were measured from dried tissue collected prior to the onset of reproduction for most individuals. Due to cost constraints, we obtained carbon isotope data for subsamples of 15 families per population per species. Samples were analyzed for carbon isotope ratios (δ) at the University of New Hampshire Stable Isotope Laboratory on a mass spectrometer (DELTAPlus XP, Thermo Fisher Scientific, Waltham, MA) interfaced to an elemental analyzer (model ECS4010, Costech Analytical Technologies Inc., Valencia, CA). Isotope ratios were converted to discrimination (Δ , ‰) according to the formula $\Delta = (\delta_a - \delta_p)/(1 + \delta_p/1000)$, where δ_a is the carbon isotope ratio of CO₂ in the air and δ_p is the carbon isotope ratio of the leaf tissue (Farquhar et al. 1989). The isotopic composition of the air was estimated from air samples taken within the greenhouse during the time of leaf development. Net discrimination against ¹³C varies with both photosynthetic demand for CO₂ and stomatal regulation of CO₂ availability, hence Δ is an estimate of the time-integrated concentration of CO₂ within the leaf relative to the atmosphere (C_i/C_a). C_i/C_a is directly related to WUE when plants experience similar respiratory and atmospheric evaporative demands (Ehleringer et al. 1993), which can be reasonably assumed for these greenhouse-grown plants. As such, Δ is inversely related

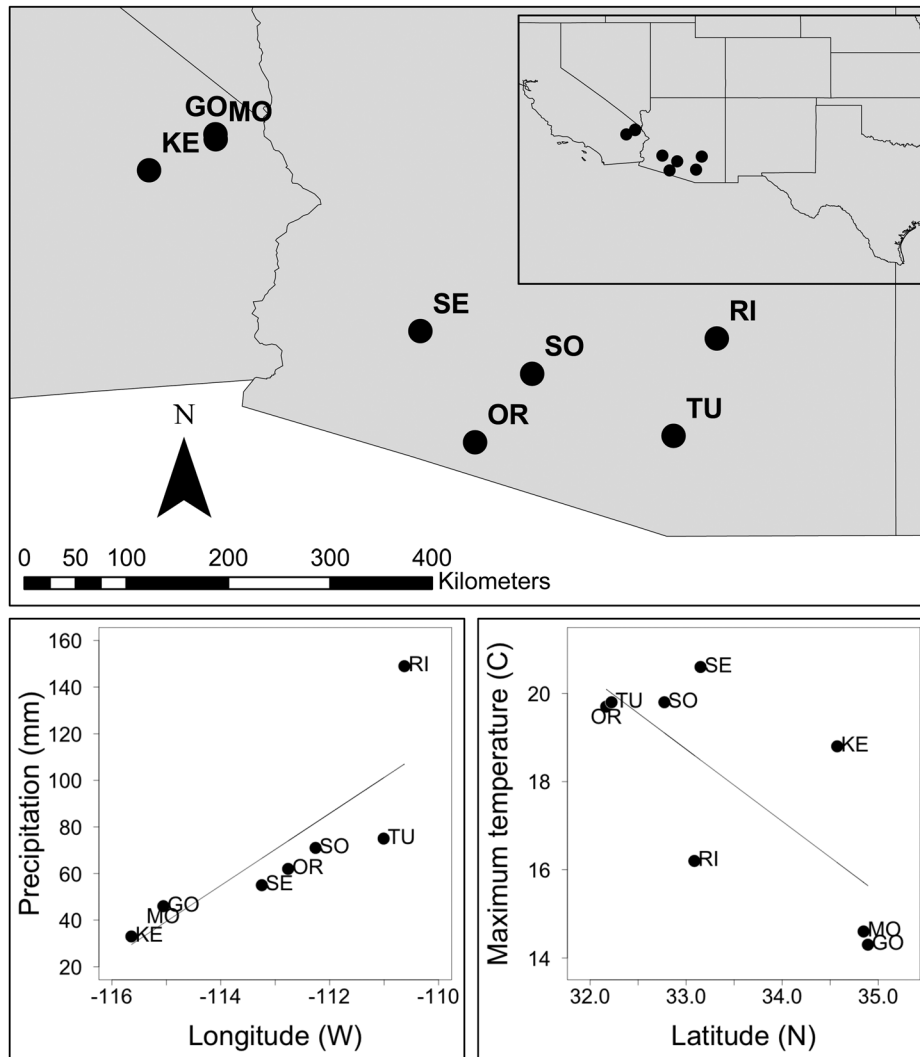


Figure 1. Map of population collection sites (top panel) encompassing a longitudinal gradient in average winter precipitation (bottom left) and a latitudinal gradient in average winter maximum temperature (bottom right). Population codes as follows: GO, Goff, California; KE, Kelbaker Road, California; MO, Mountain Springs Road, California; OR, Organ Pipe National Monument, Arizona; RI, Rincon Mountains, Arizona; SE, Sentinel, Arizona; SO, Sonoran Desert National Monument, Arizona; TU, Tumamoc Hill, Arizona.

to WUE, such that lower values of Δ indicate higher WUE. We multiplied Δ estimates by -1 so that a trade-off between RGR and WUE results in a negative correlation. Hereafter, we refer to $-\Delta$ as WUE.

STATISTICAL ANALYSIS

We used univariate analysis of variance (ANOVA) to partition variation in both traits (WUE and RGR) among species, populations, and families. To test for differences among species, we ran ANOVA with a fixed effect of species, two covariates (day of germination and day of transplant), and random effects of family nested within population and population nested within species. Population was treated as a random effect because we restricted this analysis to an unplanned set of sites from which all

four species could be sampled (TU, SO, OR; abbreviations as in Table S1). To test for differences among populations and families within each species, we ran models separately for each species and trait with a fixed effect of population and a random effect of family nested within population. These models also included day of germination or day of transplant as covariates when they improved model fit (i.e., differences in Akaike information criterion > 2 ; Burnham and Anderson 2010). Prior to each analysis, we standardized the phenotypic distributions of each trait to a mean of 0 and an SD of 1 by subtracting the grand mean and dividing by the SD of a particular dataset (e.g., for the among-species analysis, the grand mean across all species; for each within-species analysis, the grand mean across all populations of a given species). Models were fit using restricted maximum likelihood

(ReML) in Proc Mixed, SAS 9.3 (SAS Institute Inc., Cary, NC). *F*-tests for fixed effects used the Satterthwaite approximation for denominator degrees of freedom (Satterthwaite 1946). We constructed a planned contrast between least squares means estimates for the two putatively high RGR/low WUE species (*E. lanosum* and *S. micropoides*) versus the two putatively low RGR/high WUE species (*P. recurvata* and *E. texanum*). To test the significance of random effects, we used likelihood ratio tests with 1 degree of freedom to compare models with and without the random effect.

We partitioned total phenotypic variation in each trait into fractions attributable to variance among populations (V_{pop}), variance among families within populations (V_{fam}), and variance among individuals within families (V_{res}). Because population was treated as a fixed effect, V_{pop} was calculated as the variance among best linear unbiased estimates (BLUE) of population means. Variance among families is an estimate of $\frac{1}{2}$ to $\frac{1}{4}$ of the average additive genetic variation within populations, although it is a maximum estimate because it may be inflated by maternal effects and by dominance effects. For the highly self-fertilizing species *S. micropoides* and *P. recurvata*, V_{fam} estimates are likely to be closer to $\frac{1}{2}$ of the average additive genetic variation because the majority of individuals within families are full siblings. For the self-incompatible species *E. lanosum*, the V_{fam} estimate is likely to be closer to $\frac{1}{4}$ of the additive genetic variation because individuals within families are half-siblings, and for *E. texanum*, which appears to have a mixed mating system, estimates will be somewhere between $\frac{1}{2}$ and $\frac{1}{4}$ of the additive genetic variation. Thus, the degree of relationship among individuals within families varies unavoidably across species in the study, but it best reflects the structure of genetic variation within natural populations; for example, creating outcrossed families for highly selfing species would likely inflate estimates of genetic variation.

To relate trait variation among populations within each species to environmental variables, we first tested for latitudinal or longitudinal clines in each trait using Proc Reg in SAS 9.3. Latitude and longitude were examined separately because of collinearity ($r = -0.78$). We then tested for relationships with climatic variables (obtained for each population as described above) using multiple regressions. After exploring correlations among candidate climatic variables, we selected three with low collinearity (all $|r| < 0.70$; Table S3): winter (December–February) maximum temperature, winter minimum temperature, and winter precipitation. Separate models were fit for each species and trait. Family was included as a random effect to avoid pseudoreplication. The covariates, day of germination or day of transplant, were included as necessary as described above.

Following Colautti and Barrett (2011), we modeled variance and covariance in RGR and WUE with a fixed effect of population, a random effect of seed family nested within population, and repeated measurements (i.e., of multiple traits) of individuals

within families using Proc Mixed in SAS 9.3. We used “solution” statements to obtain BLUE of population means and best linear unbiased predictions (BLUP) of family means. These models used ReML to estimate the **G**-matrix, or the average (co)variance matrix among seed family means within populations, and the **R**-matrix, the residual (co)variance within seed families. We evaluated the significance of genetic correlation estimates in two ways. First, we used the Delta method (Lynch and Walsh 1998) to calculate the SE of each correlation. We report confidence intervals as ± 1.96 SE. Due to large SE, these confidence intervals are sometimes truncated at ± 1 . Second, we conducted a likelihood ratio test of a model with an unconstrained **G**-matrix and a model with the covariance between RGR and WUE constrained to be zero. To estimate the **D**-matrix, or the average (co)variance matrix among population means within species, we calculated variance and covariance among BLUE because population was treated as a fixed effect (cf. Colautti and Barrett 2011). We report confidence intervals around among-population correlations that were obtained from Fisher’s *r*-to-*z* transformation using the “fisher” option in Proc Corr, SAS 9.3 (SAS Institute Inc.).

Results

VARIATION AMONG SPECIES

Species differed in WUE and RGR (Table 1, Fig. 2), with rankings that were largely consistent with prior expectations based on field observations. RGR of *E. texanum* (a low-RGR/high-WUE species in prior work; Angert et al. 2007; Huxman et al. 2008) and WUE of *S. micropoides* (a high-RGR/low-WUE species in our prior work) were both higher than expected, but the other species rank orders were as predicted. Furthermore, the planned contrast of least squares mean estimates for *S. micropoides* and *E. lanosum* (high RGR/ low WUE) versus least squares mean estimates for *P. recurvata* and *E. texanum* (low RGR/ high WUE) was significantly different in the predicted directions for both traits (WUE, $F_{1,8} = 6.66$, $P = 0.0326$; RGR, $F_{1,8} = 7.69$, $P = 0.0242$; Fig. 2).

VARIATION AMONG POPULATIONS WITHIN SPECIES

Significant variation among populations in RGR or WUE was not detected by ANOVA for either high-RGR species, *E. lanosum* or *S. micropoides* (Table 2). One or both traits exhibited significant variation among populations for the high-WUE species, *P. recurvata* and *E. texanum* (Table 2). To further explore trait differentiation among populations, we examined relationships between traits and spatial (Table 3) or climatic (Table 4) predictor variables. Collection sites encompassed a longitudinal gradient in winter precipitation, with precipitation increasing toward the east ($b = 15.44$, $R^2 = 0.67$, $P < 0.05$) and a latitudinal gradient in winter temperature, with temperatures decreasing toward the north ($b = -1.64$, $R^2 = 0.54$, $P < 0.05$; Fig. 1). For *S. micropoides*,

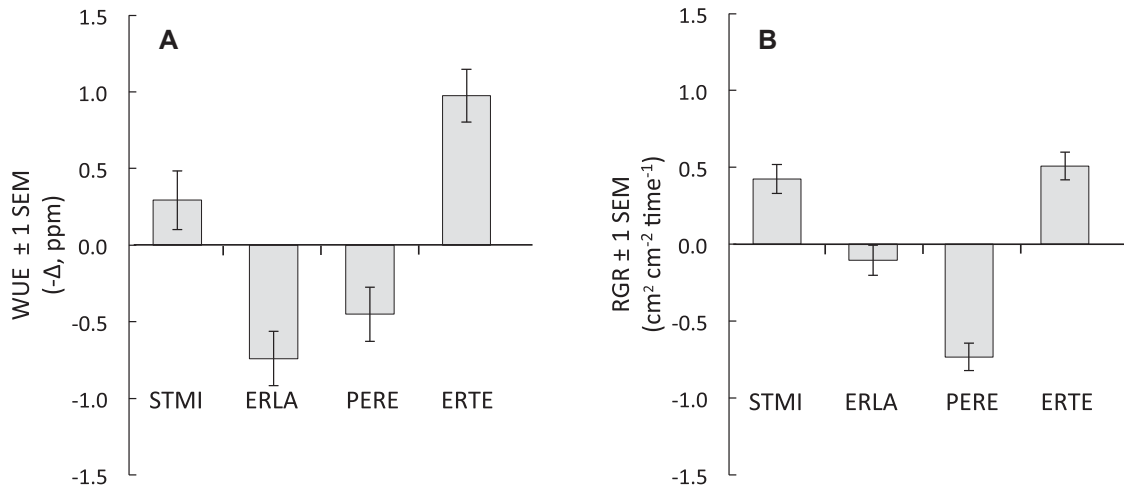


Figure 2. Least squares mean estimates of (A) water-use efficiency (WUE, measured as the inverse of carbon isotope discrimination, Δ) and (B) relative growth rate (RGR) for each species. Species are ordered based on positions along a previously documented trade-off in the field between high RGR (left) and high WUE (right). Species abbreviations are as follows: STMI, *S. micropoides*; ERLA, *E. lanosum*; PERE, *P. recurvata*; and ERTE, *E. texanum*.

a high-RGR species, WUE increased with increasing latitude (Table 3). WUE decreased with increasing winter temperatures for the low-RGR species, *P. recurvata* and *E. texanum* (Table 4), consistent with the latitudinal trends for *S. micropoides*. Together, these patterns suggest that WUE tends to be greater in populations from colder, northern sites. Variation in WUE was also related to population longitude (Table 3) and to winter precipitation (Table 4) for *S. micropoides* and *P. recurvata*, although in opposite ways for these two species. For *S. micropoides*, WUE was greater for western populations (Table 3). In contrast, WUE of *P. recurvata* increased to the east and with increasing winter precipitation (Tables 3 and 4). For *E. lanosum*, RGR was positively related to winter maximum temperature (Table 4). However, RGR was not significantly related to latitude for any species (Table 3). RGR increased toward more eastern sites (Table 3) and with increasing precipitation (Table 4) for *P. recurvata*, whereas RGR decreased with increasing precipitation for *E. lanosum* (Table 4).

VARIATION AMONG FAMILIES WITHIN POPULATIONS

RGR and WUE varied significantly among seed families within populations for only one species, the low-RGR/high-WUE *P. recurvata* (Table 2). Variance component estimates for high-RGR species tended to be higher for families than for populations, whereas the opposite was true for high-WUE species (Table 5). Estimates of broad-sense heritability ranged from 0 (WUE of *S. micropoides* and RGR of *E. texanum*) to 0.71 (WUE of *P. recurvata* and *E. texanum*) (Table 5).

COVARIATION BETWEEN RGR AND WUE AMONG POPULATIONS WITHIN SPECIES (D-MATRICES)

Estimates of the correlation between WUE and RGR among populations of *S. micropoides* and *E. texanum* were numerically

Table 5. Proportion of phenotypic variance partitioned among populations within species (V_{pop}), among seed families within populations (V_{fam}), and residual variation among individuals within families (V_{res}) for water-use efficiency (WUE) and relative growth rate (RGR).

Species	Trait	V_{pop}	V_{fam}	V_{res}	H^2
<i>S. micropoides</i>	WUE	0.0450	0	0.9568	0
	RGR	0.0338	0.1286	0.8376	0.2662
<i>E. lanosum</i>	WUE	0.0109	0.0615	0.9301	0.2400
	RGR	0.0413	0.1467	0.8120	0.6121
<i>P. recurvata</i>	WUE	0.2908	0.2500	0.4592	0.7050
	RGR	0.0958	0.1259	0.7783	0.2784
<i>E. texanum</i>	WUE	0.1585	0.1504	0.6991	0.7084
	RGR	0.0130	0	0.9870	0

Average within-population broad-sense heritabilities (H^2) are calculated as $4 \times V_{fam}/(V_{fam} + V_{res})$ for the outcrossing species *Eriophyllum lanosum* and *Erodium texanum* and as $2 \times V_{fam}/(V_{fam} + V_{res})$ for the highly selfing species *Pectocarya recurvata* and *Stylocline micropoides*. For each trait, variance components are obtained from univariate models and are standardized to sum to 1.

negative, consistent with a trade-off between RGR and WUE, but confidence intervals around these correlations contained 0 (Table 6, Fig. 3). The opposite was true for *E. lanosum* and *P. recurvata*: the correlation between RGR and WUE tended to be positive, consistent with genetic variation in the ability to acquire limited resources, but again these correlations were not significantly different from 0.

COVARIATION BETWEEN RGR AND WUE AMONG FAMILIES WITHIN POPULATIONS (G-MATRICES)

Due to estimates of zero variance in WUE among families of *S. micropoides* and in RGR among families of *E. texanum* (Table 5;

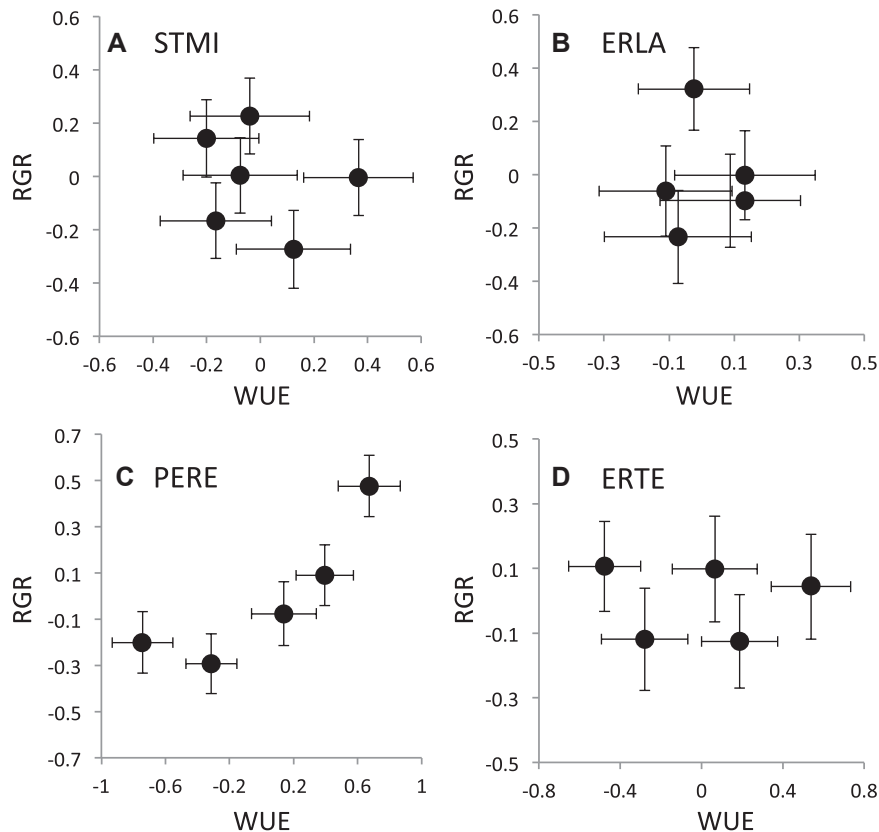


Figure 3. Best linear unbiased estimates of water-use efficiency (WUE, measured as the inverse of carbon isotope discrimination) and relative growth rate (RGR) for populations of (A) *S. micropoides* (STMI), (B) *E. lanosum* (ERLA), (C) *P. recurvata* (PERE), and (D) *E. texanum* (ERTE).

Table S4), family-level correlations between RGR and WUE were not estimable for these species. The estimates of genetic correlation for *E. lanosum* and *P. recurvata* were both strongly negative (Table 6). For *E. lanosum*, this estimate was truncated at the bounds of the distribution and had wide confidence intervals (Table 6). For *P. recurvata*, the negative correlation was significantly different from zero according to both the confidence interval calculation and likelihood ratio test (Table 6).

Discussion

We examined genetic variation within each of several, coexisting species to test whether genetic constraints shape an important community-level trade-off between growth capacity and tolerance of low resources. Within species, we found that RGR and WUE often varied clinally among populations, but genetic variation among families within populations was often undetectable. Population differentiation along latitudinal and longitudinal gradients of climate suggests the action of past natural selection. Yet, there appears to be little standing genetic variation within populations, likely hindering response to continued selection for three of the four species studied here. These results suggest that

the predominant ongoing constraint in this community is a lack of standing genetic variation in single traits. Even when genetic variation exists for particular traits, covariation between them may be antagonistic to the action of natural selection (Blows and Hoffman 2005). For the fourth species, despite significant standing genetic variation in RGR and WUE, genetic correlation estimates revealed a strong trade-off between RGR and WUE that would hinder response to selection for simultaneously high values of both of these traits, at least over the short term. Indeed, natural selection appears to favor an unobserved phenotype with high RGR and high WUE within natural populations of all four of these species (Kimball et al. 2013). Nonetheless, we do not observe native species that attain both high RGR and high WUE in the field, suggesting that pervasive genetic constraints such as those described here could be hindering further response to selection.

LOW STANDING GENETIC VARIATION IN TRAITS WITHIN MOST SPECIES

Limited standing genetic variation in RGR or WUE could pose an evolutionary constraint on attaining a high RGR and high WUE phenotype, independent of the relationship between WUE and

Table 6. Correlations between water-use efficiency (WUE) and relative growth rate (RGR).

Species	D	G
<i>S. micropoides</i>	−0.47 (−0.92, 0.58)	–
<i>E. lanosum</i>	0.21 (−0.83, 0.92)	−1.00 (−24.74, 20.57)
<i>P. recurvata</i>	0.57 (−0.67, 0.96)	−0.99* (−1.91, −0.06)
<i>E. texanum</i>	−0.73 (−0.98, 0.50)	–

Among-population correlations are calculated from variances and covariances among best linear unbiased estimates of standardized population means (i.e., from the D-matrix). Among-family correlations are estimated by restricted maximum likelihood estimates of genetic variances and covariances (i.e., from the G-matrix). Confidence intervals around correlations among population means were estimated from Fisher's *r*-to-*z* transformation using the "fisher" option in SAS Proc Corr. Confidence intervals around correlations among family means were calculated as ± 2 SEs, estimated by the Delta method (Lynch and Walsh 1998). Results from likelihood ratio tests of models with unconstrained G-matrices compared to models with the covariance between RGR and WUE constrained to be zero are given following the genetic correlation estimate (* $P < 0.05$).

RGR. We detected few significant differences among families within populations, in contrast to the frequently significant differences among populations within species (as revealed by gradients in trait values with respect to latitude, longitude, temperature, and precipitation; but see Table 2). Only for *P. recurvata* did we detect significant among-family variation in RGR and WUE. Interestingly, this is the only species for which we detected a strong, significant negative genetic correlation between RGR and WUE. The among-family component of phenotypic variance was not significant for any other species, and was even estimated to be zero for WUE of *S. micropoides* and RGR of *E. texanum*. Although C isotopes are reliable indicators of differences in WUE between species (Ehleringer et al. 1993), they might be less reliable for smaller differences within species because differences of a few ppm can be caused by factors unrelated to C_i/C_a (Warren and Adams 2006), potentially leading to type 2 errors (i.e., failure to detect genetic variation when it exists due to a low signal: noise ratio). Nonetheless, other greenhouse studies have detected genetic variation in WUE using C isotopes and similar levels of replication as our design (Condon et al. 1990; Donovan and Ehleringer 1994; Olivas-Garcia et al. 2000; Anyia et al. 2007). Maternal environmental effects, which we could not account for, could spuriously increase estimates of additive genetic variance, so it is particularly interesting that several species had little apparent among-family variation. Fitness components and fitness-related traits often exhibit low heritabilities because

selection on them reduces additive genetic variance and/or because of a greater influence of environmental variance (Fisher 1930; Kimura 1958; Price and Schluter 1991; Merilä and Sheldon 1999). Genetically based differences among populations and low standing variation within populations are both consistent with the action of past and ongoing natural selection on these key functional traits.

STRONG, NEGATIVE GENETIC CORRELATION BETWEEN RGR AND WUE FOR THE ONLY SPECIES WITH SIGNIFICANT GENETIC VARIATION IN BOTH TRAITS

Genetic correlations between RGR and WUE among families within populations reveal the structure of standing genetic covariance on which selection can act, while the magnitude of a genetic correlation that is antagonistic to the direction favored by natural selection determines the degree to which adaptive evolutionary responses might be slowed. For the two species for which we could estimate among-family genetic correlations (*P. recurvata* and *E. lanosum*), both estimates were strongly negative and antagonistic to phenotypic selection in the field (Kimball et al. 2013). However, it is equally important to note that a complete lack of detectable genetic variation in one or the other trait, even under uniform greenhouse conditions, precluded calculation of a genetic correlation for the other two species. As is often the case in studies of genetic correlation (Lynch and Walsh 1998), confidence intervals surrounding the estimates were very wide, and for only one species (*P. recurvata*) was the correlation significantly different from zero. Greater numbers of families and individuals per family will be required to estimate genetic correlations with greater precision and confidence, perhaps requiring a future focus on fewer study species. Although bivariate correlations must be very high to pose long-term evolutionary constraints on their own, as the dimensionality of the multivariate phenotype increases, the strength of pairwise correlations necessary to pose an evolutionary constraint decreases (Blows and Hoffman 2005). Our previous work has uncovered a suite of functional traits that contribute to the emergent trade-off between RGR and WUE (Angert et al. 2007, 2010; Huxman et al. 2008; Kimball et al. 2011; Gremer et al. 2012). It remains possible that weak correlations among these lower level traits constrain the joint evolution of RGR and WUE.

CONCEPTUAL AND LOGISTICAL CONSIDERATIONS WHEN ESTIMATING GENETIC CORRELATIONS

The utility of G-matrices for revealing evolutionary constraint has been questioned (Houle 1991; Pigliucci 2006) because factors such as inbreeding depression (Rose 1984) can lead to spuriously high estimates of correlation, while known allocation trade-offs might not result in strong negative genetic

correlations (Charlesworth 1990; Houle 1991). Maternal environmental effects can also impact correlation estimates, for example, by leading to spurious positive correlations when different families experience “good” versus “bad” environments (Reznick et al. 1986). Unfortunately, our use of field-collected seeds precluded estimates of maternal effects, but it is interesting to note that we did not detect strong positive correlations, as might be expected if well-provisioned seeds had both high RGR and high WUE whereas poorly provisioned seeds had low RGR and low WUE. Estimates of genetic correlations also are dependent on the environment in which they are measured (Giesel et al. 1982; Conner et al. 2003; Sgro and Hoffmann 2004), and the **G**-matrix itself can evolve (Johansson et al. 2012).

We conducted this experiment in a greenhouse setting, which has strengths and weaknesses. The major strength of a uniform greenhouse environment is that genetic differences are less likely to be obscured by environmental heterogeneity. For example, differences in resource acquisition or availability in a heterogeneous natural environment can mask trade-offs (van Noordwijk and de Jong 1986; Houle 1991; Venable 1992; Reznick et al. 2000; Worley et al. 2003). A possible drawback of a simplified greenhouse environment is that it might not capture all relevant axes of environmental variation, in which case estimated trait values might not translate to field conditions (e.g., realized RGR under competitive field conditions might be quite different than RGR in greenhouse pots). Such context dependency is of particular relevance to our study, where temporal variation in resource availability affects both the plasticity of trait expression (Angert et al. 2010) and the fitness consequences of different trait values (Kimball et al. 2012). For this reason, we grew plants with plentiful soil moisture and nutrients in warm ambient temperatures, conditions that were likely to make the trade-off between maximum growth potential (RGR) and WUE most evident. It is perhaps counterintuitive that the trade-off should be most evident under conditions of high, rather than low, resource availability, because allocation trade-offs are often most apparent when resources are limiting. However, in our study system, high-RGR species have high plasticity of leaf area and carbon gain in response to pulses of resource availability, and hence only express their high growth potential under conditions of high water availability and warm temperatures (Angert et al. 2007, 2010). Conversely, differences in WUE among species are less plastic, and high-WUE species are less able to convert extended resource pulses to biomass in warm temperatures because of respiratory load (Barron-Gafford et al. 2013). Ideally, one would estimate genetic correlations in natural environments using very large, randomized field trials, but such a design was impractical for a study on multiple species. Conner et al. (2003) reported that estimates of genetic correlations between floral characters were similar in sign, although

not necessarily in magnitude, between field and greenhouse environments.

VARIATION AMONG POPULATIONS SUGGESTS LOCAL ADAPTATION, BUT NOT LONG-TERM CONSTRAINT

Populations were sampled from south-central Arizona to south-eastern California (a distance of ~700 km) across a large portion of these species' geographic ranges, a scale at which it is common to detect genetic differentiation among populations of sessile organisms (Hereford 2009; Leimu and Fischer 2008). Clinal variation in traits along environmental gradients can be helpful for inferring local adaptation (Endler 1977), and in this case, we were particularly interested in whether divergence in RGR or WUE would entail concomitant changes in the other trait due to phenotypic constraints. Correlations among populations within species reflect the net result of long-standing constraints and past natural selection. Interestingly, these longer term outcomes can reveal evolution along “lines of least genetic resistance,” or a tendency for populations to diverge along the axes of greatest genetic covariation (Mitchell-Olds 1996; Schluter 1996; Colautti and Barrett 2011). However, in this study, simultaneous changes in both traits with respect to the same environmental gradient were not detected for any species except *P. recurvata*, and in this case, both traits increased with increasing precipitation. Consistent with the lack of concomitant change in both traits along environmental gradients, estimates of among-population correlations (**D**-matrices) were equally split between positive and negative values, and they were never significantly different from zero. Although the among-family correlation for *P. recurvata* suggests the potential for a strong constraint, the among-population correlation for this species is inconsistent with its action in the past.

Clinal variation in RGR and WUE with respect to longitude and precipitation was heterogeneous in sign and magnitude among species, whereas clinal variation with respect to temperature and latitude was more consistent among species. WUE was higher in populations from drier, western sites only for *S. micropoides*, whereas *P. recurvata* showed the opposite pattern. This suggests that populations of these species might attain high WUE in different ways. For example, the westernmost populations of *S. micropoides* might attain high WUE through stomatal regulation of water loss in highly xeric environments, as has been reported for populations of *Impatiens capensis* in eastern North America (Heschel et al. 2002), whereas high WUE in eastern populations of *P. recurvata* might be driven by high carbon gain rather than low water loss (Huxman et al. 2008), as has also been found in Patagonian species (Golluscio and Oesterheld 2007). Populations from cooler, northern sites appear to have evolved higher WUE more consistently (*P. recurvata*, *E. texanum*, and *S. micropoides*). The association

between high latitude (or low temperature) and high WUE fits with our understanding of WUE in these winter annual species. WUE appears to be attained by maximizing carbon gain during periods of low temperature, resulting in greater carbon gain relative to water loss (Huxman et al. 2008; Gremer et al. 2012; Barron-Gafford et al. 2013). This is particularly important for annuals that must grow during winter months, particularly in more northerly populations. We also found lower RGR among northern populations of *E. lanosum*, consistent with previous studies on several species of *Pinus* (Hoeksema and Thompson 2007) and *Arabidopsis thaliana* (Li et al. 1998). It is unlikely that natural selection would favor lower RGR for short-lived annual plant species, even in cooler sites, unless reduced RGR is a correlated response to selection for higher WUE or other, unmeasured traits. This provides a bit of circumstantial evidence to suggest that RGR could be constrained by some sort of trade-off.

LOWER DIFFERENTIATION AMONG POPULATIONS FOR HIGH-RGR SPECIES

We did not detect significant variation in RGR or WUE among populations of the two high-RGR species, *S. micropoides* and *E. lanosum*, using ANOVA, although some population differentiation was suggested by regressions of trait variation on latitude, longitude, and climatic variables. We did detect variation among populations of the two high-WUE species, *P. recurvata* and *E. texanum*. Variance among families tended to be relatively greater than variance among populations for the two high-RGR species, *S. micropoides* and *E. lanosum*, whereas variance among populations tended to be relatively greater than variance among families for the two low-RGR species, *P. recurvata* and *E. texanum*. Differences in mating system (e.g., outcrossing vs. selfing) could affect the amount and spatial distribution of genetic variation; for example, outcrossing could promote greater effective population sizes and genetic connectivity among populations while hindering genetic differentiation. Although the species differ in mating system, the differences do not line up in a way that relates clearly to position along the RGR-WUE trade-off or levels of differentiation among populations and families. Differences in population size also could affect levels of standing genetic variation. Interestingly, long-term demographic data from Tumamoc Hill indicate that *P. recurvata*, the only species in which we detected significant among-family genetic variation in both RGR and WUE, has the largest census population size of these four species, both in terms of plants above ground and seeds in the seed bank (Venable 2012).

High-RGR species appear to have greater phenotypic plasticity (Angert et al. 2007, 2010), which could partially substitute for adaptive differentiation among populations by enabling a given genotype to have greater fitness across a range of environments. On the other hand, adaptive plasticity could promote adap-

tive divergence by facilitating persistence in novel environments (Ghalambor et al. 2007), so the effect of plasticity on adaptive differentiation among populations remains difficult to predict. It is also possible that temporal variance in reproductive success affects selection on traits in different ways for high-RGR versus low-RGR species. Because high-RGR species have greater temporal variance in reproductive success from year to year, rare years with bumper seed crops contribute disproportionately to the genetic composition of the persistent seed bank and selection should favor specialization toward the good subset of years (Templeton and Levin 1979; Brown and Venable 1986). If “good” years with high resource abundance exert relatively similar selection pressures across sites, then adaptive differentiation might be lower than expected if considering differences in average conditions. Conversely, low-RGR species have lower variance in reproductive success, causing selection to favor less specialization toward particular year types (Templeton and Levin 1979; Brown and Venable 1986). We speculate that this creates greater opportunity for divergent adaptation to local environments, which differ in average resource availability, among populations of low-RGR species.

RELATIVE RANKINGS OF SPECIES ARE PRESERVED IN A COMMON ENVIRONMENT

We originally identified the among-species trade-off between RGR and WUE (Angert et al. 2007; Huxman et al. 2008) from a study at a natural field site, in a year with above-average precipitation, where we monitored growth with sequential harvests, analyzed leaf carbon isotopes, and measured leaf gas exchange. Subsequent investigations have bolstered our understanding of this emergent trade-off by dissecting its underlying functional basis (Angert et al. 2010; Kimball et al. 2011; Gremer et al. 2012; Barron-Gafford et al. 2013) and by demonstrating its relationship to long-term population and community dynamics (Angert et al. 2009; Kimball et al. 2010, 2012). The present study demonstrates that differences among species in RGR and WUE are by and large maintained in a greenhouse environment with uniformly high resource availability. Because the key mechanism underlying the expression of maximum RGR for any species is the ability to rapidly deploy significant leaf area, a high-resource environment is the key feature to quantifying the previously documented trade-off.

There are two exceptions to our finding that species' rank orders are consistent with prior results: RGR of *E. texanum* (previously described as a low-RGR species) and WUE of *S. micropoides* (previously described as a low-WUE species) were both higher than expected. *Erodium texanum* has a ground-hugging, rosette growth form, and leaf area expansion might be particularly constrained by neighboring plants in the field, where estimates of RGR are likely to be density-dependent and influenced by competition. We have also demonstrated experimentally that *E. texanum*

has low competitive ability, particularly in wet conditions (Gremer et al. 2013). Several studies have reported that rankings in water relations as measured in the field can differ from rankings when measured in greenhouse or field common gardens (Roy and Mooney 1982; Sandquist and Ehleringer 1997). One possible explanation for higher than expected WUE of *S. micropoides* is that the high RGR could have led to increasing stomatal restriction and hence increased WUE toward the end of the experiment as roots filled the soil volume available within the pot and sink-driven downregulation of photosynthetic carbon gain influenced leaf gas exchange. Such are the challenges of creating realistic environmental conditions in artificial settings, and it is possible that similar issues had more subtle effects on the relative rankings of populations and families.

SYNTHESIS AND FUTURE DIRECTIONS

Unique positions along the trade-off axis between RGR and WUE foster decoupled responses to environmental variation and contribute to coexistence among these species (Angert et al. 2009). Despite the ubiquity of trade-offs in natural systems, few studies have examined whether such trade-offs are fractal across multiple levels of biological organization, from families to populations to species, within the same community. We find mixed support for this idea. On the one hand, genetic correlations between RGR and WUE among families within species, when estimable, were strongly negative, consistent with the among-species trade-off. On the other hand, genetic differentiation among populations did not reveal strong trade-offs between RGR and WUE. The predominant constraint on the evolution of a high-RGR, high-WUE phenotype that is missing among the abundant, native species in this community appears to be a lack of standing genetic variation in one or both of these traits. The community-centered approach that we have taken here, focusing on distantly related species, affords us the opportunity to examine selection pressures and responses among species that co-occur in local communities and hence make connections between microevolution and community assembly. An alternative strategy would sample species within a clade and populations within those species. Such a phylogenetically nested design might provide a stronger test of concordance of within- versus between-species trade-offs by examining patterns of trait evolution from a recent common ancestor, but it would be more difficult to link within-species constraints and evolutionary dynamics to among-species coexistence mechanisms affecting community patterns. A complementary test of evolutionary constraint on these traits would be to conduct artificial selection for high RGR and high WUE to directly test the ability to construct a high-RGR and high-WUE phenotype. This would provide a comparison of realized responses to selection with field-based estimates of the strength of selection (Conner et al. 2011), and ultimately, the opportunity to test the performance of such a

phenotype within the community under natural field settings. In the end, a suite of approaches will be needed to develop a robust understanding of how functional trait evolution shapes biodiversity patterns within natural communities.

ACKNOWLEDGMENTS

We thank H. Bender, G. Barron-Gafford, T. Green, J. Horst, B. Jones, J. Matsuura, C. Nichols, G. Peterson, J. Prucha, and S. Sheth for plant care and data collection. K. Gerst collected seeds from CA populations. J. Gremer, C. Muir, and two anonymous reviewers provided constructive comments on earlier drafts. This work was supported by NSF DEB 0902173, 0844780, and 0717380. The authors have no conflicts of interest to declare.

DATA ARCHIVING

The doi for our data is 10.5061/dryad.gv682.

LITERATURE CITED

- Abràmoff, M. D., P. J. Magalhães, and S. J. Ram. 2004. Image processing with ImageJ. *Biophotonics Int.* 11:36–42.
- Amarasekare, P. 2003. Competitive coexistence in spatially structured environments: a synthesis. *Ecol. Lett.* 6:1109–1122.
- Amthor, J. S. 2000. The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. *Ann. Bot.* 86:1–20.
- Angert, A. L., T. E. Huxman, G. A. Barron-Gafford, K. L. Gerst, and D. L. Venable. 2007. Linking growth strategies to long-term population dynamics in a guild of desert annuals. *J. Ecol.* 95:321–331.
- Angert, A. L., T. E. Huxman, P. Chesson, and D. L. Venable. 2009. Functional tradeoffs determine species coexistence via the storage effect. *Proc. Natl. Acad. Sci. USA* 106:11641–11645.
- Angert, A. L., J. L. Horst, T. E. Huxman, and D. L. Venable. 2010. Phenotypic plasticity and precipitation response in Sonoran Desert winter annuals. *Am. J. Bot.* 97:405–411.
- Antonovics, J., and P. H. Van Tienderen. 1991. Ontoecogenophyloconstraints? The chaos of constraint terminology. *Trends Ecol. Evol.* 6:166–168.
- Anyia, A. O., J. J. Slaski, J. M. Nyachiro, D. J. Archambault, and P. Juskiw. 2007. Relationship of carbon isotope discrimination to water use efficiency and productivity of barley under field and greenhouse conditions. *J. Agron. Crop Sci.* 193:313–323.
- Arnold, S. J. 1992. Constraints on phenotypic evolution. *Am. Nat.* 140:S85–S107.
- Barron-Gafford, G. A., A. L. Angert, D. L. Venable, A. P. Tyler, K. L. Gerst, and T. E. Huxman. 2013. Photosynthetic temperature responses of desert annuals with contrasting resource-use efficiencies. *J. Arid Environ.* 91:95–103.
- Belyea, L. R., and J. Lancaster. 1999. Assembly rules within a contingent ecology. *Oikos* 86:402–416.
- Blows, M. W., and A. A. Hoffman. 2005. A reassessment of genetic limits to evolutionary change. *Ecology* 86:1371–1384.
- Bolker, B. M., and R. D. C. Team. 2012. bblme: tools for general maximum likelihood estimation. R package version 1.0.4.1.
- Bonsall, M. B., V. A. A. Jansen, and M. P. Hassell. 2004. Life history trade-offs assemble ecological guilds. *Science* 306:111–114.
- Brown, J. S., and D. L. Venable. 1986. Evolutionary ecology of seed-bank annuals in temporally varying environments. *Am. Nat.* 127:31–47.
- Burnham, K. P., and D. R. Anderson. 2010. Model selection and multimodel inference: a practical information-theoretic approach. 2nd ed. Springer-Verlag, New York, NY.

- Charlesworth, B. 1990. Optimization models, quantitative genetics, and mutation. *Evolution* 44:520–538.
- Chesson, P., and N. Huntly. 1988. Community consequences of life-history traits in a variable environment. *Ann. Zool. Fenn.* 25:5–16.
- Colautti, R. I., and S. C. H. Barrett. 2011. Population divergence along lines of genetic variance and covariance in the invasive plant *Lythrum salicaria* in eastern North America. *Evolution* 65:2514–2529.
- Condon, A. G., G. D. Farquhar, and R. A. Richards. 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. *Aust. J. Plant Physiol.* 17:9–22.
- Conner, J. K. 2002. Genetic mechanisms of floral trait correlations in a natural population. *Nature* 420:407–410.
- Conner, J. K., R. Franks, and C. Stewart. 2003. Expression of additive genetic variances and covariances for wild radish floral traits: comparison between field and greenhouse environments. *Evolution* 57:487–495.
- Conner, J. K., K. Karoly, C. Stewart, V. A. Koelling, H. F. Sahli, and F. H. Shaw. 2011. Rapid independent trait evolution despite a strong pleiotropic genetic correlation. *Am. Nat.* 178:429–441.
- Dawson, T. E., S. Mambelli, A. H. Plamboeck, P. H. Templer, and K. P. Tu. 2002. Stable isotopes in plant ecology. *Annu. Rev. Ecol. Syst.* 33:507–559.
- Donovan, L. A. and J. R. Ehleringer 1994. Potential for selection on plants for water-use efficiency as estimated by carbon isotope discrimination. *Am. J. Bot.* 81:927–935.
- Dudley, L. S., S. J. Mazer, and P. Galusky. 2007. The joint evolution of mating system, floral traits and life history in *Clarkia* (Onagraceae): genetic constraints vs. independent evolution. *J. Evol. Biol.* 20:2200–2218.
- Ehleringer, J., A. E. Hall, and G. D. Farquhar. 1993. Stable isotopes and plant carbon/water relations. Academic Press, San Diego, CA.
- Endler, J. A. 1977. Geographic variation, speciation, and clines. Princeton Univ. Press, Princeton, NJ.
- Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Longman, Harlow, U.K.
- Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 40:503–537.
- Fine, P. V. A., I. Mesones, and P. D. Coley. 2004. Herbivores promote habitat specialization by trees in Amazonian forests. *Science* 305:663–665.
- Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford, U.K.
- Geritz, S. A. H., E. van der Meijden, and J. A. J. Metz. 1999. Evolutionary dynamics of seed size and seedling competitive ability. *Theor. Popul. Biol.* 55:324–343.
- Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* 21:394–407.
- Giesel, J. T., P. A. Murphy, and M. N. Manlove. 1982. The influence of temperature on genetic interrelationships of life-history traits in a population of *Drosophila melanogaster*: what tangled data sets we weave. *Am. Nat.* 119:464–479.
- Golluscio, R. A., and M. Oesterheld. 2007. Water use efficiency of twenty-five co-existing Patagonian species growing under different soil water availability. *Oecologia* 154:207–217.
- Gomulkiewicz, R., and D. Houle. 2009. Demographic and genetic constraints on evolution. *Am. Nat.* 174:E218–E229.
- Gremer, J. R., S. Kimball, A. L. Angert, D. L. Venable, and T. E. Huxman. 2012. Variation in photosynthetic response to temperature in a guild of winter annual plants. *Ecology* 93:2693–2704.
- Gremer, J. R., S. Kimball, K. R. Keck, T. E. Huxman, A. L. Angert, and D. L. Venable. 2013. Water-use efficiency and relative growth rate mediate competitive interactions in Sonoran Desert winter annual plants. *Am. J. Bot.* 100:2009–2015.
- Grime, J. P. 1979. Plant strategies and vegetation processes. John Wiley and Sons, New York, NY.
- Grubb, P. J. 1977. Maintenance of species richness in plant communities: importance of regeneration niche. *Biol. Rev. Camb. Philos. Soc.* 52:107–145.
- Hereford, J. 2009. A quantitative survey of local adaptation and fitness trade-offs. *Am. Nat.* 173:579–588.
- Hermes, D. A., and W. J. Mattson. 1992. The dilemma of plants: to grow or defend. *Q. Rev. Biol.* 67:283–335.
- Heschel, M. S., K. Donohue, N. Hausmann, and J. Schmitt. 2002. Population differentiation and natural selection for water-use efficiency in *Impatiens capensis* (Balsaminaceae). *Int. J. Plant Sci.* 163:907–912.
- Hoeksema, J. D., and J. N. Thompson. 2007. Geographic structure in a widespread plant–mycorrhizal interaction: pines and false truffles. *J. Evol. Biol.* 20:1148–1163.
- Houle, D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. *Evolution* 45:630–648.
- Huxman, T. E., G. Barron-Gafford, K. L. Gerst, A. L. Angert, A. P. Tyler, and D. L. Venable. 2008. Photosynthetic resource-use efficiency and demographic variability in desert annual plants. *Ecology* 89:1554–1563.
- Huxman, T. E., S. Kimball, A. L. Angert, J. R. Gremer, G.A. Barron-Gafford, and D. L. Venable. 2013. Understanding past, contemporary, and future dynamics of plants, populations and communities using Sonoran Desert Winter Annuals. *Am. J. Bot.* 100:1369–1380.
- Johansson, F., M. I. Lind, P. K. Ingvarsson, and F. Bokma. 2012. Evolution of the G-matrix in life history traits in the common frog during a recent colonisation of an island system. *Evol. Ecol.* 26:863–878.
- Kimball, S., A. L. Angert, T. E. Huxman, and D. L. Venable. 2010. Climate change favors cold-adapted species in the Sonoran Desert. *Global Change Biol.* 16:1555–1565.
- Kimball, S., A. L. Angert, T. E. Huxman, and D. L. Venable. 2011. Differences in the timing of germination and reproduction relate to growth physiology and population dynamics of Sonoran Desert winter annuals. *Am. J. Bot.* 98:1773–1781.
- Kimball, S., J. R. Gremer, A. L. Angert, T. E. Huxman, and D. L. Venable. 2012. Fitness and physiology in a variable environment. *Oecologia* 169:319–329.
- Kimball, S., J. R. Gremer, T. E. Huxman, D. L. Venable, and A. L. Angert. 2013. Phenotypic selection favors missing trait combinations in coexisting annual plants. *Am. Nat.* 181:191–207.
- Kimura, M. 1958. On the change of population fitness by natural selection. *Heredity* 12:145–167.
- Kneitel, J. M., and M. Chase. 2004. Trade-offs in community ecology: linking spatial scales and species coexistence. *Ecol. Lett.* 7:69–80.
- Lankford, T. E. Jr., J. M. Billerbeck, and D. O. Conover. 2001. Evolution of intrinsic growth and energy acquisition rates. II. Trade-offs with vulnerability to predation in *Menidia menidia*. *Evolution* 55:1873–1881.
- Leimu, R., and M. Fischer. 2008. A meta-analysis of local adaptation in plants. *PLoS One* 3:e4010.
- Lewontin, R. C. 1974. The genetic basis of evolutionary change. Columbia Univ. Press, New York, NY.
- Li, B., J. I. Suzuki, and T. Hara. 1998. Latitudinal variation in plant size and relative growth rate in *Arabidopsis thaliana*. *Oecologia* 115:293–301.
- Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, MA.
- Merilä, J., and B. C. Sheldon. 1999. Genetic architecture of fitness and non-fitness traits: empirical patterns and development of ideas. *Heredity* 83:103–109.

- Mitchell-Olds, T. 1996. Genetic constraints on life-history evolution: quantitative-trait loci influencing growth and flowering in *Arabidopsis thaliana*. *Evolution* 50:140–145.
- Mooney, K. A., R. Halitschke, A. Kessler, and A. A. Agrawal. 2010. Evolutionary trade-offs in plants mediate the strength of trophic cascades. *Science* 327:1642–1644.
- Mooring, J. S. 2002. Experimental hybridizations of *Eriophyllum* annuals (Asteraceae, Helenieae). *Am. J. Bot.* 89:1973–1983.
- Olivas-Garcia, J. M., B. M. Cregg, and T. C. Hennessey. 2000. Genotypic variation in carbon isotope discrimination and gas exchange of ponderosa pine seedlings under two levels of water stress. *Can. J. Forest Res.* 30:1581–1590.
- Pigliucci, M. 2006. Genetic variance-covariance matrices: a critique of the evolutionary quantitative genetics research program. *Biol. Philos.* 21:1–23.
- Price, T., and D. Schluter. 1991. On the low heritability of life-history traits. *Evolution* 45:853–861.
- Rees, M., and D. L. Venable. 2007. Why do big plants make big seeds? *J. Ecol.* 95:926–936.
- Rees, M., R. Condit, M. Crawley, S. Pacala, and D. Tilman. 2001. Long-term studies of vegetation dynamics. *Science* 293:650–655.
- Reich, P. B., D. S. Ellsworth, M. B. Walters, J. M. Vose, C. Gresham, J. C. Volin, and W. D. Bowman. 1999. Generality of leaf trait relationships: a test across six biomes. *Ecology* 80:1955–1969.
- Reznick, D. N., E. Perry, and J. Travis. 1986. Measuring the cost of reproduction: a comment. *Evolution* 40:1338–1344.
- Reznick, D. N., L. Nunney, and A. J. Tessier. 2000. Big houses, big cars, superfleas and the costs of reproduction. *Trends Ecol. Evol.* 15:421–425.
- Ricklefs, R. E. 2012. Naturalists, natural history, and the nature of biological diversity. *Am. Nat.* 179:423–435.
- Robinson, B. W., D. S. Wilson, and G. O. Shea. 1996. Trade-offs of ecological specialization: an intraspecific comparison of pumpkinseed sunfish phenotypes. *Ecology* 77:170–178.
- Rose, M. R. 1984. Genetic covariation in *Drosophila* life history: untangling the data. *Am. Nat.* 123:565–569.
- Roy, J. and H. A. Mooney. 1982. Physiological adaptation and plasticity to water stress of coastal and desert populations of *Heliotropium curassavicum* L. *Oecologia* 52: 370–375.
- Ryan, M. G. 1995. Foliar maintenance respiration of subalpine and boreal trees and shrubs in relation to nitrogen content. *Plant Cell Environ.* 18:765–772.
- Sandquist, D. R., and J. R. Ehleringer. 1997. Intraspecific variation of leaf pubescence and drought response in *Encelia farinosa* associated with contrasting desert environments. *New Phytol.* 135:635–644.
- Satterthwaite, F. W. 1946. An approximate distribution of estimates of variance components. *Biometrics Bull.* 2:110–114.
- Schiesari, L., S. D. Peacor, and E. E. Werner. 2006. The growth-mortality tradeoff: evidence from anuran larvae and consequences for species distributions. *Oecologia* 149:194–202.
- Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. *Evolution* 50:1766–1774.
- Sgro, C. M., and A. A. Hoffmann. 2004. Genetic correlations, tradeoffs and environmental variation. *Heredity* 93:241–248.
- Stearns, S. C. 1992. *The evolution of life histories*. Oxford Univ. Press, Oxford, U.K.
- Suding, K. N., D. E. Goldberg, and K. M. Hartman. 2003. Relationships among species traits: separating levels of response and identifying linkages to abundance. *Ecology* 84:1–16.
- Templeton, A. R., and D. A. Levin. 1979. Evolutionary consequences of seed pools. *Am. Nat.* 114:232–249.
- Tessier, A. J., M. A. Leibold, and J. Tsao. 2000. A fundamental trade-off in resource exploitation by *Daphnia* and consequences to plankton communities. *Ecology* 81:826–841.
- Tilman, D. 1988. *Monographs in population biology*. Vol. 26. Plant strategies and the dynamics and structure of plant communities. Princeton Univ. Press, Princeton, NJ.
- . 1994. Competition and biodiversity in spatially structured habitats. *Ecology* 75:2–16.
- Tilman, D., and S. Pacala. 1993. The maintenance of species richness in plant communities. Pp.13–25 in R. E. Ricklefs and D. Schluter, eds. *Species diversity in ecological communities*. University of Chicago Press, Chicago, IL.
- Turnbull, L. A., M. Rees, and M. J. Crawley. 1999. Seed mass and the competition/colonization trade-off: a sowing experiment. *J. Ecol.* 87:899–912.
- Turnbull, M. H., D. Whitehead, D. T. Tissue, W. S. F. Schuster, K. J. Brown, and K. L. Griffin. 2003. Scaling foliar respiration in two contrasting forest canopies. *Funct. Ecol.* 17:101–114.
- van Noordwijk, A. J., and G. de Jong. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* 128:137–142.
- Vasseur, D. A., P. Amarasekare, V. H. W. Rudolf, and J. M. Levine. 2011. Eco-evolutionary dynamics enable coexistence via neighbor-dependent selection. *Am. Nat.* 178:E96–E109.
- Venable, D. L. 1992. Size-number trade-offs and the variation of seed size with plant resource status. *Am. Nat.* 140:287–304.
- . 2007. Bet hedging in a guild of desert annuals. *Ecology* 88:1086–1090.
- . 2012. Long-term population dynamics of individually mapped Sonoran Desert winter annuals from the Desert Laboratory, Tucson, AZ. Desert Annual Archive. Available at <http://www.eebweb.arizona.edu/faculty/venable/LTREB/LTREB%20data.htm>.
- Vincent, T. L. S., D. Scheel, J. S. Brown, and T. L. Vincent. 1996. Trade-offs and coexistence in consumer-resource models: it all depends on what and where you eat. *Am. Nat.* 148:1038–1058.
- Wang, T., A. Hamann, D. L. Spittlehouse, and T. Q. Murdock. 2012. ClimateWNA— high-resolution spatial climate data for western North America. *J. Appl. Meteorol. Climatol.* 61:16–29.
- Warren, C. R., and M. A. Adams. 2006. Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis. *Plant Cell Environ.* 29:192–201.
- Weiher, E., D. Freund, T. Bunton, A. Stefanski, T. Lee, and S. Bentivenga. 2011. Advances, challenges and a developing synthesis of ecological community assembly theory. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 366:2403–2413.
- Werner, E. E., and B. R. Anholt. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *Am. Nat.* 142:242–272.
- Wolf, M., G. S. van Doorn, O. Leimar, and F. J. Weissing. 2007. Life-history trade-offs favor the evolution of animal personalities. *Nature* 447:581–584.
- Worley, A. C., D. Houle, and S. C. H. Barrett. 2003. Consequences of hierarchical allocation for the evolution of life-history traits. *Am. Nat.* 161:153–167.
- Zera, A. J., and L. G. Harshman. 2001. The physiology of life history trade-offs in animals. *Annu. Rev. Ecol. Syst.* 32:95–126.

Associate Editor: J. Etterson

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Appendix S1. Effect of seed origin on relative growth rate.

Table S1. Location and climatic profiles of population collection sites.

Table S2. Sample sizes of families for each population and species.

Table S3. Correlation matrix among the climatic variables average winter (December–February) maximum temperature (Tmax), average winter minimum temperature (Tmin), and average winter precipitation (PPT), obtained for the period 1961–1990 in the ClimateWNA database (Wang et al. 2006), based on the latitude and longitude of the study populations.

Table S4. Variance-covariance matrices for water-use efficiency (WUE) and relative growth rate (RGR) among populations (**D**-matrices) and among seed families (**G**-matrices) generated from repeated-measures mixed models.