

**Water-use efficiency may influence the distribution of
Ipomopsis aggregata, *I. tenuituba*, and their natural hybrids
along an environmental gradient**

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Abstract

In flowering plants, environment-mediated hybrid fitness plays a large role in hybrid zone dynamics. The relationship of physiological traits to hybrid fitness, however, has not been described for many plant systems. In this study, we investigated the effects of soil moisture on water-use efficiency (WUE), above-ground relative growth rate (RGR), and other leaf traits for *Ipomopsis aggregata*, *I. tenuituba*, and their natural hybrids from two contact sites in Gunnison County, Colorado. In nature, hybrids occur in extremely dry, hot conditions, while *I. tenuituba* and *I. aggregata* occur in relatively dry and moist conditions, respectively. One contact site (Poverty Gulch), furthermore, appears to be generally drier than the other (Deadman's Gulch). During the study, all plant types increased WUE as soil moisture decreased, but the effect for hybrids was especially strong. WUE, however, only affected RGR for *I. tenuituba*, where it dramatically reduced above-ground growth. Differences in site of origin alternately constrained plasticity of leaf traits or became less important in determining leaf traits over the study period. Specific leaf area, for example, was consistently higher for plants from Poverty Gulch than plants from Deadman's Gulch, but initial site differences in chlorophyll *a* index disappeared by the end of the study. The fact that contact sites differed in mean temperature and relative humidity, and that site of origin affected several plant traits, suggests that these environmental factors drive at least some fitness trait differences in *Ipomopsis* parents and hybrids. However, because *I. tenuituba* naturally occurs in dry conditions, where it has the lowest RGR, WUE cannot entirely explain the distribution of *Ipomopsis* parents and hybrids, if above-ground biomass controls fitness. It is possible, however, that WUE has positive effects on other fitness traits, such as below-ground biomass.

Keywords: Water-use efficiency, *Ipomopsis*, Hybrid zone dynamics

Introduction

Hybridization in flowering plants can initiate many kinds of evolutionary change. Some hybrid zones lead to a blending of the parent species (Levin et al. 1996), while in other cases, strong species differences are maintained due to genomic incompatibilities or the absence of “hybrid” habitat (Dobzhansky 1937, Johnston et al. 2001). Often, however, hybrids coexist with parent species in a stable hybrid zone. These hybrid populations can be maintained by a balance between gene flow and low hybrid fitness (Barton and Hewitt 1985, Servedio and Kirkpatrick 1997), by greater fitness than parents in marginal habitats (Moore 1977), or by variable fitness in different environments, due to genetic recombination (Johnston et al. 2001). Although hybrid fitness has been studied in several plant genera, little is known about how physiological traits influence hybrid fitness in nature (Ludwig et al. 2004).

Ipomopsis populations (Polemoniaceae) in the Central Rockies of Colorado readily hybridize along elevational gradients. In the well-studied Poverty Gulch hybrid zone (Gunnison County, CO), *I. aggregata* occurs in moist sites up to 2900m, *I. tenuituba* occurs in dry sites above 3100m, and hybrids occur at extremely dry intermediate elevations (Campbell et al. 1997). Campbell and Waser (2001) investigated survival of all three plant types in each habitat. Surprisingly, both parent species had high survival in the hybrid zone, and certain hybrids survived well in both parent zones. These results led to research on physiological traits that affect not just survival, but also quality of the survivors—the ability to accrue the biomass necessary for vigorous flowering and high contribution to the next generation, also known as lifetime reproductive success (Wu and Campbell 2006). Physiological specialization to certain soil moisture regimes may shape the natural distribution of *Ipomopsis* parents and hybrids.

Adaptation to low soil moisture can be achieved by high water-use efficiency (WUE), which is the ratio of photosynthetic rate (A) to transpiration (E) (Heschel et al. 2004). A study on the annual grass *Avena barbata*, however, found that it is possible for high WUE to benefit plants only in mesic environments (Sherrard and Maherali 2006). In that case, WUE may be selectively neutral under

drought conditions, or there may be pressure to reduce WUE by lowering A , due to the costs of maintaining photosynthetic enzymes. Therefore, there is not a consistent relationship between WUE and fitness traits.

Reflecting its dry habitat in Poverty Gulch, *I. tenuituba* has much higher water-use efficiency (WUE) than *I. aggregata* (Campbell et al. 2005). Hybrids also have high WUE (Campbell et al. 2005), which may allow them to outcompete *I. aggregata* in arid zones (Wu and Campbell 2006). The soil moisture regimes associated with parents and hybrids in Poverty Gulch, however, are not necessarily consistent from site to site: at other contact sites, such as Deadman's Gulch, the two species show a different distribution with elevation and so may occupy different kinds of soil conditions (Aldridge 2005). To understand if high WUE is an adaptive trait for *I. tenuituba* and hybrids in the habitats they occupy, it is necessary to investigate the relationship between WUE and fitness for all plant types in different soil moisture regimes. This will indicate not only if high WUE is adaptive in the habitats where these plant types are found, but also the soil moisture at which fitness is optimized for each plant type, which could influence *Ipomopsis* hybrid zone dynamics.

In this study, we ask the following specific questions: Do *Ipomopsis* parents and hybrids show different growth and physiological responses to water availability? Are these differences reflected in the natural distribution of these plants across certain soil moisture regimes? How are other plant traits (specific leaf area, relative water content, and chlorophyll a index) affected by soil moisture regime? Are *Ipomopsis* physiological traits conserved within each plant type, or do they differ between Poverty Gulch and Deadman's Gulch populations? To investigate these questions, we transplanted vegetative rosettes of *I. tenuituba*, *I. aggregata*, and their hybrids from Poverty Gulch and Deadman's Gulch into a common garden. The plants were grown under ambient, wet, and dry soil moisture treatments, and we recorded changes in biomass, photosynthetic rate, transpiration, relative water content, specific leaf area, and leaf reflectance spectra.

Materials and Methods

Study System—*Ipomopsis aggregata* and *I. tenuituba* occur extensively throughout the high alpine regions of the Rocky Mountains. The species rarely grow at the same sites but usually hybridize along the elevational gradients between parental populations. Despite strong altitudinal segregation, each parent species can possibly exist either above or below a given hybrid site. Most hybrid zones are structured like the one at Poverty Gulch, a high mountain valley that drains into the Slate River Valley of Gunnison County, CO. At Poverty Gulch, populations of *I. aggregata* are found on relatively moist, low-grade slopes up to 2900m in elevation. *I. tenuituba* populations occur on drier, high-grade slopes down to 3100m. Isolated hybrid populations exist at intermediate elevations, typically on high-grade talus slopes that are hotter and drier than the *I. tenuituba* sites (Wu and Campbell 2007).

At Deadman's Gulch, however, *I. tenuituba* contacts the hybrid zone at lower elevations, while *I. aggregata* is found at higher elevations. Deadman's Gulch is a steep-sided valley that drains into Taylor Canyon of Gunnison County, CO, and *I. tenuituba* populations occur on slopes up to 2970m. *I. aggregata* populations exist on slopes down to 3220m. In addition to abiotic differences between Poverty Gulch and Deadman's Gulch, the two *Ipomopsis* populations appear to differ morphologically: in Poverty Gulch, leaves on vegetative rosettes are bright green, lacy, and hairless, while in Deadman's Gulch they are dark green, robust, and hairy.

We investigated the physiology of *Ipomopsis* populations from both Deadman's Gulch and Poverty Gulch. To quantify the environmental conditions at both sites, we recorded temperature and relative humidity for much of the summer. We placed temperature and relative humidity data loggers at Deadman's Gulch (both parent sites and the hybrid site) and Poverty Gulch (hybrid site) from July 20 to August 5. The data loggers took readings every 15 minutes, and these data were used to correlate

initial physiological measurements to the prevailing conditions at both Poverty Gulch and Deadman's Gulch.

Common Garden Setup and Water Treatments—We grew the plants in a common garden, where we could manipulate water availability while keeping soil conditions similar. We potted 18 vegetative rosettes of each type from Deadman's Gulch (June 25) and Poverty Gulch (July 2). The plants were kept in screenhouses and watered daily until being planted in the common garden (July 3). The common garden was a 3 x 3 grid of 1m² plots in an open meadow at the Rocky Mountain Biological Lab, Gothic, CO. We cleared the plots of vegetation and separated them by a margin of 1m to minimize interference between water treatments. We planted a total of 12 rosettes in each plot—two of each plant type from each location—ordering them randomly and spacing them 20-25cm apart. We watered all plots at least daily from planting (July 3) to July 10. On July 11, we began the water treatments. Three replicates of each water treatment (wet, dry, and ambient) were assigned to the nine plots.

Three plots ("wet") initially received 50% more rainfall than the July average (5.4 cm, SD = 4.0 cm, from 1989 to 2006, EPA precipitation data), equivalent to an additional 27L water distributed in 3.5L increments every four days. We maintained this treatment from July 11 to July 23, and then doubled the frequency of watering for the remainder of the study, providing wet plots with 3.5L water every two days.

We covered three plots ("dry") with rainout shelters to intercept roughly 50% of rainfall. We constructed rainout shelters according to the design in Yahdjian and Sala (2002). The shelters measured 1.6m x 1.6m and were centered over the dry plots to minimize unfiltered rainfall blowing in from the sides of the shelter. Although Yahdjian and Sala (2002) recommended that the partial roof be made of acrylic strips, for their optical clarity, we instead used strips of clear, corrugated greenhouse roofing. In direct sunlight, the greenhouse roofing removed 37% of photosynthetically active radiation (PAR), but the net effect was less dramatic because the slat shadows moved throughout the day and did not always

cover the plants. Nevertheless, we monitored fluctuations in PAR over the study period by suspending temperature/light data loggers 30cm above the center of six plots (two replicates of each treatment) from August 4 to August 14. These data indicated that light conditions, on average, were very similar across all plots.

The remaining three plots (“ambient”) remained under natural conditions and generally received water only from rainfall. Due to high plant stress during a period of low rainfall in early July, however, we simulated natural rainfall on July 17 and July 22, providing wet and ambient plots with 2.5L water and dry plots with 1.25L. Nevertheless, there was 11% mortality during this dry period, mostly affecting the Poverty Gulch plants, which had had less time to recover from transplant shock before the treatment began. We replaced three of the dead rosettes with plants of appropriate type and origin. We measured soil moisture content in each of the nine plots throughout the summer to quantify the effects of the treatments (Figure 1).

Response Measurements—We removed a leaf from each plant to measure physical leaf traits (area, wet mass, dry mass, reflectance spectrum at 400-800nm). With these data we determined relative water content ($RWC = [\text{wet mass} - \text{dry mass}] / \text{wet mass}$), specific leaf area ($SLA = \text{dry mass} / \text{area}$), and the chlNDI. This index equals $(R_{750} - R_{705}) / (R_{750} + R_{705})$, where R indicates reflectance at a particular wavelength. This index is usually correlated with chlorophyll *a* concentration (Richardson et al. 2001, Sims and Gamon 2002). Dry mass was found after drying leaves in a 60°C oven for 2hr. Physical leaf traits were measured just prior to the treatment period (July 8-9) and twice more throughout the summer (July 22-23 and August 4-5). We measured photosynthetic traits using an infrared gas analyzer Li-Cor 6400 Portable Photosynthesis System. All measurements were made with saturating light conditions (PAR = 1800), a leaf temperature of 27°C, and a sample CO₂ concentration of 375ppm (Wu and Campbell 2007). *Ipomopsis* spp. tend to stop photosynthesizing in the early afternoon, so photosynthetic traits were measured from 0800 to 1200 hours. Photosynthetic rate (A)

and stomatal conductance (g) were recorded for each plant by clamping a leaf in the Li-Cor chamber and recording exchange of CO_2 and water vapor. To standardize A and g measurements we removed the leaves used in the Li-Cor and determined their area. Water-use efficiency (WUE) was calculated as A/g , a measure of intrinsic water-use efficiency that is comparable to A/E , where E represents transpiration rate. We also recorded soil moisture near each plant when these measurements were taken.

Photosynthetic traits were measured three times throughout the treatment (July 13-15, July 29-31, and August 10-12). As the final response variable, we approximated aboveground biomass of each plant as the product of the number of leaves and length of the longest leaf (Campbell and Waser 2001). For individuals having multiple rosettes, which occurs when herbivory breaks apical dominance, these characters were recorded for each rosette and the products were summed to find biomass. Biomass was measured just prior to the treatment (July 7) and three times more throughout the water treatments (July 20, August 3, and August 14). For the second, third, and fourth measurement periods, the number of leaves included the leaves removed during analysis of physical leaf traits and photosynthetic traits throughout the study period.

Statistical Analyses—The common garden followed a replicated split-plot design, with water replicated at the plot level and plant type replicated within plots. We used a split-plot ANOVA to test the effects and interactions among water treatment (wet, ambient, dry), plant type (*I. aggregata*, *I. tenuituba*, hybrid), and site of origin (Deadman Gulch, Poverty Gulch) on relative growth rate, A , g , A/g , relative water content, specific leaf area, and chlNDI. The model had factors of water treatment, plot nested within water treatment, plant type, site, and the interactions of treatment*plant type, treatment*site, and plant type*site. We did not include the three-way interaction; preliminary analyses with three-way interactions included never detected statistical significance. We also used ANCOVA to analyze the relationship between individual plant soil moisture and A , g , and A/g . The analysis had categorical factors of site, plant type, and the site*plant type interaction, along with soil moisture as a

covariate. We also estimated correlation coefficients between relative growth rate, A , g , relative water content, and specific leaf area.

Results

Environmental Conditions at Deadman's Gulch and Poverty Gulch—Similar to the prevailing conditions in Poverty Gulch, the Deadman's Gulch *I. tenuituba* site was hotter and drier (mean temp = 14.04°C, mean RH = 64.51%) than the *I. aggregata* site (mean temp = 13.87°C, mean RH = 71.12%), and the hybrid site was the hottest and driest of all (mean temp = 14.85°C, mean RH = 61.39%). The Poverty Gulch hybrid site, however, was even hotter and drier (mean temp = 15.13°C, mean RH = 42.54%), suggesting that Poverty Gulch is generally drier than Deadman's Gulch.

Specific Leaf Area—At each point in the study period, specific leaf area was significantly affected by site of origin (Collection 1: $F_{1,88} = 85.56$, $P < .0001$, Collection 2: $F_{1,78} = 45.39$, $P < .0001$, Collection 3: $F_{1,77} = 17.31$, $P < .0001$), with plants from Deadman's Gulch having much denser leaves (mean SLA = 121.1 cm/g) than those from Poverty Gulch (mean SLA = 147.1 cm/g, Figure 2). Over the course of the study, mean SLA decreased for plants from both sites (Figure 2). Plant type significantly affected SLA in Collection 2 ($F_{2,78} = 5.17$, $P = .0078$), with *I. aggregata* having significantly higher SLA than both hybrids (Tukey a posteriori comparison, difference between means = 13.489) and *I. tenuituba* (difference between means = 12.577). Plant type did not affect SLA in Collection 1 ($F_{2,88} = 1.12$, $P = .3301$) or 3, however ($F_{2,77} = 1.64$, $P = .2007$). Neither water treatment, nor its interaction with plant type or site, had any detectable effect on SLA. Water treatment had generated consistent differences in soil moisture between the dry treatment and the others, but not always between the ambient and wet treatments (Figure 1).

Relative Water Content—Initial relative water content differed significantly by site ($F_{1,88} = 26.21$, $P < .0001$), with Deadman's Gulch plants containing less water (RWC = .7521) than those from Poverty

Gulch (RWC = .7745, Figure 3). The site-specific difference in RWC, however, disappeared after the first data collection (Collection 2: $F_{1,78} = .03$, $P = .8689$, Collection 3: $F_{1,77} = .27$, $P = .6069$), suggesting that the initial difference could have been due to transplant stress in Poverty Gulch plants, which had had less time to acclimate before the water treatment began. Unlike specific leaf area, relative water content appears to be conserved between Poverty Gulch and Deadman's Gulch populations. Water treatment, however, had a significant effect on RWC in Collection 3 ($F_{2,6} = 6.86$, $P = .0282$), with plants in the wet treatment having higher RWC than those in the dry treatment (difference between means = .06692, Figure 3).

Chlorophyll *a* Concentration—At the start of the experiment, the spectral estimate of chlorophyll *a* concentration differed significantly between plants from different sites ($F_{1,88} = 15.62$, $P = .0002$), with plants from Deadman's Gulch containing more chlorophyll *a* (chlNDI = .3697) than those from Poverty Gulch (chlNDI = .3244, Figure 4). See Figure 5 for sample leaf reflectance spectra and the wavelengths used to estimate chlorophyll *a*. The site-specific difference in chlNDI was maintained through Collection 2 ($F_{1,78} = 21.08$, $P < .0001$) but disappeared in Collection 3 ($F_{1,77} = .72$, $P = .3978$). Accordingly, there appeared to be less of a difference in leaf pigments between Deadman's Gulch and Poverty Gulch plants by the end of the period in the common garden, but there was still no treatment effect on chlorophyll *a* concentration ($F_{2,6} = 1.59$, $P = .2799$). In Collection 2, there was a significant type x site interaction on chlorophyll *a* concentration ($F_{2,78} = 3.27$, $P = .0432$, Figure 4). *I. aggregata* plants had very similar chlNDI regardless of which site they came from, while there were large site differences for the other two types of plants.

Relative Growth Rate—Growth over the entire study period was significantly affected by site of origin ($F_{1,74} = 47.69$, $P < .0001$), with plants from Deadman's Gulch growing much more (RGR = .5867) than those from Poverty Gulch (RGR = .1082). Water treatment did not have a significant effect on RGR (split-plot ANOVA, $F_{2,6} = 4.37$, $P = .0673$), but plant type and water treatment interacted in their effect on

RGR ($F_{4,74} = 5.39$, $P = .0007$), with *I. tenuituba* having much higher growth rates in the wet treatment than either *I. aggregata* or hybrids (Figure 6). There was a strong overall negative relationship between mean WUE and RGR (Pearson's correlation $r = -.3428$, $P = .0008$). Furthermore, in analysis of covariance, WUE interacted with plant type in its effect on RGR ($F_{2,82} = 4.21$, $P = .0182$). When separate slopes were estimated for the three plant types, the negative relationship was significant only for *I. tenuituba* (slope = $-.009322$, $P = .0005$, Figure 6). Photosynthetic rate and conductance both had strong, positive correlations with RGR (A : $r = .5904$, $P < .0001$, g : $r = .5619$, $P < .0001$). Conductance interacted with plant type in its effect on RGR ($F_{2,82} = 4.58$, $P = .0130$), indicating that the positive effect of conductance on RGR differed by plant type; when separate slopes were estimated for each type of plant, they were significant only for hybrids (slope = $.6383$, $P = .0368$) and *I. tenuituba* (slope = 1.5392 , $P < .0001$, Figure 7).

Water-Use Efficiency—Like relative growth rate, water-use efficiency was significantly affected by site of origin ($F_{1,74} = 6.76$, $P = .0112$), with Poverty Gulch plants being more water-use efficient ($WUE = 82.17$) than those from Deadman's Gulch ($WUE = 74.17$). Deadman's Gulch plants, however, achieved higher mean RGR, perhaps because they maintained higher rates of photosynthesis ($A = 14.61 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) than those from Poverty Gulch ($A = 12.45 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Furthermore, site of origin interacted with plant type in its effect on WUE ($F_{2,74} = 4.19$, $P = .0188$), and plant type interacted with water treatment ($F_{4,74} = 5.25$, $P = .0009$). Specifically, all plants increased their WUE in dry soil, but hybrids increased it by far more than either parent species (Figure 6).

When WUE was analyzed against soil moisture readings, rather than water treatment, plant type was found to have a significant effect on WUE ($F_{2,85} = 3.57$, $P = .0326$). While water treatment did not affect WUE in the split-plot ANOVA (probably because of low statistical power for that main effect), soil moisture as a covariate did (ANCOVA, $F_{1,85} = 25.63$, $P < .0001$), with WUE decreasing significantly with soil moisture (slope = -2.3725 , $P = .0500$). This relationship was especially pronounced for hybrids

(slope = -5.3294, $P = .0073$), leading to an interaction between soil moisture and type of plant ($F_{2,85} = 4.03$, $P = .0212$). Relative growth rate increased significantly with soil moisture (slope in ANCOVA = .04622, $P = .0002$).

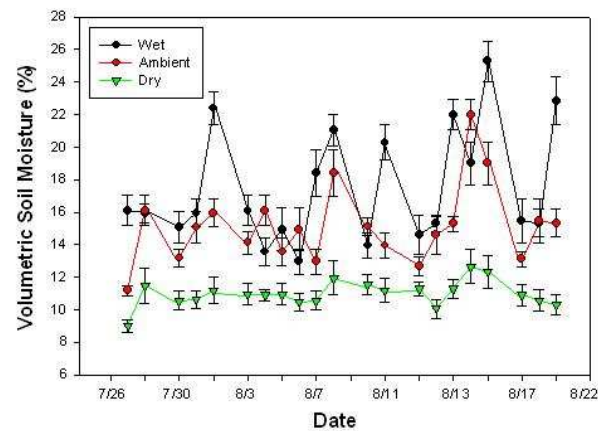


Figure 1. Volumetric soil moisture as a function of water treatment, from July 26 to August 20. Data shown are means and standard errors across a total of 18 measurements in each treatment.

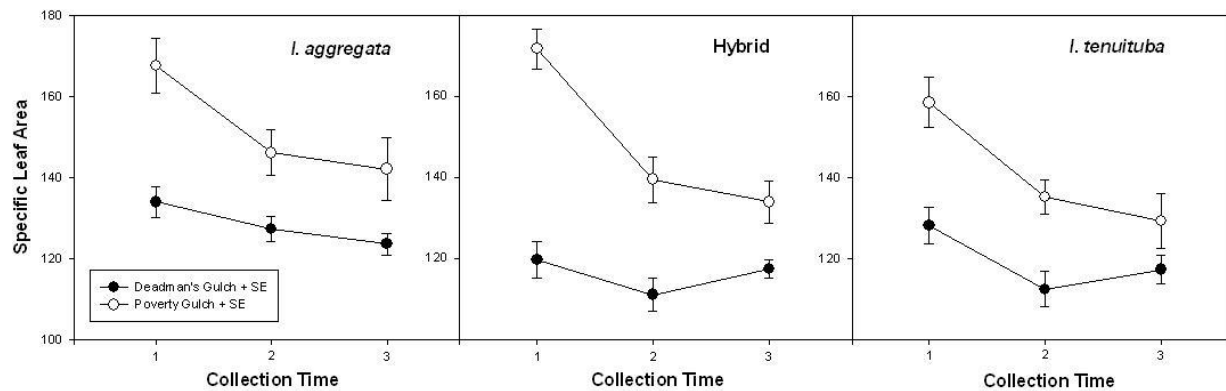


Figure 2. Specific leaf area as a function of collection time and site of origin, shown separately for each type of plant.

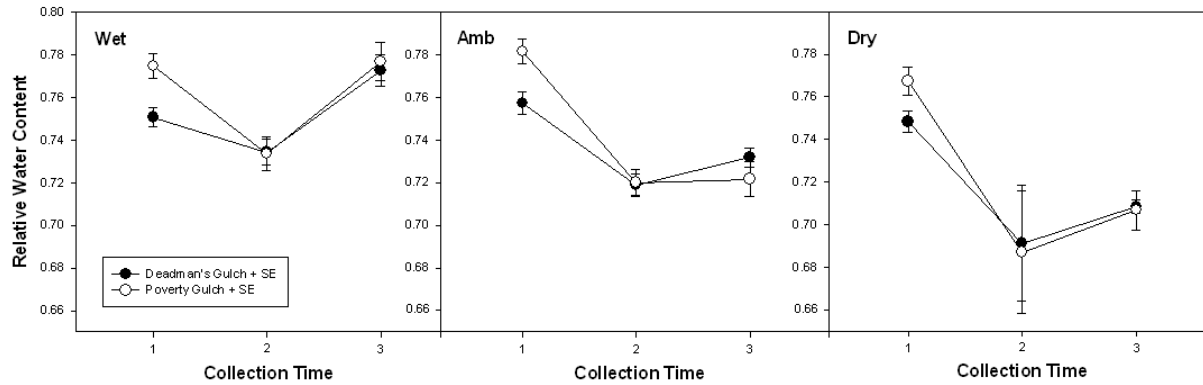


Figure 3. Relative water content by collection time and site of origin, shown separately for each water treatment.

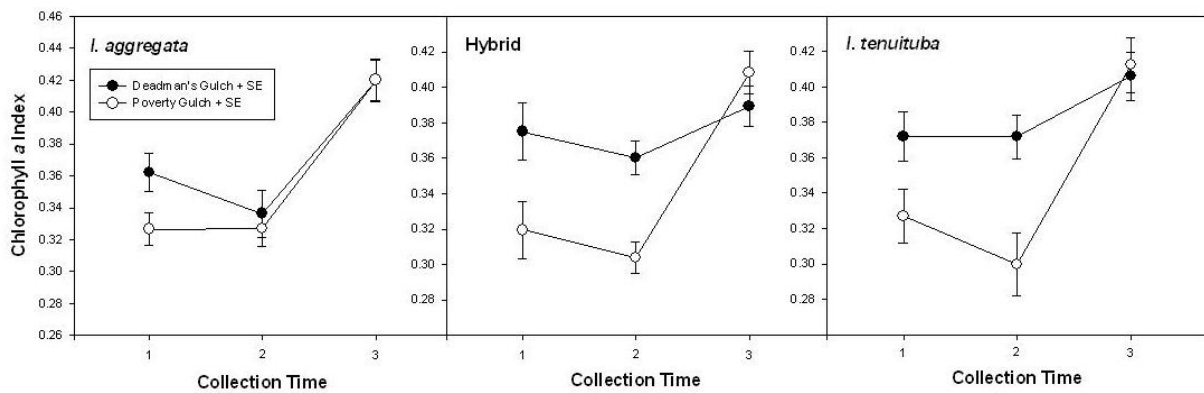


Figure 4. Chlorophyll *a* index by collection time and site of origin, shown separately for each type of plant.

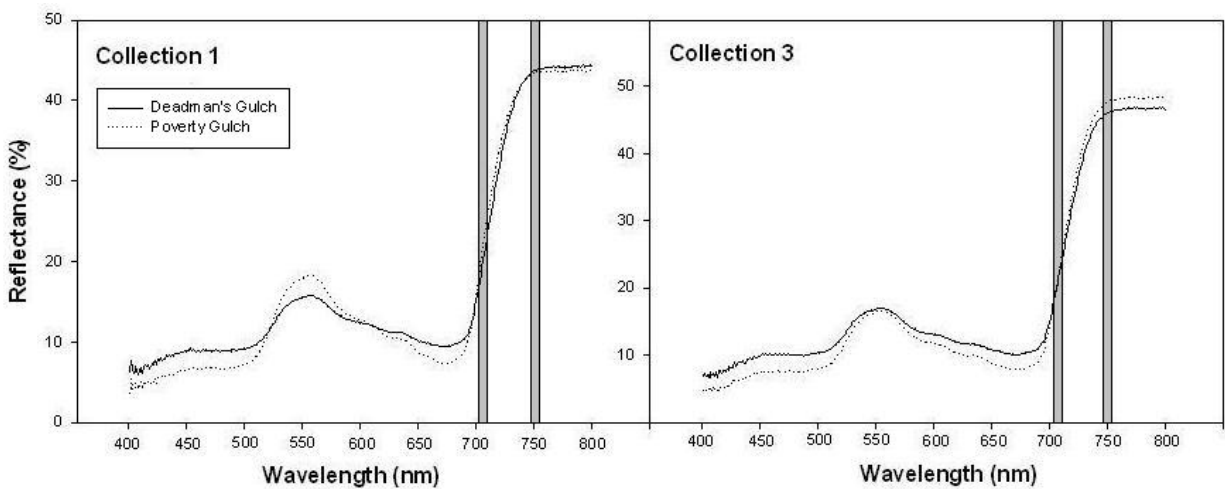


Figure 5. Leaf reflectance spectra for Poverty Gulch and Deadman's Gulch, for both Collection 1 and Collection 3. Gray bars show the wavelengths (750nm and 705nm) used to calculate chlNDI.

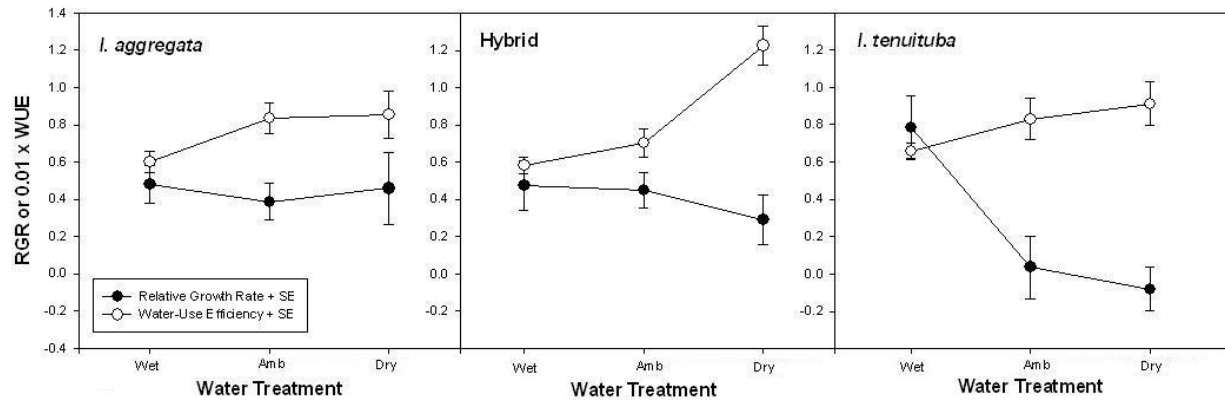


Figure 6. Relative growth rate and water-use efficiency as a function of water treatment, shown separately for each type of plant.

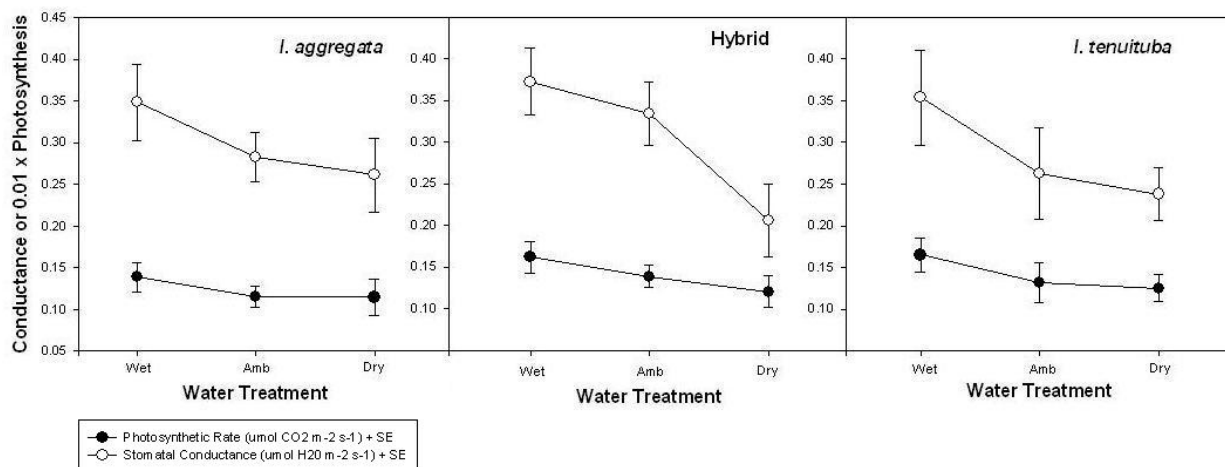


Figure 7. Conductance and photosynthetic rate as a function of water treatment, shown separately for each type of plant.

Discussion

Do *Ipomopsis* parents and hybrids show different growth and physiological responses to water availability? Investigating the physiological responses to soil moisture among *Ipomopsis* parents and hybrids revealed significantly different responses for each plant type. All plant types responded to low soil moisture by decreasing photosynthetic rate and stomatal conductance, thereby increasing their water-use efficiency, but hybrids lowered their conductance much more than either parent species.

This resulted in unusually high WUE for hybrids in dry soil, which supports previous work that found high WUE for hybrids under the relatively dry natural conditions at Poverty Gulch (Campbell et al. 2005).

Photosynthetic rate and stomatal conductance were positively correlated with relative growth rate, and the relationship with conductance was especially strong for hybrids and *I. tenuituba*. *I. tenuituba*, in fact, derived twice as much of a growth benefit from high conductance as hybrids did. As a result, dry-treatment hybrids with high WUE (due to low conductance) did not significantly increase RGR, and wet-treatment *I. tenuituba* with low WUE (due to high conductance) had dramatically high growth rates. Thus, *I. tenuituba* appeared to benefit from low WUE in the wet conditions.

Are these differences reflected in the natural distribution of these plants across certain soil moisture regimes? Although *Ipomopsis* parents and hybrids all responded to drought by reducing photosynthetic rate and conductance, thereby increasing water-use efficiency, the magnitude of these responses differed by plant type. Furthermore, growth responded in different ways, with the most extreme difference with soil moisture seen for *I. tenuituba*. Therefore, the growth component of fitness for *I. aggregata*, hybrids, and *I. tenuituba* is likely optimized in different soil moisture regimes. Water-use efficiency had no effect at all on growth rate in *I. aggregata*, suggesting that this species is a generalist and could grow at similar rates in a variety of soil moisture regimes. The exaggerated effect of conductance on growth rate in *I. tenuituba*, suggests that these plants would grow best in moist soils, where conductance and photosynthetic rate are maximized. Interestingly, *I. tenuituba* is not found in moister conditions in nature.

At both Poverty Gulch and Deadman's Gulch, *I. aggregata* is found in much moister soil than *I. tenuituba*. At both sites, hybrids grow in much drier conditions than either parent species. This pattern is puzzling, as all plants responded to dry soil by lowering stomatal conductance, and *I. tenuituba* showed reduced growth under these conditions. However, above-ground growth rate is only one component of fitness, and the remarkably high water-use efficiency that hybrids achieve in dry soil may

have beneficial consequences elsewhere. For example, high WUE may allow hybrids to increase their below-ground biomass, which could improve their ability to over-winter and grow vigorously the following spring. The occurrence of *I. aggregata* in relatively moist soils is not unusual, considering that water-use efficiency did not have consequences for growth in this species. However, it is strange that both populations of *I. tenuituba* occur in relatively dry soil, where high water-use efficiency severely reduces growth rate. As with *Ipomopsis* hybrids, however, it is possible that high WUE does not have negative consequences for overall fitness. Although *I. tenuituba* consistently grows in soil moisture regimes that do not optimize growth, its dominance at these sites from year to year suggests that high WUE generates some form of fitness benefit.

How are other plant traits (specific leaf area, relative water content, and chlorophyll a index) affected by soil moisture regime? The three leaf traits measured in conjunction with relative growth rate showed interesting responses to either water treatment, plant type, or a combination of the two. Specific leaf area decreased significantly throughout the study period but remained drastically different for plants from different sites, with Deadman's Gulch plants having denser leaves than Poverty Gulch plants. Thick leaves with low specific leaf area often have higher concentrations of photosynthetic proteins (Brock and Galen 2005), which may help explain why Deadman's Gulch plants also had higher estimates of chlorophyll and photosynthetic rate. Water treatment, however, did not have any effect on SLA, so the reason for the decrease in SLA over the study period remains unknown. It is possible that the plants were responding to a constant abiotic factor in the common garden, such as soil nutrients, light intensity, or temperature. Regardless of these unknowns, the data suggest that SLA is a plastic trait, but that variation is constrained by genetic differences between Deadman's Gulch and Poverty Gulch populations.

Relative water content differed between Poverty Gulch and Deadman's Gulch plants only in Collection 1, suggesting that this difference was due to unequal transplant shock rather than genetic

constraint. Furthermore, since temperature and relative humidity data suggest that Poverty Gulch is drier than Deadman's Gulch, it is likely that the higher initial RWC in Poverty Gulch plants does not represent the natural state of those traits. RWC was significantly affected by water treatment, with dry soil reducing RWC relative to ambient and wet soil. These data suggest that RWC is environment-mediated, with the magnitude of the response conserved between Deadman's Gulch and Poverty Gulch populations.

Chlorophyll *a* index differed significantly between sites for both Collection 1 and 2, but in Collection 3 all plants—regardless of type or site of origin—were statistically indistinguishable with regard to this index. Although water treatment did not affect chlorophyll *a* index, the fact that these indices initially differed by site and ultimately stabilized at a common value suggests that chlorophyll *a* index—like relative water content—may depend on a different abiotic factor, such as soil nutrients, light intensity, or temperature. In a greenhouse study, chlorophyll concentration in *Ipomopsis* increased with addition of soil nitrogen (D. Campbell, pers. comm.).

Are *Ipomopsis* physiological traits conserved within each plant type, or do they differ between Poverty Gulch and Deadman's Gulch populations? *Ipomopsis* parents and hybrids had higher relative growth rates in Deadman's Gulch and higher water-use efficiency in Poverty Gulch. These trends are understandable given that Poverty Gulch is drier than Deadman's Gulch, and that soil moisture is inversely proportional to WUE and proportional to RGR. Furthermore, plants from Deadman's Gulch had consistently denser leaves (lower SLA) than plants from Poverty Gulch. There were initial site differences in relative water content and chlorophyll *a* index, with plants from Deadman's Gulch containing more chlorophyll *a* and plants from Poverty Gulch retaining more water.

In conclusion, the slightly moister conditions in Deadman's Gulch seem to support *Ipomopsis* populations that, compared to Poverty Gulch, are less water-use efficient and have higher relative growth rates, greater concentration of chlorophyll *a*, and denser leaves. Each of these traits

demonstrated plasticity over the course of this study, but not necessarily in response to changes in soil moisture. Therefore, while water-use efficiency is affected by both plant type and water treatment, other abiotic factors likely have strong impacts on the distribution of *Ipomopsis* parents and hybrids in nature.

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